PI: **CHUPP, GEOFFREY L**	Title: Next Generation Endotyping of Asthma Heterogeneity	
Received: 10/01/2015	FOA: AI15-032	Council: 05/2016
Competition ID: FORMS-C	FOA Title: ASTHMA AND ALLERGIC DISEASES COOPERATIVE RESEARCH CENTERS (U19)	
1 U19 AI125281-01	Dual:	Accession Number: 3861454
IPF: 9420201	Organization: YALE UNIVERSITY	
Former Number:	Department:	
IRG/SRG: ZAI1 LAR-I (M1)	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 900,000 Year 2: 900,000 Year 3: 900,000 Year 4: 900,000 Year 5: 900,000	Animals: N Humans: Y Clinical Trial: N Current HS Code: 20 HESC: N	New Investigator: N Early Stage Investigator: N
Senior/Key Personnel:	*Organization:*	*Role Category:*
GEOFFREY CHUPP	YALE UNIVERSITY	PD/PI

Appendices

APPX_2_Adenotonsillectomy_Study_HIC_1009007345,Appx_3_Submitted_manuscript_Bothwell_Immunity,APPX_1_YCAAD_Study_HIC_010201226

OMB Number: 4040-0001
Expiration Date: 06/30/2016

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	**State Application Identifier**

1. TYPE OF SUBMISSION*

❍ Pre-application ● Application ❍ Changed/Corrected Application

4.a. Federal Identifier

b. Agency Routing Number

c. Previous Grants.gov Tracking Number

2. DATE SUBMITTED | **Application Identifier**

5. APPLICANT INFORMATION

Organizational DUNS*: 0432075620000

Legal Name*: YALE UNIVERSITY
Department:
Division:
Street1*: OFFICE OF SPONSORED PROJECTS
Street2: 25 Science Park
City*: NEW HAVEN
County:
State*: CT: Connecticut
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 065208237

Person to be contacted on matters involving this application

Prefix: First Name*: Marybeth Middle Name: Last Name*: Brandi Suffix:
Position/Title: Proposal Manager
Street1*: 25 Science Park
Street2: 150 Munson Street
City*: New Haven
County:
State*: CT: Connecticut
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 06520-8237
Phone Number*: 203-737-3495 Fax Number: Email: marybeth.brandi@yale.edu

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** 1060646973A1

7. TYPE OF APPLICANT* O: Private Institution of Higher Education

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

● New ❍ Resubmission
❍ Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).

❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Next Generation Endotyping of Asthma Heterogeneity

12. PROPOSED PROJECT

Start Date* Ending Date*
07/01/2016 06/30/2021

13. CONGRESSIONAL DISTRICTS OF APPLICANT
CT-003

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name*: GEOFFREY Middle Name: L Last Name*: CHUPP Suffix:
Position/Title: Associate Professor
Organization Name*: YALE UNIVERSITY
Department:
Division:
Street1*: 300 Cedar Street, TAC S441B
Street2: PO Box 208057
City*: New Haven
County:
State*: CT: Connecticut
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 065208057
Phone Number*: 203-785-3627 Fax Number: 203-785-3826 Email*: geoffrey.chupp@yale.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$7,479,199.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$7,479,199.00
d. Estimated Program Income*	$0.00

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ❍ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:
DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR

❍ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: First Name*: Marybeth Middle Name: Last Name*: Brandi Suffix:
Position/Title*: Proposal Manager
Organization Name*: Yale University
Department:
Division:
Street1*: 25 Science Park
Street2: 150 Munson Street
City*: New Haven
County:
State*: CT: Connecticut
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 06520-8237
Phone Number*: 203-737-3495 Fax Number: Email*: gcat5@yale.edu

Signature of Authorized Representative*
Marybeth Brandi

Date Signed*
10/01/2015

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:

424 R&R and PHS-398 Specific Table Of Contents

Component Summary

Components	Component Project Title	Organization Name	Contact PD/PI Name or Project Lead Name
Overall	Next Generation Endotyping of Asthma Heterogeneity	YALE UNIVERSITY	CHUPP, GEOFFREY L
Admin-Core-001 (113)	Administrative Core	YALE UNIVERSITY	CHUPP, GEOFFREY L
Core-001 (294)	Clinical Recruitment and Biostatistics Core	YALE UNIVERSITY	COHN, LAUREN E
Core-002 (055)	Precision Profiling Core	YALE UNIVERSITY	MONTGOMERY, RUTH R
Core-003 (780)	IOF Management Core	YALE UNIVERSITY	MONTGOMERY, RUTH R
Project-001 (246)	Single Cell Mechanisms of YKL-40 and Transcriptomic Endotypes of Asthma	YALE UNIVERSITY	CHUPP, GEOFFREY L
Project-002 (217)	Novel Immune Pathways Drive Asthma Heterogeneity	YALE UNIVERSITY	Craft, Joseph Edgar
Project-003 (552)	Asthma MAP: Computational Tools and Clustering for the Study of Asthma Heterogeneity	YALE UNIVERSITY	Gerstein, Mark Bender

Project/Performance
Site Location(s) Summary

Applicant Organization	City	State/Province	Country
YALE UNIVERSITY	NEW HAVEN	CT	UNITED STATES

Organization Name	City	State/Province	Country	Component
YALE UNIVERSITY	NEW HAVEN	CT	UNITED STATES	Admin-Core-001 (113)
YALE UNIVERSITY	NEW HAVEN	CT	UNITED STATES	Admin-Core-001 (113)
YALE UNIVERSITY	NEW HAVEN	CT	UNITED STATES	Core-001 (294)
YALE UNIVERSITY	NEW HAVEN	CT	UNITED STATES	Core-001 (294)
YALE UNIVERSITY	NEW HAVEN	CT	UNITED STATES	Core-002 (055)
YALE UNIVERSITY	NEW HAVEN	CT	UNITED STATES	Core-002 (055)
YALE UNIVERSITY	NEW HAVEN	CT	UNITED STATES	Core-003 (780)
YALE UNIVERSITY	NEW HAVEN	CT	UNITED STATES	Core-003 (780)
YALE UNIVERSITY	NEW HAVEN	CT	UNITED STATES	Overall
YALE UNIVERSITY	NEW HAVEN	CT	UNITED STATES	Project-001 (246)
YALE UNIVERSITY	NEW HAVEN	CT	UNITED STATES	Project-001 (246)
YALE UNIVERSITY	NEW HAVEN	CT	UNITED STATES	Project-002 (217)
YALE UNIVERSITY	NEW HAVEN	CT	UNITED STATES	Project-002 (217)
YALE UNIVERSITY	NEW HAVEN	CT	UNITED STATES	Project-003 (552)
YALE UNIVERSITY	NEW HAVEN	CT	UNITED STATES	Project-003 (552)

Human Subjects
Clinical Trial
Human Embryonic Stem Cells
Vertebrate Animals
Summary

Components	Human Subjects	Clinical Trial	HESC Involved	Vertebrate Animals
Overall	Y	N	N	N
Admin-Core-001 (113)	N	N	N	N
Core-001 (294)	Y	N	N	N
Core-002 (055)	Y	N	N	N
Core-003 (780)	N	N	N	N
Project-001 (246)	Y	N	N	N
Project-002 (217)	Y	N	N	N
Project-003 (552)	Y	N	N	N

Composite Application Budget Summary

Categories	Budget Period 1	Budget Period 2	Budget Period 3	Budget Period 4	Budget Period 5	TOTALS
Salary, Wages and Fringe Benefits	588,353	618,404	633,299	637,088	661,476	3,138,620
Equipment	20,000	0	0	0	0	20,000
Travel	15,950	15,950	15,950	15,950	15,950	79,750
Participant/Trainee Support Costs	0	0	0	0	0	0
Other Direct Costs (excluding Consortium)	275,697	265,646	250,751	246,962	222,574	1,261,630
Consortium Costs	0	0	0	0	0	0
Direct Costs	900,000	900,000	900,000	900,000	900,000	4,500,000
Indirect Costs	585,200	598,499	598,500	598,500	598,500	2,979,199
Total Direct and Indirect Costs	1,485,200	1,498,499	1,498,500	1,498,500	1,498,500	**7,479,199**

Total Direct Costs less Consortium F&A

NIH policy (NOT-OD-05-004) allows applicants to exclude consortium/contractual F&A costs when determining if an application falls at or beneath any applicable direct cost limit. When a direct cost limit is specified in an FOA, the following table can be used to determine if your application falls within that limit.

Category	Budget Period 1	Budget Period 2	Budget Period 3	Budget Period 4	Budget Period 5	TOTALS
Total Direct Costs less Consortium F&A	900,000	900,000	900,000	900,000	900,000	**4,500,000**

Component Budget Summary

Components	Categories	Budget Period 1	Budget Period 2	Budget Period 3	Budget Period 4	Budget Period 5	TOTALS
Admin-Core-001 (113)	Salary, Wages and Fringe Benefits	29,645	30,174	30,719	31,280	31,859	153,677
	Equipment	0	0	0	0	0	0
	Travel	7,000	7,000	7,000	7,000	7,000	35,000
	Participant/Trainee Support Costs	0	0	0	0	0	0
	Other Direct Costs (excluding Consortium)	3,500	3,500	3,500	3,500	3,500	17,500
	Consortium Costs	0	0	0	0	0	0
	Direct Costs	40,145	40,674	41,219	41,780	42,359	206,177
	Indirect Costs	26,696	27,048	27,410	27,784	28,168	137,106
TOTALS	Total Direct and Indirect Costs	66,841	67,722	68,629	69,564	70,527	**343,283**
Core-001 (294)	Salary, Wages and Fringe Benefits	120,681	128,887	132,754	125,182	137,869	645,373
	Equipment	0	0	0	0	0	0
	Travel	0	0	0	0	0	0
	Participant/Trainee Support Costs	0	0	0	0	0	0
	Other Direct Costs (excluding Consortium)	46,799	36,405	31,535	30,750	20,000	165,489
	Consortium Costs	0	0	0	0	0	0
	Direct Costs	167,480	165,292	164,289	155,932	157,869	810,862
	Indirect Costs	111,374	109,919	109,252	103,695	104,983	539,223
TOTALS	Total Direct and Indirect Costs	278,854	275,211	273,541	259,627	262,852	**1,350,085**

Core-002 (055)	Salary, Wages and Fringe Benefits	97,223	99,276	101,389	103,567	105,809	507,264
	Equipment	0	0	0	0	0	0
	Travel	950	950	950	950	950	4,750
	Participant/Trainee Support Costs	0	0	0	0	0	0
	Other Direct Costs (excluding Consortium)	69,202	58,808	57,153	62,771	58,013	305,947
	Consortium Costs	0	0	0	0	0	0
	Direct Costs	167,375	159,034	159,492	167,288	164,772	817,961
	Indirect Costs	111,305	105,757	106,063	111,246	109,574	543,945
TOTALS	Total Direct and Indirect Costs	278,680	264,791	265,555	278,534	274,346	**1,361,906**
Core-003 (780)	Salary, Wages and Fringe Benefits	0	0	0	0	0	0
	Equipment	0	0	0	0	0	0
	Travel	0	0	0	0	0	0
	Participant/Trainee Support Costs	0	0	0	0	0	0
	Other Direct Costs (excluding Consortium)	0	0	0	0	0	0
	Consortium Costs	0	0	0	0	0	0
	Direct Costs	0	0	0	0	0	0
	Indirect Costs	0	0	0	0	0	0
TOTALS	Total Direct and Indirect Costs	0	0	0	0	0	**0**
Project-001 (246)	Salary, Wages and Fringe Benefits	119,195	121,330	123,529	125,794	128,127	617,975
	Equipment	0	0	0	0	0	0
	Travel	3,000	3,000	3,000	3,000	3,000	15,000
	Participant/Trainee Support Costs	0	0	0	0	0	0

	Other Direct Costs (excluding Consortium)	52,805	50,670	48,471	46,206	43,873	242,025
	Consortium Costs	0	0	0	0	0	0
	Direct Costs	175,000	175,000	175,000	175,000	175,000	875,000
	Indirect Costs	116,375	116,375	116,375	116,375	116,375	581,875
TOTALS	Total Direct and Indirect Costs	291,375	291,375	291,375	291,375	291,375	**1,456,875**
Project-002 (217)	Salary, Wages and Fringe Benefits	82,524	84,280	86,088	87,950	89,868	430,710
	Equipment	0	0	0	0	0	0
	Travel	0	0	0	0	0	0
	Participant/Trainee Support Costs	0	0	0	0	0	0
	Other Direct Costs (excluding Consortium)	92,476	90,720	88,912	87,050	85,132	444,290
	Consortium Costs	0	0	0	0	0	0
	Direct Costs	175,000	175,000	175,000	175,000	175,000	875,000
	Indirect Costs	116,375	116,375	116,375	116,375	116,375	581,875
TOTALS	Total Direct and Indirect Costs	291,375	291,375	291,375	291,375	291,375	**1,456,875**
Project-003 (552)	Salary, Wages and Fringe Benefits	139,085	154,457	158,820	163,315	167,944	783,621
	Equipment	20,000	0	0	0	0	20,000
	Travel	5,000	5,000	5,000	5,000	5,000	25,000
	Participant/Trainee Support Costs	0	0	0	0	0	0
	Other Direct Costs (excluding Consortium)	10,915	25,543	21,180	16,685	12,056	86,379
	Consortium Costs	0	0	0	0	0	0
	Direct Costs	175,000	185,000	185,000	185,000	185,000	915,000
	Indirect Costs	103,075	123,025	123,025	123,025	123,025	595,175

TOTALS	Total Direct and Indirect Costs	278,075	308,025	308,025	308,025	308,025	**1,510,175**
TOTALS		**1,485,200**	**1,498,499**	**1,498,500**	**1,498,500**	**1,498,500**	**7,479,199**

Categories Budget Summary

Categories	Components	Budget Period 1	Budget Period 2	Budget Period 3	Budget Period 4	Budget Period 5	TOTALS
R&R Budget - Senior/Key Person Funds Requested	Admin-Core-001 (113)	12,006	12,006	12,006	12,006	12,006	60,030
	Core-001 (294)	49,507	55,578	57,246	58,963	75,766	297,060
	Core-002 (055)	28,814	28,814	28,814	28,814	28,814	144,070
	Core-003 (780)	0	0	0	0	0	0
	Project-001 (246)	67,590	68,177	68,782	69,404	70,045	343,998
	Project-002 (217)	24,012	24,012	24,012	24,012	24,012	120,060
	Project-003 (552)	26,887	27,423	27,975	28,545	29,131	139,961
TOTALS		208,816	216,010	218,835	221,744	239,774	**1,105,179**
R&R Budget - Other Personnel Funds Requested	Admin-Core-001 (113)	17,639	18,168	18,713	19,274	19,853	93,647
	Core-001 (294)	71,174	73,309	75,508	66,219	62,103	348,313
	Core-002 (055)	68,409	70,462	72,575	74,753	76,995	363,194
	Core-003 (780)	0	0	0	0	0	0
	Project-001 (246)	51,605	53,153	54,747	56,390	58,082	273,977
	Project-002 (217)	58,512	60,268	62,076	63,938	65,856	310,650
	Project-003 (552)	112,198	127,034	130,845	134,770	138,813	643,660
TOTALS		379,537	402,394	414,464	415,344	421,702	**2,033,441**
R&R Budget - Section A & B. Total Salary, Wages and Fringe Benefits (A+B)	Admin-Core-001 (113)	29,645	30,174	30,719	31,280	31,859	153,677

	Core-001 (294)	120,681	128,887	132,754	125,182	137,869	645,373
	Core-002 (055)	97,223	99,276	101,389	103,567	105,809	507,264
	Core-003 (780)	0	0	0	0	0	0
	Project-001 (246)	119,195	121,330	123,529	125,794	128,127	617,975
	Project-002 (217)	82,524	84,280	86,088	87,950	89,868	430,710
	Project-003 (552)	139,085	154,457	158,820	163,315	167,944	783,621
TOTALS		588,353	618,404	633,299	637,088	661,476	**3,138,620**
R&R Budget - Section C. Total Equipment	Admin-Core-001 (113)	0	0	0	0	0	0
	Core-001 (294)	0	0	0	0	0	0
	Core-002 (055)	0	0	0	0	0	0
	Core-003 (780)	0	0	0	0	0	0
	Project-001 (246)	0	0	0	0	0	0
	Project-002 (217)	0	0	0	0	0	0
	Project-003 (552)	20,000	0	0	0	0	20,000
TOTALS		20,000	0	0	0	0	**20,000**
R&R Budget - Domestic Travel	Admin-Core-001 (113)	7,000	7,000	7,000	7,000	7,000	35,000
	Core-001 (294)	0	0	0	0	0	0
	Core-002 (055)	950	950	950	950	950	4,750
	Core-003 (780)	0	0	0	0	0	0
	Project-001 (246)	3,000	3,000	3,000	3,000	3,000	15,000
	Project-002 (217)	0	0	0	0	0	0
	Project-003 (552)	5,000	5,000	5,000	5,000	5,000	25,000

TOTALS		15,950	15,950	15,950	15,950	15,950	**79,750**
R&R Budget - Foreign Travel	Admin-Core-001 (113)	0	0	0	0	0	0
	Core-001 (294)	0	0	0	0	0	0
	Core-002 (055)	0	0	0	0	0	0
	Core-003 (780)	0	0	0	0	0	0
	Project-001 (246)	0	0	0	0	0	0
	Project-002 (217)	0	0	0	0	0	0
	Project-003 (552)	0	0	0	0	0	0
TOTALS		0	0	0	0	0	**0**
R&R Budget - Section D. Total Travel	Admin-Core-001 (113)	7,000	7,000	7,000	7,000	7,000	35,000
	Core-001 (294)	0	0	0	0	0	0
	Core-002 (055)	950	950	950	950	950	4,750
	Core-003 (780)	0	0	0	0	0	0
	Project-001 (246)	3,000	3,000	3,000	3,000	3,000	15,000
	Project-002 (217)	0	0	0	0	0	0
	Project-003 (552)	5,000	5,000	5,000	5,000	5,000	25,000
TOTALS		15,950	15,950	15,950	15,950	15,950	**79,750**
R&R Budget - Tuition/Fees/Health Insurance	Admin-Core-001 (113)	0	0	0	0	0	0
	Core-001 (294)	0	0	0	0	0	0
	Core-002 (055)	0	0	0	0	0	0
	Core-003 (780)	0	0	0	0	0	0
	Project-001 (246)	0	0	0	0	0	0

	Project-002 (217)	0	0	0	0	0	0
	Project-003 (552)	0	0	0	0	0	0
TOTALS		0	0	0	0	0	**0**
R&R Budget - Stipends	Admin-Core-001 (113)	0	0	0	0	0	0
	Core-001 (294)	0	0	0	0	0	0
	Core-002 (055)	0	0	0	0	0	0
	Core-003 (780)	0	0	0	0	0	0
	Project-001 (246)	0	0	0	0	0	0
	Project-002 (217)	0	0	0	0	0	0
	Project-003 (552)	0	0	0	0	0	0
TOTALS		0	0	0	0	0	**0**
R&R Budget - Trainee Travel	Admin-Core-001 (113)	0	0	0	0	0	0
	Core-001 (294)	0	0	0	0	0	0
	Core-002 (055)	0	0	0	0	0	0
	Core-003 (780)	0	0	0	0	0	0
	Project-001 (246)	0	0	0	0	0	0
	Project-002 (217)	0	0	0	0	0	0
	Project-003 (552)	0	0	0	0	0	0
TOTALS		0	0	0	0	0	**0**
R&R Budget - Subsistence	Admin-Core-001 (113)	0	0	0	0	0	0
	Core-001 (294)	0	0	0	0	0	0
	Core-002 (055)	0	0	0	0	0	0

	Core-003 (780)	0	0	0	0	0	0
	Project-001 (246)	0	0	0	0	0	0
	Project-002 (217)	0	0	0	0	0	0
	Project-003 (552)	0	0	0	0	0	0
TOTALS		0	0	0	0	0	**0**
R&R Budget - Other Participants/Trainee Support Costs	Admin-Core-001 (113)	0	0	0	0	0	0
	Core-001 (294)	0	0	0	0	0	0
	Core-002 (055)	0	0	0	0	0	0
	Core-003 (780)	0	0	0	0	0	0
	Project-001 (246)	0	0	0	0	0	0
	Project-002 (217)	0	0	0	0	0	0
	Project-003 (552)	0	0	0	0	0	0
TOTALS		0	0	0	0	0	**0**
R&R Budget - Section E. Total Participants/Trainee Support Costs	Admin-Core-001 (113)	0	0	0	0	0	0
	Core-001 (294)	0	0	0	0	0	0
	Core-002 (055)	0	0	0	0	0	0
	Core-003 (780)	0	0	0	0	0	0
	Project-001 (246)	0	0	0	0	0	0
	Project-002 (217)	0	0	0	0	0	0
	Project-003 (552)	0	0	0	0	0	0
TOTALS		0	0	0	0	0	**0**
R&R Budget - Materials and Supplies	Admin-Core-001 (113)	3,500	3,500	3,500	3,500	3,500	17,500

	Core-001 (294)	37,049	26,655	21,785	21,000	18,000	124,489
	Core-002 (055)	69,202	58,808	57,153	62,771	58,013	305,947
	Core-003 (780)	0	0	0	0	0	0
	Project-001 (246)	49,805	47,670	45,471	43,206	40,873	227,025
	Project-002 (217)	92,476	90,720	88,912	87,050	85,132	444,290
	Project-003 (552)	10,915	25,543	21,180	16,685	12,056	86,379
TOTALS		262,947	252,896	238,001	234,212	217,574	**1,205,630**
R&R Budget - Publication Costs	Admin-Core-001 (113)	0	0	0	0	0	0
	Core-001 (294)	0	0	0	0	0	0
	Core-002 (055)	0	0	0	0	0	0
	Core-003 (780)	0	0	0	0	0	0
	Project-001 (246)	3,000	3,000	3,000	3,000	3,000	15,000
	Project-002 (217)	0	0	0	0	0	0
	Project-003 (552)	0	0	0	0	0	0
TOTALS		3,000	3,000	3,000	3,000	3,000	**15,000**
R&R Budget - Consultant Services	Admin-Core-001 (113)	0	0	0	0	0	0
	Core-001 (294)	0	0	0	0	0	0
	Core-002 (055)	0	0	0	0	0	0
	Core-003 (780)	0	0	0	0	0	0
	Project-001 (246)	0	0	0	0	0	0
	Project-002 (217)	0	0	0	0	0	0
	Project-003 (552)	0	0	0	0	0	0

TOTALS		0	0	0	0	0	**0**
R&R Budget - ADP/Computer Services	Admin-Core-001 (113)	0	0	0	0	0	0
	Core-001 (294)	0	0	0	0	0	0
	Core-002 (055)	0	0	0	0	0	0
	Core-003 (780)	0	0	0	0	0	0
	Project-001 (246)	0	0	0	0	0	0
	Project-002 (217)	0	0	0	0	0	0
	Project-003 (552)	0	0	0	0	0	0
TOTALS		0	0	0	0	0	**0**
R&R Budget - Subawards/Consortium/Contractual Costs	Admin-Core-001 (113)	0	0	0	0	0	0
	Core-001 (294)	0	0	0	0	0	0
	Core-002 (055)	0	0	0	0	0	0
	Core-003 (780)	0	0	0	0	0	0
	Project-001 (246)	0	0	0	0	0	0
	Project-002 (217)	0	0	0	0	0	0
	Project-003 (552)	0	0	0	0	0	0
TOTALS		0	0	0	0	0	**0**
R&R Budget - Equipment or Facility Rental User Fees	Admin-Core-001 (113)	0	0	0	0	0	0
	Core-001 (294)	0	0	0	0	0	0
	Core-002 (055)	0	0	0	0	0	0
	Core-003 (780)	0	0	0	0	0	0

	Project-001 (246)	0	0	0	0	0	0
	Project-002 (217)	0	0	0	0	0	0
	Project-003 (552)	0	0	0	0	0	0
TOTALS		0	0	0	0	0	**0**
R&R Budget - Alterations and Renovations	Admin-Core-001 (113)	0	0	0	0	0	0
	Core-001 (294)	0	0	0	0	0	0
	Core-002 (055)	0	0	0	0	0	0
	Core-003 (780)	0	0	0	0	0	0
	Project-001 (246)	0	0	0	0	0	0
	Project-002 (217)	0	0	0	0	0	0
	Project-003 (552)	0	0	0	0	0	0
TOTALS		0	0	0	0	0	**0**
R&R Budget - Other Direct Cost 1	Admin-Core-001 (113)	0	0	0	0	0	0
	Core-001 (294)	9,750	9,750	9,750	9,750	2,000	41,000
	Core-002 (055)	0	0	0	0	0	0
	Core-003 (780)	0	0	0	0	0	0
	Project-001 (246)	0	0	0	0	0	0
	Project-002 (217)	0	0	0	0	0	0
	Project-003 (552)	0	0	0	0	0	0
TOTALS		9,750	9,750	9,750	9,750	2,000	**41,000**
R&R Budget - Other Direct Cost 2	Admin-Core-001 (113)	0	0	0	0	0	0
	Core-001 (294)	0	0	0	0	0	0

	Core-002 (055)	0	0	0	0	0	0
	Core-003 (780)	0	0	0	0	0	0
	Project-001 (246)	0	0	0	0	0	0
	Project-002 (217)	0	0	0	0	0	0
	Project-003 (552)	0	0	0	0	0	0
TOTALS		0	0	0	0	0	**0**
R&R Budget - Other Direct Cost 3	Admin-Core-001 (113)	0	0	0	0	0	0
	Core-001 (294)	0	0	0	0	0	0
	Core-002 (055)	0	0	0	0	0	0
	Core-003 (780)	0	0	0	0	0	0
	Project-001 (246)	0	0	0	0	0	0
	Project-002 (217)	0	0	0	0	0	0
	Project-003 (552)	0	0	0	0	0	0
TOTALS		0	0	0	0	0	**0**
R&R Budget - Section F. Total Other Direct Cost	Admin-Core-001 (113)	3,500	3,500	3,500	3,500	3,500	17,500
	Core-001 (294)	46,799	36,405	31,535	30,750	20,000	165,489
	Core-002 (055)	69,202	58,808	57,153	62,771	58,013	305,947
	Core-003 (780)	0	0	0	0	0	0
	Project-001 (246)	52,805	50,670	48,471	46,206	43,873	242,025
	Project-002 (217)	92,476	90,720	88,912	87,050	85,132	444,290
	Project-003 (552)	10,915	25,543	21,180	16,685	12,056	86,379
TOTALS		275,697	265,646	250,751	246,962	222,574	**1,261,630**

R&R Budget - Section G. Total Direct Cost (A thru F)	Admin-Core-001 (113)	40,145	40,674	41,219	41,780	42,359	206,177
	Core-001 (294)	167,480	165,292	164,289	155,932	157,869	810,862
	Core-002 (055)	167,375	159,034	159,492	167,288	164,772	817,961
	Core-003 (780)	0	0	0	0	0	0
	Project-001 (246)	175,000	175,000	175,000	175,000	175,000	875,000
	Project-002 (217)	175,000	175,000	175,000	175,000	175,000	875,000
	Project-003 (552)	175,000	185,000	185,000	185,000	185,000	915,000
TOTALS		900,000	900,000	900,000	900,000	900,000	**4,500,000**
R&R Budget - Section H. Indirect Costs	Admin-Core-001 (113)	26,696	27,048	27,410	27,784	28,168	137,106
	Core-001 (294)	111,374	109,919	109,252	103,695	104,983	539,223
	Core-002 (055)	111,305	105,757	106,063	111,246	109,574	543,945
	Core-003 (780)	0	0	0	0	0	0
	Project-001 (246)	116,375	116,375	116,375	116,375	116,375	581,875
	Project-002 (217)	116,375	116,375	116,375	116,375	116,375	581,875
	Project-003 (552)	103,075	123,025	123,025	123,025	123,025	595,175
TOTALS		585,200	598,499	598,500	598,500	598,500	**2,979,199**
R&R Budget - Section I. Total Direct and Indirect Costs (G +H)	Admin-Core-001 (113)	66,841	67,722	68,629	69,564	70,527	343,283
	Core-001 (294)	278,854	275,211	273,541	259,627	262,852	1,350,085
	Core-002 (055)	278,680	264,791	265,555	278,534	274,346	1,361,906
	Core-003 (780)	0	0	0	0	0	0
	Project-001 (246)	291,375	291,375	291,375	291,375	291,375	1,456,875
	Project-002 (217)	291,375	291,375	291,375	291,375	291,375	1,456,875

	Project-003 (552)	278,075	308,025	308,025	308,025	308,025	1,510,175
TOTALS		1,485,200	1,498,499	1,498,500	1,498,500	1,498,500	**7,479,199**

Senior/Key Personnel
Summary

Name	Organization	Role on Project	Components
CHUPP, GEOFFREY L	YALE UNIVERSITY	PD/PI(Contact)	Overall
Bothwell, Alfred LM	YALE UNIVERSITY	Other: Co-Lead	Project-002 (217)
CHUPP, GEOFFREY L	YALE UNIVERSITY	Other: Project Lead	Admin-Core-001 (113)
CHUPP, GEOFFREY L	YALE UNIVERSITY	Other: Project Lead	Project-001 (246)
COHN, LAUREN E	YALE UNIVERSITY	Other: Project Lead	Core-001 (294)
Craft, Joseph Edgar	YALE UNIVERSITY	Other: Project Lead	Project-002 (217)
Flavell, Richard A.	YALE UNIVERSITY	Other: Co-Lead	Project-002 (217)
Gerstein, Mark Bender	YALE UNIVERSITY	Other: Project Lead	Project-003 (552)
Gomez-Villalobos, Jose Luis	YALE UNIVERSITY	Other: Co-Lead	Project-001 (246)
Krishnaswamy, Smita	YALE UNIVERSITY	Other: Co-Lead	Project-003 (552)
MANE, SHRIKANT M	YALE UNIVERSITY	Other: Co-Lead	Core-002 (055)
MONTGOMERY, RUTH R	YALE UNIVERSITY	Other: Project Lead	Core-002 (055)
MONTGOMERY, RUTH R	YALE UNIVERSITY	Other: Project Lead	Core-003 (780)
YAN, XITING	YALE UNIVERSITY	Other: Co-Lead	Core-001 (294)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Alfred L.M. Bothwell

eRA COMMONS USER NAME (credential, e.g., agency login): vertical

POSITION TITLE: Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Washington University, St. Louis MO	A.B.cum laude	05/1971	Biology
Yale University, New Haven CT	Ph.D.	05/1975	Biology
Cold Spring Harbor Lab, Cold Spring Harbor NY	Postdoc	06/1976	Virology
MIT, Cambridge MA	Postdoc	08/1982	Immunology

A. Personal Statement

I have supervised a productive research lab at the Yale Medical School and have expertise, training and great enthusiasm for the proposed studies of Wnt antagonist regulation of asthma. Much of my career has been devoted to understanding how T lymphocytes regulate immunity. We are expert in the study of mouse and human T lymphocyte function *in vitro* and in *in vivo* models. My lab has significant studies on the regulation of endogenous complement inhibitors on the immune system and vascular activation. We have also characterized the activation of vascular endothelium that occurs in allografts in humanized mouse models. Our research has shifted in the last 3 years to inflammation and its regulation via Wnt signaling. We discovered a unique role of platelet derived Dkk-1 in the development of an inflammatory allergic response in the lung to house dust mite. This protein has unexpected remarkable immunoregulatory capacity, skewing the lymphocyte response to a type 2 phenotype. We collaborate with Dr. John Hwa in the Cardiology Section in the analysis of platelets, who will provide human platelets for *in vitro* studies and with Dr. Steven Ziegler who has had a long history of contributions to the role of TSLP in asthma. In this U19 grant application I will collaborate with all members of the project. The microarray data on asthma patients suggests the pathways we have uncovered in mice are significant contributors to human asthma. I have listed recent publications on inflammation and *in vivo* mouse models (also see Contributions 4 and 5 below).

a. Kidane, D., Chae, W.J., Czochor, J., Eckert, K.A., Glazer, P.M., **Bothwell, A.L.M.,** Sweasy, J.B. (2014). Links between DNA repair and inflammation. *Crit. Rev. Biochem*. Mol. Biol., 49:116-139. PMCID: PMC4300235.
b. Senejani, A., Liu, Y., Kidane, D., Maher, S.E., Zeiss, C.J., Park, H.-J., Kashgarian, M., McNiff, J.M., **Bothwell, A.L.M.** and Sweasy, J.B. (2014). Mutation of DNA Polymerase Beta Results in Lupus. *Cell Reports* 6:1-8. PMCID: PMC3916967.
c. Ehrlich, A., Castilho, T., Goldsmith-Pestana, K., Chae, W.-J., **Bothwell, A.L.M.,** Sparwasser, T., and Diane McMahon-Pratt, D. (2014). The immunotherapeutic role of regulatory T cells in Leishmania (Viannia) panamensis infection. *J. Immunol*. 193:2961-2970. PMCID: PMC4170189.
d. Park, H.-J., Choi, J.-Y., Senejani, A.G., Tobiasova, Z., Kim, D.-H., ***Bothwell, A.L.M.** and *Choi, J.-M. (2014).The nuclear receptor PPARγ regulates T cell tolerance and germinal center formation via follicular helper T cells. *PLoS One* 9(6): e99127. *joint senior authors. PMCID: PMC4170189.

B. Positions and Honors

Positions and Employment

1975 -1978 Jane Coffin Childs Fellowship

1978 -1981	NIH, NRSA Fellowship
1982 -1988	Assistant Professor, Dept. of Pathology, Yale Medical School, New Haven, CT
1982 -1998	Lecturer, Assistant Professor, Associate Professor, Dept. of Biology, Yale University
1982 -1991	Associate Investigator, Howard Hughes Medical Institute, Yale Medical School
1986 -1989	Director of Graduate Studies for Immunobiology, Yale Medical School
1988 - 2001	Associate Professor, Section of Immunobiology, Yale Medical School
1989 -1990	President's Council on Priorities and Planning, Yale University
1989 -1992	Dean's Council on Priorities and Planning for Yale Medical School
1992 -1997	Director of Medical Studies for Immunobiology Yale Medical School
2001 -pres	Professor, Dept. of Immunobiology and Interdepartmental Programs in Vascular Biology and Transplantation (VBT) and Human Translational Immunology (HTI), Yale Medical School
2003 -pres	Director of Graduate Studies for Immunobiology, Yale Medical School

C. Contribution to Science

1. Antibody Diversity and Immunoglobulin Genes

I entered immunology when it was being transformed by molecular genetics and was thus one of the first molecular immunologists. The primary research goal was to understand the molecular basis of affinity maturation of an antibody response. It was necessary to clone the cDNAs for the expressed antibody genes from a real immune response but that had not been done before. Indeed, cloning mammalian cDNA was prohibited for a time. I began by cloning for the first time all the cDNAs for all the mouse heavy and light chain immunoglobulin (Ig) genes including all the lambda light chains and J chain Ig by subtractive hybridization. I developed methods for transforming the enfeebled bacteria for mammalian cDNA cloning which were critical and contributed to mammalian cDNA cloning in general (see the acknowledgement in cloning of an expressible form of insulin in bacteria in *PNAS* 75:3727, 1978). From the cloning of antibody genes, many new structural properties were evident from the DNA sequences that suggested functional significance. Characterization of light chain structure and expression predicted that the light chains must be synthesized in a completely functional form to achieve Ig chain allelic exclusion. Differences in membrane vs secreted IgM resided in the 3' end of the cDNA, and comparison of C57Bl/6 and BALB/c heavy chain sequences suggested the basis of allotype differences and the contribution of gene conversion to the evolution of IgG2 constant regions. Full length cDNA sequences of IgM vs IgG cDNAs from a primary vs secondary immune response hybridomas showed the basis of somatic hypermutation at the DNA level for the first time. A large number of the C57Bl/6 VH germline genes were cloned and sequenced and defined the molecular genetic basis of the NP^b and NP^a idiotypic responses. This is still one of the most highly utilized antibody model systems used to study B cell responses. Prior to starting my faculty position, I published 42 papers, many in high quality journals (e.g., 6 in *Cell*, 4 in *PNAS*, 3 in *JBC*, 2 in *Nature*).

a. Alt, F.W., **Bothwell, A.L.M.,** Knapp, M., Siden, E., Mather, E., Koshland, M. and Baltimore, D. (1980). Synthesis of secreted and membrane-bound immunoglobulin Mu heavy chains is directed by mRNAs that differ at their 3' ends. *Cell* 20: 293-301. PMID 6771018.
b. **Bothwell, A.L.M.,** Paskind, M., Reth, M., Imanishi-Kari, T., Rajewsky, K. and Baltimore, D. (1981). Heavy chain variable region contribution to the NP^b family of antibodies: Somatic mutation evident in a γ2a variable region. *Cell* 24: 625-637. PMID:6788376
c. **Bothwell, A.L.M**., Paskind, M., Schwartz, R.C., Sonenshein, G.E., Gefter, M. and Baltimore, D. (1981). Dual expression of λ genes in the MOPC-315 plasmacytoma. *Nature* 290: 65-67. PMID:6259534
d. **Bothwell, A.L.M.,** Paskind, M., Reth, M., Imanishi-Kari, T., Rajewsky, K. and Baltimore, D. (1982). Somatic variants of murine λ light chains. *Nature* 298: 380-382. PMID:6283385

2. B cell lineage development during immune responses

Once we observed the increased mutation in the NP antibody from a hyperimmune response compared with a primary response it was critical to further analyze the development of B cell lineage development and somatic hypermutation (SHM). We also studied T cell dependent (TD) and T cell independent (TI) responses to NP on a protein carrier and coupled to ficoll, respectively. This was the first analysis of a T independent response of this type and we gained new insight into the B cell repertoire utilized and the effects on subsequent TD responses. Our analysis of the primary anti-NP TD repertoire showed a striking oligoclonality. Mutation of certain important residues were noted and their effects on affinity maturation as

the primary response progressed. Studies of the TD secondary response revealed several fundamental properties. The early secondary response was also quite oligoclonal, and related clones of mutations marked their development. A quantitative analysis could be performed and it was clear that SHM must have occurred in the peripheral immune system outside the germinal center. These data provided an understanding of the cellular events in the maturation of an antibody response. Extensive analysis of VH gene analysis suggested a model for SHM involving DNA replication errors.

a. **Bothwell, A.L.M.** (1984). The genes encoding anti-NP antibodies in inbred strains of mice. In: The Biology of Idiotypes, M.I. Greene and A. Nisonoff (eds.) Plenum Publishing Corp., New York, pp 19-34.
b. Maizels, N. and **Bothwell, A.** (1985). The T cell independent immune response to the hapten NP uses a large repertoire of heavy chain genes. *Cell* 43: 715-720. PMID:2416469
c. Blier, P.R. and **Bothwell, A.L.M.** (1988). The immune response to the hapten NP in C57BL/6 mice: Insights into the structure of the B cell repertoire. *Immunol. Rev.* 105: 27-43. PMID:3058576
d. Tao, W. and **Bothwell, A.L.M.** (1990). Development of B cell lineages during a primary anti-hapten immune response. *J. Immunol.* 145:3216-3222. PMID:2230115

3. T cell receptor structure/function

Studies of T cells were initiated by cloning an antigen that was initially defined as Ly-6A/E but was also known as T cell activation protein and soon after the cloning as Sca-1 for Stem Cell Antigen-1. My research characterized the molecular genetics of this multigene family in a large number of publications and this has served as an invaluable marker for studies of hemopoietic stem cell differentiation and other stem cell populations. I also cloned and characterized the Ly-6C gene that is hypomorphic in some autoimmune prone strains. This is now a widely utilized cell surface marker for studies of myeloid cells and development of memory. There are two characteristic features of this gene family that were extensively characterized by us, the interferon response elements for both IFN-α,β and IFN-γ. We identified the first GAS site (STAT site for IFN-γ with J. Darnell) in the Ly-6E promoter and studied the tissue specific transcriptional elements regulating expression. The Ly-6E antigen was also one of the first GPI-linked proteins to be characterized via cDNA, and we studied the molecular signals for anchor addition and the properties of the membrane anchor on cell surface mobility with other cell surface receptors. Based on this knowledge, we fused the T cell receptor cDNAs to the signal sequences for the GPI anchor to create soluble T cell receptors that could be enzymatically cleaved from the cell surface of transfected T cells. This was used to measure the first affinity of completely soluble TcR/MHC peptide complexes using Biacor technology. We were also the first to clone the human homologue CD59 which turned out to be one of the final complement regulatory proteins to be identified. There were 23 publications in the Ly6/CD59 fields that contributed to issued patents that were critical to the formation of Alexion Pharmaceuticals. We studied the biochemical mechanisms of CD59 inhibition of formation of the membrane attack complex. This work stimulated significant interest in xenotransplantation because it was hoped it could offer an explanation for homologous restriction of complement.

a. LeClair, K.P., Palfree, R.G.E., Flood, P.M., Hammerling, U. and **Bothwell, A.** (1986). Isolation of a murine Ly-6 cDNA reveals a new multigene family. *EMBO J.* 5: 3227-3234. PMID:3028776; PMCID:PMC1167316
b. Philbrick, W.M., Palfree, R.G.E., Maher, S., Bridgett, M.M., Sirlin, S. and **Bothwell, A.L.M.** (1990). The CD59 antigen is a structural homologue of murine Ly-6 antigens but lacks interferon inducibility. *Eur. J. Immunol.* 20: 87-92. PMID:1689664
c. Philbrick, W.M., Maher, S., Bridgett, M.M. and **Bothwell, A.L.M.** (1990). A recombination event in the 5' flanking region of the Ly-6C gene correlates with impaired expression in the NOD, NZB and ST strains of mice. *EMBO J.* 9: 2485-2492. PMID:2164472; PMCID:PMC552277
d. Corr, M., Slanetz, A.E., Boyd, L.F., Jelonek, M.T., Al-Ramadi, B.K., Kim, Y.S., Maher, S.E., **Bothwell, A.L.M. and** Margulies, D.H. (1994). T cell receptor/MHC class I-peptide interactions: Affinity, kinetics and specificity. *Science* 265:946-949. PMID 8052850.

4. Regulatory T cell and Th17 cell control of autoimmunity

Regulatory T cells (Treg) were definitively shown to be important regulators of autoimmunity in the mid-90s, and in 2000 Foxp3 was shown to be a critical transcription factor in Treg. Treg are also important in regulation of immune responses that control development of cancer. To gain molecular insight into Treg

function, we studied mutant forms of Foxp3 and showed that one critical amino acid was necessary for the dimerization of Foxp3 that was required for function. We also showed that it is possible to deliver a recombinant form of Foxp3 fused with a short protein transduction domain made in bacteria to the nucleus of cells *in vivo*. This recombinant protein was able to reverse the severe autoimmunity in the scurfy mouse which lacks Foxp3. Intestinal inflammation can lead to inflammatory bowel disease and intestinal polyps in the Apc/Min mouse. We have probed the role of Treg and Th17 cells in both of these models. Genetic deletion of IL-17 inhibits the ability of Treg to inhibit effector T cell mediated colitis and the development of intestinal polyps. These studies of mutation in the Apc gene function in these mice have led to studies of the wnt signaling pathway notably in peripheral T cells. We have identified a remarkable contribution of wnt signaling to innate and adaptive immunity. This is accomplished via interactions with wnt antagonist derived from platelets and various lymphocyte populations.

a. Chae, W.-J., Henegariu, O., Lee, S.-K. and **Bothwell, A.L.M.** (2006). Mutant leucine zipper domain impairs both dimerization and suppressive function of Foxp3 in T cells. *PNAS USA* 103:9631-9636, PMID: 16769892.
b. Choi, J.-M., Shin, J.-H., Sohn, M.-H., Harding, M.J., Park, J.-H., Tobiasova, Z., Kim, D.-Y., Maher, S.E., Chae, W.-J., Park, S.-H., Lee, C.-G., Lee, S.-K. and **Bothwell, A.L.M.** (2010). Cell permeable Foxp3 protein alleviates autoimmune disease associated with inflammatory bowel disease and allergic airway inflammation. *PNAS USA* 107:18575-18580. PMCID: PMC2972952.
c. Chae, W.-J., Gibson, T.F., Zelterman, D., Hao, L., Henegariu, O. and **Bothwell, A.L.M.** (2010). Ablation of IL17A abrogates progression of spontaneous intestinal tumorigenesis. *PNAS USA* 107:5540-5544. PMCID: PMC2851824.
d. Park, H.-J., Choi, J.-Y., Senejani, A.G., Tobiasova, Z., Kim, D.-H., * **Bothwell, A.L.M.** and * Choi, J.-M., (2014).The nuclear receptor PPARγ regulates T cell tolerance and germinal center formation via follicular helper T cells. *PLoS One* 9(6): e99127. *joint senior authors. PMCID:PMC4170189.

5. Mechanisms of autoimmunity and alloreactivity in novel humanized mouse models

Studies of the human immune system were initially stimulated by our cloning of human CD59 which led us into functional studies of human immune responses to allogeneic vs xenogeneic endothelial cells. For the last 20 years, we have developed increasingly complex humanized mouse models. We discovered that Bcl2 transduction could render endothelial cells resistant to both *in vitro* and *in vivo* CTL mediated lysis. Initial reconstitution of human hemopoietic cells in immunodeficient mice was achieved by introduction of peripheral blood mononuclear cells (PBMC). Subsequently we have utilized CD34 hemopoietic progenitor cells as well as different mouse strains to optimize the biologic system. We began with studies of human skin grafts and synthetic microvessel grafts and then developed the human arterial grafts models to study both acute and chronic allograft rejection. These models can be utilized to study human cell interactions that occur. For instance, we were able to study the mechanisms by which T cells can transmigrate across an arterial cell wall. We are now developing humanized mouse models to study type1 diabetes and cancer.

a. Murray, A., Khodadoust, M.M., Maher, S.E., Pober, J.S. and **Bothwell, A.L.M.** (1994). Porcine aortic endothelial cells activate human T cells: Direct presentation of MHC antigens and costimulation by ligands for human CD2 and CD28. *Immunity* 1:57-63. PMID:7889399
b. Zheng, L., Gibson, T.F., Schechner, J.S., Pober, J.S. and **Bothwell, A.L.M.** (2004). Bcl-2 transduction protects human endothelial cell synthetic microvessel grafts from allogeneic T cells in vivo. *J. Immunol.* 173:3020-3026, PMID: 15322161.
c. Tobiasova, Z., Zhang, L., Yi, T., Qin, L., Kulkarni, S., Rodriguez, F.C., Choi, J.-M., Tellides, G., Pober, J.S., Kawikova, I. and **Bothwell, A.L.M.** (2011). PPARγ agonists prevent in vivo remodeling of human artery induced by alloreactive T cells. *Circulation* 124:196-205, PMCID: PMC3347866.
d. Viehmann Milam, A.A., Maher, S.E., Gibson, J.A., Lebastchi, J., Wen, L., Ruddle, N.H., Herold, K.C. and **Bothwell, A.L.M.** (2014). A humanized mouse model of autoimmune insulitis. *Diabetes*, 63:1712-1724. PMCID: PMC3994947.

Complete List of Published Work in My Bibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/alfred.bothwell.1/bibliography/41166763/public/?sort=date&direction=ascending

D. Research Support

Ongoing Research Support

R01 CA168670-01A1 (Bothwell) 03/01/14 – 02/28/19
NIH
Regulatory T cell Control of Intestinal Tumorigenesis
Goals: To address the role of Dkk1 in intestinal tumorigenesis in mice by generating Dkk1 deficient Treg. Effects of wildtype and mutant Dkk1 on Treg *in vitro* will also be evaluated and compared with Wnt3a. We have shown dramatic effects of IL-17A signaling on tumors in Apc/Min mice. The function of the tumor microenvironment will be analyzed with IL17AR conditional mice. We will study the effect of IL-17 signaling on intestinal stem cells (ISC) via ISC deficient IL-17RA cells in Apc/Min mice. Finally, we will analysis FAP and CRC in humanized mice.
Role: PI

WHRY (Women's Health Research at Yale (Bothwell) 07/01/14 – 06/30/16
Endogenous Immune Interactions to Uterine Tumors
Goals: To characterize the lymphocyte subpopulations in uterine serous patients. We will test the hypothesis that a subset of USC tumors that are hypermutated are due to a mutant DNA polymerase E (*POL E*) which is beneficial for the patient.
Role: PI

Completed Research Support (last 3 years)

Yale Skin Center SPORE Pilot grant (Bothwell) 10/01/13 – 08/31/15
Humanized mouse model of melanoma for evaluation of treatment options
Goals: To develop a humanized mouse model to evaluate patient treatments for melanoma using tumor and lymphocytes from the same patient.
Role: PI

R21 AI107957-01A1 (Bothwell) 06/01/13 – 05/30/15
NIH
Regulatory T cell mediated control of colitis by Dkk1
Goals: To characterize the function of Treg from Dkk-1 deficient (doubleridge) vs wildtype mice *in vitro* and *in vivo.* In addition the effects of wnt signaling on wnt receptor (Lrp5/6) deficient effector CD4 T cells will be characterized to assess whether the canonical wnt pathway is utilized.
Role: PI

Mary Kay Foundation (Bothwell, Sweasy,Chagpar) 07/01/12 – 6/30/14
Modeling Human Immune System Interaction with breast tumors and treatments in mice.
Goals: To establish an orthotopic breast cancer model in immunodeficient mice combined with lymphocytes from the same patient. To study the effects of irradiation on the breast cancer model.
Role: PI

R01 HL088258 (Bothwell) 04/01/08 – 03/31/13
NIH
PPAR-Mediated Inhibition of Vascular Remodeling
Goals: The goal of this project is to characterize the *in vivo* and *in vitro* role of PPARgamma ligand induced effects in T cells and endothelial cells.
Role: PI

JDRF (Bothwell) 03/01/08 – 02/28/13
JDRF Center for Developing Immune Therapies for Type I Diabetes
Project 4: Functional analysis of islet specific human T cells
Goals: To characterize the function *in vitro* and homing properties of human islet specific (insulin and GAD) T cells in humanized models of diabetes.
Role: PI

BIOGRAPHICAL SKETCH

NAME: Geoffrey Lowell Chupp

eRA COMMONS USER NAME: (credential, e.g., agency login): GCHUPP

POSITION TITLE: Professor of Medicine
Director, Yale Center for Asthma and Airway Disease
Co-Director, Center for Precision Pulmonary Medicine

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	MM/YYYY	FIELD OF STUDY
Tufts University, Boston, MA	BS	05/1986	Biology
George Washington School of Medicine, Washington DC	MD	05/1990	Medicine

A. Personal Statement

I have the knowledge experience and expertise to serve as the overall Principal Investigator (PI), the Core A PI (Administrative Core), and Project 1 PI (Single Cell Mechanisms of YKL-40 and Transcriptomic Endotypes of Asthma) for this Asthma and Allergic Diseases Cooperative Research Center (ADCRC) to lead this team of gifted scientists to define endotypes of asthma heterogeneity. Our translational asthma research program is focused on understanding the pathogenesis of asthma and asthma heterogeneity through the study of genotype-phenotype-endotype relationships in populations of asthmatics. As the director of the Yale Center for Asthma and Airways Disease (YCAAD), I have developed a translational research program integrated into an active clinical program that is focused on the management of patients with asthma. Enrolled subjects (up to 150 per year) complete a phenotyping protocol and contribute biologic specimens (from sputum induction, and blood) to a biorepository (Core B, being led by Dr. Cohn) and are available for longitudinal studies. Our team demonstrated that YKL-40 is increased in the serum and lung of a subset of severe asthmatics from this cohort (Chupp et al, *NEJM* 2007) and then identified polymorphisms in the *CHI3L1* promoter that correlated with YKL-40 levels in the blood, asthma prevalence, bronchial hyperresponsiveness and reduced lung function (Ober et al, *NEJM* 2008, Gomez et al, *JACI* 2015). These studies validated the relevance of the YKL-40 pathway in inflammation and remodeling in asthma and demonstrated the capacity to identify endotypes in a complex inflammatory disease, such as asthma, by measuring biomarkers in the circulation. These discoveries also laid the groundwork for the development of an anti-YKL-40 biologic therapy (Yaklizumab) as an NIH sponsored Center for Advanced Diagnostics and Experimental Therapeutics (CADET), of which I am Principal Investigator, which does not allow any human studies as part of the award. In addition, using this phenotyping system, we conducted an analysis of the airway transcriptome using genome-wide gene expression microarrays and identified three Transcriptomic Endotypes of Asthma (TEA) clusters that correlate with near fatal asthma and hospitalizations for asthma, again demonstrating capacity of transcriptomics to identify clinically meaningful endotypes of disease through the evaluation of mixed cell populations isolated from the sputum (Yan et al, *AJRCCM* 2015). This infrastructure will be the hub for enrollment of asthma subjects for the studies outlined in this proposal. The research plan proposed in this application is the natural extension of the "team" science approach we have developed to pursue basic pathogenesis research in human asthma. This team will achieve the goals of this proposal and has all the facilities and resources to enroll subjects from our cohort of over 800 well phenotyped asthmatics. Ultimately, the translational studies outlined in these projects will break new ground in the field of asthma pathogenesis and novel biologic based stratifications of disease.

B. Positions and Honors

Professional Experience

1990-1993 Resident, Internal Medicine, Tufts-New England Medical Center, Boston, MA
1993-1997 Fellowship, Pulmonary & Critical Care Medicine, Boston University School of Medicine
1997-1998 Clinical Instructor, Division of Pulmonary & Critical Care Medicine, Yale University School of Medicine, New Haven, CT

1998-2005	Assistant Professor, Division of Pulmonary and Critical Care Medicine, Yale University School of Medicine
1997-	Director, Pulmonary Function Laboratory, Yale New Haven Hospital, New Haven, CT
2000-	Director Yale Center for Asthma & Airways Disease, Yale-New Haven Hospital
2001-2003	Director of Bronchoscopy, Yale New Haven Hospital
2001-2008	Director, Pulmonary and Critical Care Fellowship Program, Yale University School of Medicine
2005-2015	Associate Professor of Medicine, Division of Pulmonary and Critical Care Medicine, Yale University School of Medicine
2010-	Associate Director, Human Research Unit, Yale Center for Clinical Investigation, Yale University School of Medicine
2015-	Professor of Medicine, Division of Pulmonary and Critical Care Medicine, Yale University School of Medicine

Grants and Honors

1990	Kane-King Dodek Society, George Washington University School of Medicine
1990	Alpha Omega Alpha, George Washington University School of Medicine
1994	Outstanding Fellow Award, Dept. of Medicine, Boston University Medical Center
1996	Institutional National Research Service Award, National Heart, Lung, and Blood Institutes
1994	Research Fellowship Award, American Lung Association
1998	Parker B. Francis Fellowship Award in Pulmonary Research
1999	Research Career Award (KO-8), National Heart Lung and Blood Institute
2002	Excellence in Teaching Award, Division of Pulmonary and Critical Care Medicine, Yale School of Medicine
2006	Excellence in Teaching Award, Division of Pulmonary and Critical Care Medicine, Yale School of Medicine
2006	Distinguished Service Award, Yale New Haven Hospital
2007	Distinguished Service Award, Yale New Haven Hospital
2008	Distinguished service Award, Yale New Haven Hospital
2008-2010	YCCI Pilot Project Grant, Translational and Interdisciplinary Research Pilot Projects “Genotype Phenotype Relationships of the Chitinase-Like Protein CHI3L1/YKL-40 in Childhood and Adult Asthma and the Establishment of the Yale Center for Asthma and Airways Disease (YCAAD) Research Group”
2009	National Institutes of Health Loan Repayment Award for Clinical Research

Professional Organizations

1993-	Member, American Thoracic Society
1993-	Member, American College of Chest Physicians
2000-	Member, Subcommittee on Allergy and Immunology, American Thoracic Society
2002-	Member, American Association of Pulmonary and Critical Care Medicine Program Directors
2003-	Member, Association of Subspecialty Professors

C. Contributions to Science

1. The role of chitinases and chitinase-like proteins in human asthma, airway remodeling and lung function decline. My laboratory discovered that chitinases and chitinase-like-proteins are elevated in the circulation of asthmatics. Chitinases are 18 family hydrolases that are defined by their ability to bind or hydrolyze chitin, the second most common polysaccharide in nature (the most common is cellulose). In the seminal study, we determined that YKL-40 levels in the circulation are elevated in subjects with asthma, compared to controls, and showed in three different populations (Yale, Wisconsin, and Paris), that the levels in the blood correlate with severity. We also conducted the first GWAS study linking a polymorphism in *CHI3L1* to asthma. We have also discovered 2 novel polymorphisms; one in the *CHI3L1* promoter and one in the open reading frame. The promoter polymorphism is associated with YKL-40 levels in the blood, asthma prevalence, and lung function, and the open reading frame polymorphism is associated with sputum levels of YKL-40 and asthma severity. The findings were validated multiple populations enrolled at YCAAD and through the NHLBI

sponsored Severe Asthma Research Program (SARP). Ultimately, these studies demonstrate have laid the groundwork defined the CHI3L1/YKL-40 endotype of disease that will be studied mechanistically in Project 1 of this U19 proposal.

a. **Chupp GL,** Lee CG, Jarjour N, Shim YM, Holm CT, He S, Dziura JD, Reed J, Coyle AJ, Kiener P, Cullen M, Grandsaigne M, Dombret MC, Aubier M, Pretolani M, Elias JA. A chitinase-like protein in the lung and circulation of patients with severe asthma. New Engl J Med, 2007 Nov 15;357(20):2016-27. PMID:18003958
b. Ober C, Tan Z, Sun Y, Possick JD, Pan L, Nicolae R, Radford S, Parry RR, Heinzmann A, Deichmann KA, Lester LA, Gern JE, Lemanske RF, Nicolae DL, Elias JA, **Chupp GL.** Effect of variation in CHI3L1 on serum YKL-40 level, risk of asthma, and lung function. *New Engl J Med,* 2008, 358 (16):1682-91. PMID:18403759; PMCID:PMC2629486
c. Gavala ML, Kelly EA, Esnault S, Kukreja S, Evans MD, Bertics PJ, **Chupp GL**, Jarjour NN. Segmental allergen challenge enhances chitinase activity and levels of CCL18 in mild atopic asthma. *Clin Exp Allergy*. 2013 Feb; 43(2):187-97. PMID: 23331560; PMCID:PMC3623278
d. Gomez GL, Crisafi GM, Holm CT, He X, Cohn L, Meyers DA, Hawkins GA, Bleecker ER, Jarjour N for the SARP Investigators, **Chupp GL**. Genetic variation in *CHI3L1* Contributes to asthma severity and airway expression of YKL-40. *J Allergy Clin Immunol* 2015; 136(1):51-58.e10. PMID:25592985

2. Non-invasive translational research on asthma heterogeneity. My research program is focused on understanding the pathogenesis of asthma and asthma heterogeneity through the study of genotype-phenotype relationships in populations of asthmatics. As the director of the Yale Center for Asthma and Airways Disease (YCAAD), our group of scientists and coordinators have developed a research infrastructure that is integrated with an active clinical program. Asthma subjects complete a phenotyping protocol and contribute biologic specimens (from bronchoscopy, sputum induction, and blood) to the biorepository on a continual basis. As part of the NHLBI Molecular Phenotyping of Lung Disease Study Group (R01 HL118346-01) our multi-disciplinary team of scientists developed a system to analyze genome-wide gene expression in the sputum and identified several transcriptomic endophenotypes of asthma that are associated with near fatal asthma, and hospitalizations for asthma that also have elevated levels of YKL-40 in the sputum. This the first study to use a novel pathway based unsupervised cluster analysis of induced sputum gene expression to discriminate subgroups of asthma. The study also identified a 50 gene profile that can measured in the blood to determine an individual's subgroup and risk. We have collaborated with multiple investigators to provide samples from the YCAAD biorepository. We will pursue mechanistic studies at the single cell level in these TEA clusters.

a. Ramaprakash H, Shibata T, Duffy KE, Ismailoglu UB, Bredernitz RM, Moreira AP, Coelho AL, Das AM, Fursov N, **Chupp GL,** Hogaboam CM. Targeting ST2L potentiates CpG-mediated therapeutic effects in a chronic fungal asthma model. *Am J Pathol* 2011, 179(1):104-15. PMID:21640974; PMCID:PMC3123853
b. Shibata T, Ismailoglu UB, Kittan NA, Moreira AP, Coelho AL, **Chupp GL,** Kunkel SL, Lukacs NW, and Hogaboam CM. Role of growth arrest-specific gene 6 in the development of fungal allergic airway disease in mice. *Am J Respir Cell Mol Biol.* 2014 Nov; 51: 615-625. PMID: 24810144
c. Lee N, You S, Shin MS, Lee WW, Kang KS, Kim SH, Kim WU, Homer RJ, Kang MJ, Montgomery RR, Dela Cruz CS, Shaw AC, Lee PJ, **Chupp GL,** Hwang D, and Kang I. IL-6 receptor alpha defines effector memory CD8+ T cells producing Th2 cytokines and expanding in asthma. *Am J Respir Crit Care Med.* 2014 Dec; 190 (12): 1383-1394. PMID: 25390970; PMCID:PMC4299645
d. Yan X, Chu J-H, Gomez JL, Koenigs M, Holm CT, He X, Zhao H, Mane S, Martinez FD, Ober C, Nicolae DL, Barnes KC, London SJ, Gilliland F, Weiss ST, Raby BA, Cohn L, **Chupp GL.** Non-invasive analysis of the airway transcriptome discriminates clinical phenotypes of asthma. *Am J Respir Crit Care Med.* 2015 May 15;191(10):1116-1125. PMID:25763605

3. Pre-clinical development of an anti-YKL-40 biologic for severe asthma. Based on the discoveries outlined above, we have been awarded a Center for Advanced Diagnostics and Therapeutics (CADET) to formulate and manufacture an anti-YKL-40 biologic for the treatment of severe asthma. Following the

discoveries in humans outlined above, in collaboration with Brown University, we defined the biologic effects of YKL-40 in animal models and identified its receptors (IL-13Rα2 and CRTH2), and characterized its relevance to the pathobiology of severe asthma and airway remodeling. These studies have validated the relevance of this pathway in inflammation and remodeling, making it an ideal therapeutic target in severe asthma.

a. Lee CG, Hartl D, Lee GR, Koller B, Matsuura H, Da Silva CA, Sohn MH, Cohn L, Homer RJ, Kozhich AA, Humbles A, Kearley J, Coyle A, **Chupp G,** Reed J, Flavell RA, and Elias JA. Role of breast regression protein 39 (BRP-39)/chitinase 3-like-1 in Th2 and IL-13-induced tissue responses and apoptosis. *J Exp Med* 2009, 206(5):1149-66. PMID: 19414556; PMCID: PMC2715037
b. Ahangari F, Sood A, Ma B, Takyar S, Schuyler M, Qualls C, Dela Cruz CS, **Chupp GL,** Lee CG, Elias JA. Chitinase 3-like-1 Regulates both Visceral Fat Accumulation and Asthma-like Th2 Inflammation. *Am J Respir Crit Care Med.* 2015 Apr 1;191(7):746-57. PMID: 25629580

4. Discoveries related to Pulmonary Physiology. In my role as Director of the Pulmonary Function Laboratory at Yale New Haven Hospital I have made significant contributions to understanding to the field of Pulmonary physiology. I have been the Director of the LIFE Study spirometer reading center in collaboration with Tom Gill, the Director of the Pepper Center in the Yale Program on Aging, and Carlos Fragoso. We have overseen the conduct of thousands of spirometry studies as part of the LIFE study and we have built a large biorepository (over 5,000 samples) of blood RNA from the LIFE study for the study of exercise capacity in the elderly. As an ancillary study, we received approval to longitudinally collect blood RNA samples from LIFE study subjects to pursue functional genomic research on exercise capacity decline in the elderly: the Yale LIFE RNA Biorepository. Our broad goal is to define the transcriptomic signatures in the blood that are associated with exercise capacity, physical performance, and response to physical training.

a. Pahor M, Guralnik JM, Ambrosius WT, Blair S, Bonds DE, Church TS, Espeland MA, Fielding RA, Gill TM, Groessl EJ, King AC, Kritchevsky SB, Manini TM, McDermott MM, Miller ME, Newman AB, Rejeski WJ, Sink KM, Williamson JD; LIFE Study Investigators **(Chupp GL).** Effect of structured physical activity on prevention of major mobility disability in older adults: the LIFE study randomized clinical trial. *JAMA*, 2014, Jun 18;311(23):2387-96. PMID: 24866862.
b. Vaz Fragoso CA, Beavers DP, Hankinson JL, Flynn G, Berra K, Kritchevsky SB, Liu CK, McDermott MM, Manini TM, Rejeski WJ, Gill TM; Lifestyle Interventions Independence for Elders Study Investigators **(Chupp GL).** Respiratory impairment and dyspnea and their associations with physical inactivity and mobility in sedentary community-dwelling older persons. *J Am Geriatr Soc* 2014;62(4): 622-8. PMID: 24635756.
c. Vaz Fragoso CA, Hsu FC, Brinkley T, Church T, Liu CK, Manini T, Newman AB, Stafford RS, McDermott MM, Gill TM; LIFE Study Group **(Chupp GL).** Combined reduced forced expiratory volume in 1 second (FEV1) and peripheral artery disease in sedentary elders with functional limitations. *J Am Med Dir Assoc*. 2014 Sep;15(9):665-70. PMID: 24973990
d. Assi R, Wong DJ, Boffa DJ, Detterbeck FC, Wang Z, **Chupp GL,** Kim AW. Hospital readmission after pulmonary lobectomy is not affected by surgical approach. *Ann Thorac Surg*. 2015 Feb;99(2):393-8. Epub 2014 Dec 12. PMID: 25497070

Link to Dr. Chupp's publications:
http://www.ncbi.nlm.nih.gov/sites/myncbi/geoffrey.chupp.1/bibliography/47770570/public/?sort=date&direction=ascending

D. Research Support

Ongoing Research Support

1 R01 HL118346-01 (Chupp) 04/01/13 – 03/31/17
NIH / NHLBI
Longitudinal Analysis of Transcriptomic Endophenotypes in Asthma

Using an integrative functional genomics method to identify subgroups of patients, study will evaluate gene expression in sputum from asthmatics. These studies will improve our understanding of the molecular diversity of asthma and will identify patients with similar gene networks. The results will generate new molecular diagnoses of asthma that can then be used to sub-classify patients for future research and therapeutic studies.
Role: PI

1 R01 HL118346-supplement (Chupp) 09/01/14 – 08/01/16
NIH / NHLBI
(Research Supplements to Promote Diversity in Health-Related Research)
Longitudinal Analysis of Transcriptomic Endophenotypes in Asthma
This research project explores the role of microRNAs on the sputum of individuals with asthma and its effect on asthma heterogeneity.
Role: PI

1 UH2 HL 123876-01 (Chupp/Elias) 07/01/14 –06/30/19
NIH / NHLBI
Preclinical Development of a Novel Anti-YKL-40 Biologic to Treat Severe Asthma
We will complete the pre-clinical development of a humanized monoclonal antibody against the chitinase-like protein, YKL-40, for the treatment of severe asthma. A companion diagnostic test will also be developed to measure YKL-40 in the serum that is drug-bound or free for monitoring the bioavailability, dosing, and biologic effect. The results will generate a novel therapeutic against a validated mediator of inflammation and remodeling in asthma and will lead to an Investigational New Drug (IND) application following the conclusion of the award.
Role: PI

U01 AG022376 (subaward) (Gill) 09/01/11 – 11/30/15
NIH / NHLBI
Yale LIFE Spirometry Reading Center
The aim of this proposal is to serve as the Reading Center for the spirometric measures that are being obtained in the LIFE Study
Role: Director and Co-Investigator

<u>Completed Research Support</u> (last 3 years)
R01 HL 095390 (Chupp) 09/24/08 – 07/31/13
NIH / NHLBI
Gene Expression Profiling in Asthma Severity: CHI3L1 Genotypes and Serum YKL-40
This grant characterized the genome wide expression profile associated with asthma severity and YKL 40, CHI3L1 genotypes and phenotypes. By correlating these profiles in the lung and peripheral blood, we defined novel pathways associated with asthma severity and chitinases and developed gene profiles that will have practical use in the clinical management of asthma.
Role: PI

R01 HL 093017 (Elias) 01/18/10 – 06/30/14 (NCE)
NIH / NHLBI
BRP-39/YKL-40 in Th2 Inflammation and Asthma
Characterize expression, localization and regulation of BRP-39 in antigen-challenged and transgenic murine lung and the role by which BRP-39 contributes to Th2 inflammation.
Role: Co-Investigator

BIOGRAPHICAL SKETCH

NAME: Lauren E. Cohn

eRA COMMONS USER NAME (credential, e.g., agency login): LECOHN

POSITION TITLE: Associate Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Smith College, Northampton MA	BA	1982	Biology
University of Minnesota Medical School, Minneapolis MN	MD	1987	Medicine
Columbia College of Physicians and Surgeons, New York NY	Postdoc Fellow	1993	Immunology

A. Personal Statement

This proposal will define the transcriptional endophenotypes of asthma. I am a clinician with expertise in the diagnosis and management of asthma with a practice dominated by severe asthmatics. As the Co-Director of the Yale Center for Asthma and Airway Diseases, I have helped to build our clinical database through recruitment of subjects, acquisition of patient samples through sputum induction and bronchoscopy, and intellectual commitment. I have worked closely with Dr. Chupp on his study, "*Gene Expression Profiling in Asthma Severity,*" on which I serve as a Co-Investigator. In addition, I have a bench-based research program focused on allergic airway inflammation and immunology. This particular focus on biology provides a critical basis for understanding pathways identified in our human studies. In this project I will assist with recruitment and characterization of subjects and analyze expression and immunophenotyping data based on my expertise in inflammation and immunobiology. I have a demonstrated record of successful and productive research projects in the field of airway disease, including extensive collaborations with Dr. Chupp and many other investigators.

B. Positions and Honors

Professional Experience

1987-1990 Resident in Internal Medicine, Columbia-Presbyterian Medical Center, New York, NY
1990-1991 Clinical Fellow, Pulmonary Division, Columbia University College of Physicians and Surgeons
1990-1993 Clinical Assistant Physician, Columbia-Presbyterian Medical Center
1991-1993 Post-Doctoral Research Fellow, Department of Medicine, Columbia University College of Physicians and Surgeons
1991-1993 Assistant in Clinical Medicine, Columbia University College of Physicians and Surgeons
1993-1995 Associate Research Scientist, Departments of Immunobiology and Internal Medicine, Yale University School of Medicine
1993-present Attending Physician, Yale-New Haven Hospital and V.A. Connecticut Health Care System
1996-1998 Medical Director, Department of Respiratory Care, V. A. Connecticut Health Care System
1995-2001 Assistant Professor, Department of Internal Medicine, Section of Pulmonary and Critical Care, Yale University School of Medicine
2001-present Associate Professor, Department of Internal Medicine, Section of Pulmonary, Critical Care and Sleep Medicine, Yale University School of Medicine
2005-present Co-Director, Yale Center for Asthma and Airway Diseases

Other Experience and Professional Membership

1999 Ad hoc Reviewer, NIH, NIAID, Special Emphasis Panel

2001	Ad hoc reviewer, NIH, NHLBI, Lung Biology and Pathology Study Section
2001-2006	Program Committee, American Thoracic Society, Allergy, Immunology and Inflammation Assembly
2002-2012	Reviewer, NIH, Center for Scientific Review, multiple (>10) Special Review Committees and Special Emphasis Panels.
2003-2015	Data Safety Monitoring Board, MannKind Corporation
2005 & 2008	Nominating Committee, Allergy, Immunol. Inflammation Assembly, American Thoracic Society
2005-2008	Consulting Editor, Journal of Clinical Investigation
2006-2010	Research Fellowship Peer Review Committee, American Lung Association
2008-2014	Editorial Board, American Journal of Respiratory and Critical Care Medicine
2009-present	Editorial Board, American Journal of Respiratory Cell and Molecular Biology
2015-	Co-Chair, Pulmonary Workgroup, Yale-New Haven Health Systems Clinical Integration Steering Committee

Honors and Awards

1991-1992	Sarnoff Scholarship
1995	Clinical Investigator Development Award, NIH/ NHLBI
1995	Parker B. Francis Fellowship, award declined
1998	Hellman Family Fellowship, Yale University
1998-2000	Junior Faculty Research Award, Yale University, Department of Internal Medicine
1998	Mentored Clinical Scientist Award, NHLBI, 50th Anniversary
2000-2010	R01 HL 64040-07, NIH/NHLBI, “T Cell Control of Airway Mucus”
2004-2007	American Lung Association, Career Investigator Award
2009-2011	R21 AI083475-01, NIH/NIAID “Immune Regulation in the Respiratory Tract by the Epithelial Protein PLUNC”

C. Contribution to Science

1. As Co-Director of the Yale Center for Asthma and Airway Diseases and a leader in the field of immune mechanisms of asthma, I have been active in translational research in asthma and helped to establish novel methods to define disease heterogeneity.

a. **Cohn L**, Elias JA, Chupp GL. Asthma: Mechanisms of disease persistence and progression. *Annu Rev Immunol,* 2004, 22:789-815. PMID:15032597
b. **Cohn L,** Woodruff PG. Update in asthma 2013. *Am J Respir Crit Care Med,* 2014, 189(12):1487-93. Review. PMID: 24930529.
c. Gomez JL, Crisafi GM, Holm CT, Meyers DA, Hawkins GA, Bleecker ER, Jarjour N; Severe Asthma Research Program (SARP) Investigators, **Cohn L,** Chupp GL. Genetic variation in chitinase 3-like 1 (CHI3L1) contributes to asthma severity and airway expression of YKL-40. *J Allergy Cin Immunol,* 2015, 136(1):51-58.e10. PMID:25592985; PMCID:PMC4494869
d. Yan X, Chu JH, Gomez J, Koenigs M, Holm C, He X, Perez MF, Zhao H, Mane S, Martinez FD, Ober C, Nicolae DL, Barnes KC, London SJ, Gilliland F, Weiss ST, Raby BA, **Cohn L,** Chupp GL. Noninvasive analysis of the sputum transcriptome discriminates clinical phenotypes of asthma. Am *J Respir Crit Care Med,* 2015, 191(10):1116-25.PMID:25763605

2. Defined Th2 cell and Th2 cytokine effects in models of allergic asthma, including essential role in mucus induction and eosinophilia.

a. **Cohn L**, Homer RJ, Marinov A, Rankin J, Bottomly K. Induction of airway mucus production by Th2 cells: A critical role for IL-4 in inflammation but not mucus production. *J Exp Med*, 1997, 186: 1737-1747. PMID:9362533
b. **Cohn L**, Homer R, MacLeod H, Mohrs M, Brombacher F, Bottomly K. Th2 induced airway mucus production is dependent on ILRα, but not on eosinophils. *J Immunol,* 1999, 162:6178-83. PMID:10229862

c. Whittaker L, Niu N, Temann A, Stoddard A, Flavell R, Ray A, Homer R, **Cohn L**. IL-13 mediates a fundamental pathway for airway epithelial mucus induced by CD4 T cells and IL-9. *Am, J Respir Cell Mol Biol,* 2002, 27: 593-602. PMID:12397019
d. Zhu Z, Zheng T, Homer RJ, Kim Y-K. Chen NY, **Cohn L**. Hamid Q, Elias JA. Essential role of acidic mammalian chitinase (AMCase) in asthmatic Th2 inflammation and IL-13 effector pathway activation. *Science*, 2004, 304:1678-1682. PMID: 15192232.

3. Defined pulmonary physiological responses in mouse models of acute and chronic lung diseases.

a. **Cohn L**, Tepper JS, Bottomly K. IL-4 independent induction of airway hyperresponsiveness by Th2, but not Th1, cells. *J Immunol,* 1998, 161:3813-3816. PMID: 9780144
b. Zhang X, Shan P, Jiang G, **Cohn L**, Lee PJ. Toll-like receptor 4 deficiency causes pulmonary emphysema. *J Clin Invest,* 2006, 116: 3050–3059. PMCID:PMC1616193
c. Caceres AI, Brackmann M, Elia MD, Bessac BF, Del Camino D, D'Amours M, Witek JS, Fanger CM, Chong JA, Hayward NJ, Homer RJ, **Cohn L**, Huang X, Moran MM, Jordt SE. A sensory neuronal ion channel essential for airway inflammation and hyperreactivity in asthma. *Proc Natl Acad Sci* USA, 2009, 106:9099-104. PMCID:PMC2684498
d. Takyar S, Vasavada H, Zhang JG, Ahangari F, Niu N, Liu Q, Lee CG, **Cohn** L, Elias JA. VEGF controls lung Th2 inflammation via the miR-1-Mpl (myeloproliferative leukemia virus oncogene)-P-selectin axis. *J Exp Med,* 2013, Sep 23;210(10):1993-2010. PMCID:PMC3782056.

4. Defined pathways that regulate allergic airway inflammation, including the mechanisms of Th1 cell, IFN-g inhibition of and dendritic cell and MHC II control of allergic airway disease.

a. **Cohn L**, Homer R, Niu N, Bottomly K. Th1 cells and IFN-gamma regulate allergic airway inflammation and mucus production. *J Exp Med,* 1999, 190:1309-1317. PMID: 10544202
b. Zhang DH, Yang L, **Cohn L**, Parkyn L, Homer R, Ray P, Ray A. Inhibition of allergic inflammation in a murine model of asthma by expression of a dominant-negative mutant of GATA-3. *Immunity,* 1999, 11:473-482. PMID:10549629
c. Niu N, Laufer T, Homer RJ, **Cohn L**. Cutting Edge: Limiting MHC class II expression to DC alters the ability to develop Th2-dependent allergic airway inflammation. *J Immunol*, 2009, 183: 1523-1527. PMID:19596982
d. Mitchell C, Provost K, Niu N, Homer RJ, **Cohn L**. Interferon-gamma acts on the airway epithelium to inhibit local and systemic pathology in allergic airway disease. *J Immunol,* 2011, 187:3815-20. PMID:21873527; PMCID:PMC3178669

5. Defined pathways that regulate adaptive and innate immune responses in the lung.

a. Niu N, Le Goff MK, Li F, Rahman M, Homer RJ, **Cohn L**. A novel pathway that regulates inflammatory disease in the respiratory tract. *J Immunol,* 2007, 178:3846-3855. PMID:17339484
b. Bessac BF, Sivula M, von Hehn CA, Escalera J, **Cohn L**, et al.TRPA1 is a major oxidant sensor in murine airway sensory neurons. *J Clin Invest,* 2008; 118(5):1899-910.PMID:18398506; PMCID:PMC2289796
c. Wright PL, YU J, Di P, Homer RJ, Elias JA, **Cohn L**, Sessa,W. Epithelial reticulon-4B (Nogo-B) is an endogenous regulator of asthmatic inflammation. *J Exp Med,* 2010, 207:2595-2607. PMCID:PMC2989775
d. Britto CJ, Liu Q, Curran DR, Patham B, DelaCruz CS, **Cohn L**, SPLUNC1 is a tightly regulated airway sensor in innate and adaptive immunity. *Am J Respir Cell Mol Biol,* 2013, Jun;48(6):717-24. PMID:23470624; PMCID:PMC3727874

A complete listing of my peer-reviewed publications:
http://www.ncbi.nlm.nih.gov/sites/myncbi/lauren.cohn.1/bibliograpahy/41150898/public/?sort=date&direction=ascending

D. Research Support

Ongoing Research Support

1 R01 HL118346-01 (Chupp) 08/15/13 – 08/14/17
NIH / NHLBI
Longitudinal Analysis of Transcriptomic Endophenotypes in Asthma
Project Goals: To perform validation study of transcriptional endophenotype clusters of asthma, perform immunophenotyping of TEA 1, 2, and 3, and perform a longitudinal evaluation of sputum TEA clusters in asthma.
Role: Co-Investigator

1 UH2 HL123876-01 (Chupp) 09/01/14 – 08/30/19
NIH / NHLBI
Pre-Clinical Development of a Novel Anti-YKL-40 Biologic to Treat Severe Asthma
Goals: To develop an anti-YKL-40 biological therapy for severe asthma for IND submission.
Role: Co-Investigator

UH2 HL123886-01 (Kaminski) 07/01/14 – 06/30/19
NIH / NHLBI
Mir-29 Mimicry as a Therapy for Pulmonary Fibrosis
Project Goal: To develop miR-29 mimicry as a long-term, efficient and personalized anti-fibrotic therapy.
Role: Co-Investigator

Completed Research Support (last 3 years)

U01 ES015674-01 (Jordt) 09/29/06 – 04/30/15
NIH / National Institute of Environmental Health Sciences
Targeting Injury Pathways to Counteract Pulmonary Agent and Vesicant Toxicity
Project Goals: Identify and characterize sensory neural molecular targets for chlorine and chlorine-containing warfare agents using mice lacking a novel sensory receptor in C-fibers, TRPA-1.
Role: Co-Investigator

W.W. Winchester Fund Award 2013 (Cohn) 10/01/12 – 09/30/14
Yale-New Haven Hospital
Protective Function of the Airway Epithelium in Mycobacterial Infection
Project Goals: To characterize IFN-γ-dependent airway epithelial effects on immunity and inflammation in *M. bovis* pulmonary infection and define airway epithelial cell-driven protective functions in *M. bovis* pulmonary infection.
Role: PI

R01 HL 081160-01 (Cohn) 01/01/08 – 12/31/12
NIH / NHLBI
Airway Inflammation-Related Inhibition of Disease (AIRID)
Project Goals: To define mechanisms that control induction of and mediate inhibition of a novel immunoregulatory pathway in the lung.
Role: PI

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Joseph E. Craft

eRA COMMONS USER NAME (credential, e.g., agency login): JOE_CRAFT

POSITION TITLE: Paul B. Beeson Professor of Medicine, Professor of Immunobiology, Chief of Rheumatology, Director, Investigative Medicine Program

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of North Carolina, Chapel Hill	A.B.	1969-1973	Chemistry
University of North Carolina School of Medicine	M.D.	1973-1977	Medicine
Yale University School of Medicine	Postdoctoral	1982-1985	Rheumatology/Immunology

A. Personal Statement

My research investigates the pathogenesis of systemic autoimmunity, focusing upon the activation and differentiation of CD4 T cell subsets in mice and in humans. My lab has characterized CD4 T cells that help B cells in murine models of lupus and in conventional immune responses, with the idea that information gleaned from the latter studies could be applied to our understanding of autoimmunity. We have also characterized separable CD4 T cell subsets that promote antibody and autoantibody help in secondary lymphoid organs and inflammation, including analysis of the transcriptional control and function of follicular helper and effector T cells, with additional studies on the role of CD4 T cell help for CD8 T cell development and function. Our current studies involve further characterization of these cells in mice and in humans, and dissection of the mechanisms that lead to their activation, development, and effector function in inflammation and autoimmunity.

B. Positions and Honors

Positions and Employment

1977-80 Intern & Asst. Resident, Internal Medicine, Yale-New Haven Hospital (Chief, Samuel Their, MD)
1980-82 Instructor & Asst. Professor of Medicine (General Medicine), Yale University
1985-97 Assistant, Associate & Associate (tenure) Professor of Medicine (Rheumatology), Yale University
1991- Chief, Section of Rheumatology, Yale School of Medicine
1997 Professor of Medicine, Yale University
1999 Professor of Immunobiology, Yale University
2004- Director, Investigative Medicine Program (Ph.D. program for physician-scientists), Yale University

Other Experience and Professional Memberships

1980 ABIM certified, Internal Medicine; 1988 ABIM certified, Rheumatology; recertified 2008
1989-93 Arthritis Foundation: Applied Immunology Study Section; 1997-00, Cell. Immunol. Study Section
1993- American Association of Immunologists
1995-00 NIAMS (NIH) Special Grants Review Committee, Chair 1997-00
2000-04 NIH, Immunological Sciences Study Section (now HAI Study Section), Chair 2002-04
1991-00 *The Journal of Immunology*, Associate & Section Editor
1997-09 *Arthritis & Rheumatism*, Advisory Editor
2003-04 Arthritis Foundation: Member, Executive Committee & Chair, Medical & Scientific Council
2000-10 ABIM Subspecialty Board in Rheumatology, Chair 2006-08, Chair Test Committee 2008-10
2000-08 Director of Medical Studies, Department of Immunobiology, Yale School of Medicine
2002- Board of Lupus Clinical Trials Consortium
2003-07 Chair, Scientific Advisory Board, Alliance for Lupus Research
2006-11 Immune Tolerance Network, Steering Committee
2007-10 Arthritis Foundation: Member, Research Committee
2009- Course Director (with Karen Anderson, PhD) IMED 680: Topics in Human Investigation
2010-14 Chair, Board of Scientific Counselors, NIAMS
2015- *Arthritis & Rheumatology*, Co-Editor (Basic Science of SLE)

Honors

1972 Phi Beta Kappa, University of North Carolina, Chapel Hill
1976 Alpha Omega Alpha, University of North Carolina School of Medicine
1984 Postdoctoral Fellow, Arthritis Foundation
1985 Fellow, American College of Rheumatology
1985-89 Pew Scholar in the Biomedical Sciences
1994 Elected, American Society for Clinical Investigation; 1996 Elected, Interurban Clinical Club
1998 M.A. (Honorary) Yale University
1998 Elected Fellow, American Association for the Advancement of Science
2000 NIH (NIAMS) MERIT Award
2001 Elected, Kunkel Society; 2002-05 Kirkland Scholar
2004 Bohmfalk Teaching Prize (for outstanding teaching in the basic sciences), Yale School of Medicine
2004- Society of Distinguished Teachers, Yale School of Medicine
2010 Paul B. Beeson Professor of Medicine
2014 Randy T. Fischer Award for Excellence in Basic Research at Lupus 2014
2015 Co-Chair (with Rachel Caspi, PhD) FASEB Scientific Research Conference “Autoimmunity”

C. Contribution to Science

1. Characterization of novel autoantigens and the role of CD4 T cells in autoantibody promotion. My early work was devoted to dissecting targets of the systemic autoimmune response. We identified several novel autoantigens, including the RNA processing enzyme RNase MRP (and its relationship to one of the first two-described RNA enzymes, RNase P), known as the Th autoantigen, while determining for the first time its nucleolar localization; the U2 small nuclear RNP; the human Ro^{hY5} particle; and RNA polymerase II (a scleroderma autoantigen). We were then among the first groups to characterize T cell responses to autoantigens, including collaborative studies with Mark Mamula at Yale, and their necessity, and that of their cytokines, in genesis of pathogenic autoantibodies in SLE. A representative sample of these papers, among many, include:

a. Gold H, Topper JN, Clayton DA, **Craft J**. 1989. The human RNA processing enzyme RNase MRP is identical to the Th ribonucleoprotein autoantigen and related to RNase P. *Science.* 245:1377-1380. PMID:2476849
b. Hirakata M, Okano Y, Pati U, Suwa A, Medsger TA, Hardin JA, **Craft J**. 1993. Identification of autoantibodies to RNA polymerase II: Occurrence in systemic sclerosis and association with autoantibodies to RNA polymerases I and III. *J Clin Invest*. 91:2665-2672. PMID:8390487; PMCID:PMC443330
c. Mamula MJ, Fatenejad S, **Craft J**. 1994. B cells process and present lupus autoantigens that initiate autoimmune T cell responses. *J Immunol*. 152:1453-1461. PMID:8301145
d. Peng S, Moslehi J, **Craft J**. 1997. Roles of interferon-gamma and interleukin-4 in murine lupus. *J Clin Invest*. 99:1936-1946. PMID:9109438; PMCID:PMC508018

2. Tolerance and autoimmunity and lupus. A major focus of the lab has been the regulation of CD4 T cell tolerance in normal homeostatic conditions, and how this goes awry in systemic autoimmunity. These studies led to the first identification of naturally occurring, self-reactive thymic Th17 cells that have the capacity to regulate peripheral inflammatory events, as well as to the cell-intrinsic events that lead to T cell tolerance loss in lupus, and the first demonstration of the ICOS-dependence of peripherally autoreactive CDT cells in lupus, as well as the recent characterization and disease association of follicular helper T cells in humans with SLE. A representative sampling of this work, among other studies, include:

a. Vratsanos G, Jung S, Park Y-M, **Craft J**. 2001. CD4+ T cells from lupus-prone mice are hyper-responsive to low-affinity ligands. *J Exp Med*. 193:329-337. PMID:11157053; PMCID:PMC2195926
b. Odegard J, DiPlacido L, Greenwald L, Kashgarian M, Dong C, Flavell R, **Craft J**. 2009. ICOS controls effector function, but not trafficking receptor expression, of kidney-infiltrating effector T cells in murine lupus. *J Immunol*. 182:4076-4084. PMCID: PMC2746004
c. Marks BR, Nowyhed H, Choi J-Y, Poholek AC, Odegard J, Flavell RA, **Craft J**. 2009. Thymic self-reactivity selects natural interleukin 17–producing T cells that can regulate peripheral inflammation. *Nat Immunol.* 10:1125-1132. PMCID: PMC2751862. (Accompanied by News and Views article entitled “The importance of being earnestly selfish” by Cheroutre H, Mucida D & Lambolez F, published in the same issue.) Also see Corrigendum, *Nature Immunol.* 2010. 11:97.
d. Choi JY, Ho JH, Pasoto SG, Bunin V, Kim ST, Carrasco S, Borba EF, Gonçalves CR, Costa PR, Esper GK, Bonfa E*, **Craft** J*. 2015. Circulating follicular helper-like T cells in systemic lupus erythematosus:

Association with disease activity. *Arth Rheum*. 67:988–999. (*co-senior and co-corresponding authors). PMCID: PMC4450082

3. Signals for Tfh cell development and migration. T follicular helper cells (Tfh) are required for B cell maturation in T-dependent immunity and autoimmunity. Jared Odegard, a student in my lab, was the first to characterize T cells that promote extrafollicular B cell responses in immunity and autoimmunity, revealing their close developmental relationship to Tfh cells, with both dependent upon ICOS signaling. He also uncovered the role for downregulation of the P-selectin ligand, PSGL1, in Tfh cell migration to the B cell follicle and germinal center, and PSGL1 downregulation as a novel marker of these cells, studies corroborated by Amanda Poholek in the lab (see next section, Poholek, *et al*., *J Immunol.* 2010. 185:313-326). Subsequent work by Jason Weinstein dissected the relative contributions of Ag-MHC and B-cell delivered ICOS stimulation in Tfh cell differentiation, studies Jason continued with dissection of their cytokine expression in collaborative work led by Ziv Shulman in Michel Nussenzweing's group. John Ray in my lab also led studies exploring the metabolic regulation of Tfh cells, compared to their Th1 counterparts.

a. Odegard J, Marks B, Eardley L, Poholek A, Kono D, Dong C, Favell R, **Craft J**. 2008. ICOS-dependent extrafollicular helper T cells elicit IgG production via IL-21 in systemic autoimmunity. *J Exp Med*. 205:2873-2886. PMCID: PMC2585848
b. Weinstein J, Bertino SA, Hernandez SG, Poholek AC, Teplitzky TB, Nowyhed HN, **Craft J**. 2014. B cells in T follicular helper cell development and function: Separable roles in delivery of ICOS ligand and antigen. *J Immunol*. 192:3166-3179. PMCID: PMC3991608
c. Shulman Z, Gitlin AD, Weinstein JS, Lainez B, Esplugues E, Flavell RA, **Craft J**, Nussenzweig M. 2014. Dynamic signaling by T follicular helper cells during germinal center B cell selection. *Science.* 345:1058-1062. PMCID: PMC4519234
d. Ray JP, Staron MM, Shyer JA, Ho P-C, Marshall HD, Gray SM, Laidlaw BJ, Araki K, Ahmed R, Kaech SM*, **Craft J***. 2015. The interleukin-2/mTORc1 axis designates the reciprocal signaling, differentiation, and metabolism of T helper 1 and follicular helper T cells. *Immunity* (in press). (*co-senior and co-corresponding authors)

4. Transcriptional regulation of Tfh cells. We found, in studies by Amanda Poholek in my lab in collaboration with Shane Crotty and his group at La Jolla Institute of Allergy and Immunology, that the transcription factor Bcl6 is both necessary and sufficient for Tfh cell genesis. Subsequent work by John Ray in my lab deciphered the competing cytokine and transcriptional events that dictate Tfh cell differentiation compared to that of Th1 cells. Jason Weinstein in my group, in collaboration with Pat Gallagher from Yale, then explored the genetic regulation of human Tfh cells using transcriptome analyses and chromatin immunoprecipitation followed by massively parallel sequencing, enabling construction of genome-wide maps of their candidate enhancers.

a. Johnson RJ*, Poholek AC*, Yusuf I, DiToro D, Eto D, Barnett B, Dent AL, **Craft J**, Crotty S. 2009. Bcl6 and Blimp-1 are reciprocal and antagonistic regulators of follicular helper (T_{FH}) CD4 T cell differentiation. *Science.* 325:1006-1010 (*co-first authors). PMCID: PMC2766560 (Accompanied by a *Perspectives* article entitled "The Yin and Yang of follicular helper T cells" by Awasthi A & Kuchroo VJ, published in the same issue.)
b. Poholek AC, Hansen K, Hernandez S, Eto D, Chandele A, Weinstein J, Dong X, Odegard JM, Kaech SM, Dent AL, Crotty S, **Craft J**. 2010. *In vivo* regulation of Bcl6 and T follicular helper cell development. *J Immunol.* 185:313-326. PMCID: PMC2891136.
c. Ray JP, Marshall HD, Laidlaw BJ, Staron MW, Kaech SM, **Craft J**. 2014. The transcription factor STAT3 and type I Interferons are mutually repressive insulators for differentiation of follicular helper and T helper 1 cells. *Immunity.* 40:367-377. PMCID: PMC3992517 (Accompanied by a *Perspectives* article entitled "Tfh cell differentiation: Missing Stat3 uncovers interferons' interference" by Edelmann SL and Heissmeyer V, published in the same issue.)
d. Weinstein JS, Lezon-Geyda K, Maksimova Y, Craft S, Zhang Y, Su M, Schultz VP, **Craft J***, Gallagher PG*. 2014. Global transcriptome analysis and enhancer landscape of human primary T follicular helper and T effector lymphocytes. *Blood.* 124:3719-3729 (*co-senior and co-corresponding authors). PMCID: PMC4263981

5. CD4 T cell regulation of memory T cell development. Memory T cells mediate protection against pathogen challenge. Deciphering the signals important for the induction of memory T cells is a subject of active investigation with important implications for vaccine design. We have investigated the roles of CD4 T helper cells

and CD4 regulatory T cells in memory development. For example, CD4 T cells are important in memory CD8 T cell development in the context of systemic infection; but their role in the formation of resident memory (T_{RM}) cells that mediate protection against infection at mucosal sites is unclear. In collaboration with Susan Kaech at Yale, our joint graduate student Brian Laidlaw demonstrated that CD4 T cell help is critical for the ability of T_{RM} cells to upregulate CD103 and be maintained to the lung airways following influenza infection, with the transcription factor T-bet critical for controlling T_{RM} cell formation. In related studies, we investigated the events regulating memory CD8 T cell formation during the resolution of infection, by contrast to other work in the field that has focused upon these events during infection priming. We identified a critical window during resolution of acute viral infection when regulatory CD4 T cell derived IL-10 is necessary for promotion of memory CD8 T cell maturation. These latter findings uncovered a previously unrecognized role in regulating the maturation of memory CD8 T cells, through insulation of CD8 T cells from proinflammatory signals present during the resolution phase of infection.

a. Laidlaw BJ, Zhang N, Marshall HD, Staron MM, Guan T, Hu Y, Cauley LS, **Craft J***, Kaech SM*. 2014. $CD4^+$ T cell help guides formation of $CD103^+$ lung-resident memory $CD8^+$ T cells during influenza viral infection. *Immunity*. 41:633-645. (*co-senior and co-corresponding authors). (Accompanied by a *Perspectives* article entitled “CD4 helpers put tissue-resident memory cells in their Place” by Mackay LK & Carbone FR, published in the same issue.) PMCID: PMC5324721
b. Laidlaw BJ, Cui W, Amezquita R, Gray SM, Guan T, Lu Y, Kobayashi Y, Flavell RA, Kleinstein S, **Craft J***, Kaech SM*. 2015. IL-10 production by regulatory $CD4^+$ T cells during resolution of infection promotes memory $CD8^+$ T cell maturation. *Nature Immunol* Aug;16(8):871-9. (*co-senior and co-corresponding authors). PMID:26147684

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/josep0h.craft.1/bibliography/40351952/public/?sort=date&direction=ascending

D. Research Support

Ongoing Research Support

5 R01 AR40072 (Craft) 8/31/90 – 4/30/16
NIH/NIAMS **Immune Responses in Lupus** NCE
The aims of this grant are to determine the roles of Bcl6 in PSGL1 regulation in the initial events that lead to Tfh cell migration and development, and to further dissect the requirements for Tfh cell differentiation and function. These studies are to be conducted in mice. Competitive renewal submitted. Role: PI

Target Identification in Lupus (TIL) (Craft) 2/1/15 – 1/31/18
Alliance for Lupus Research (ALR)
Characterization and Function of CD4 T Cell Subsets in Lupus
The goals of this proposal are to dissect the signals required for effector T cell development and maintenance in lupus using novel cytokine reporter and gene-mutant mice. Role: PI

1R21 AR068662 (Craft) 6/1/15 – 5/31/17
NIH/NIAMS **Follicular Helper T Cells in Immunity, Allergy, and Autoimmunity**
The aims of this proposal are to identify the mechanisms regulating germinal center Tfh-cell maturation from IL-21 to IL-4 secretion in helminth infection, and investigate the impact of this GC Tfh-cell transition on GC B-cell development. Role: PI

YA-002 Research Grant (Craft) 5/20/13 – 5/19/17
AbbVie Yale Collaboration in Immunobiology
Clinical Assessment and Therapeutic Blockade of T - B Cell Collaboration in Autoimmunity
The goals of this proposal are track circulating T follicular helper-like (cTfh-like) cells in lupus patients in relationship to disease activity, and ask if abrogation of Tfh cell development by therapeutic intervention also alters the numbers and phenotypes of cTfh-like cells in SLE. Role: PI

5 P30 AR053495 Craft (PI) 3/1/06 – 8/31/17
NIH/NIAMS **Yale Rheumatic Disease Research Core Center**
The goal of this center application is to support novel cores in generation and preservation of genetically modified mice and in *in vivo* microscopy. The PI does not receive project support. Role: PI

Rheumatology Research Foundation (Craft, Kang, Park) 7/1/14 – 6/30/16
Studying Monocytes and iPS cells in RA

The goals of this project are to derive iPSCs from peripheral blood mononuclear cells (PBMCs) of RA patients bearing the RA-risk *IRF5* SNP rs2004640; generate isogenic iPSCs by editing the RA-risk *IRF5* SNP rs2004640 to the non-risk SNP, using CAS9 (CRISPR-associated) nuclease and CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) gene editing; differentiate monocytes (MO) from iPSCs and isogenic iPSCs; define the function and transcriptomes of MO differentiated from iPSCs and isogenic iPSCs; transplant iPSCs into humanized mice, followed by assessment of their phenotype and function, and their role in induction and as therapeutic targets in inflammatory arthritis. Role: PI

Connecticut Innovations, Inc. (Craft, Kang, Park) 1/1/15 – 12/31/18 .
Studying the Therapeutic Role of IPSCs in Human Lupus
The goals of this proposal are to test whether iPSCs from lupus patients bearing the SLE-risk allele *IRF5* rs2004640 can be edited into isogenic iPSCs bearing the non-risk variant, resulting in the improvement of inflammation with the normalization of aberrant cytokine production from iPSC-differentiated monocytes. We will also ask if monocytes derived from iPSCs, and macrophages derived from them, are an important source of inflammatory cytokines in lupus. Role: PI

YA-010 Research Grant (Kaech, Craft, Herold) 7/10/14 – 7/9/16
AbbVie Yale Collaboration in Immunology
Harnessing PD-1 Inhibitory Checkpoints to Treat Autoimmune Disease
The goal of this two-year research project is to evaluate to develop reagents that can stimulate PD-1 on Tfh cells or deplete PD-1+ T cells to quell their tissue-damaging effector functions. Role: PI

5 UL1 TR000142 (Sherwin) 9/30/06 – 6/30/16
NIH/NCATS **Clinical and Translational Science Award**
This project will develop educational and research programs in translational investigation at Yale. Dr. Craft is included on this award as Director of the Investigative Medicine Program. He does not receive project support.
Role: Director, Investigative Medicine PhD Program

5 T32 AR07107 (Craft) 7/1/76 – 8/31/16
NIH/NIAMS
Training Program in Investigative Rheumatology
The goal of this project is to train five M.D. and Ph.D. postdoctoral fellows yearly for careers in investigative rheumatology and immunology. The PI does not receive salary or project support. Role: PI

Sponsored

Jason Weinstein, PhD (co-sponsor with Patrick Gallagher, MD), NIH/NIAMS K01 AR067892 4/1/15-3/31/20
Edward Herman, BS, NIH/NHLBI F30 HL120497 1/1/14-12/31/16
Brian Laidlaw, BS (co-sponsor, with Susan Kaech, PhD), NIH/NIA F31 AG047777 9/1/14-8/31/17
Abhinav Seth, MD/PhD, Scientist Development Award Rheumatology Research Foundation 7/1/13-6/30/16

Completed Research Support (last 3 years)

5 R01 AR44076-16 (Craft) 7/1/96 – 2/29/13
NIH/NIAMS **Genetic Analysis of T Cells in Lupus**
The aims of this project are to dissect the nature of the defect(s) in B cell maturation that occurs in the absence of inducible costimulator (ICOS), to characterize $CD4^+$ T cells that promote B cell maturation and peripheral inflammation in lupus-prone mice, and to determine the role of B7RP-1 in these events. Role: PI

Target Identification in Lupus (TIL) (Craft) 3/1/11 – 2/28/14
Alliance for Lupus Research (ALR)
Follicular Helper T Cells: Characterization and Function
The goal of this proposal is to characterize follicular helper cells and their ability to help B cells. Role: PI

5 R21 AR062842-02 (Craft) 4/1/12 – 3/31/14
NIH/NIAMS **A Novel B Cell Marker and Therapeutic Target in Lupus**
The goal of this project is to test the notion that PSGL-1 expression on antibody-secreting cells plays an important role in their trafficking to the bone marrow and splenic red pulp, niches for their survival, and that PSGL-1 expressing plasmablasts are expanded in the blood of patients with SLE. Role: PI

5 R21 AR063942-02 (Craft) 9/7/12 – 8/31/14
NIH/NIAMS **Manipulation of Follicular Helper T Cells in Immunity and Autoimmunity**
The goal of this proposal is genetically manipulate Tfh cells, and genes of interest within them, to evaluate their respective roles in normal and pathogenic immune responses. Role: PI

BIOGRAPHICAL SKETCH

NAME: Richard A. Flavell

eRA COMMONS USER NAME (agency login): RFLAVELL

POSITION TITLE: Sterling Professor, Chairman

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
University of Hull	BS	05/1967	Biochemistry
University of Hull	PHD	10/1970	Biochemistry

A. Personal Statement

My laboratory uses mouse reverse genetics to study innate and adaptive immunity, T cell tolerance, apoptosis and autoimmunity, and the regulation of T cell differentiation. Recent studies include the NLR family of innate sensors and their role in anti-infective responses and inflammatory disease. Most recently we have established the connection between inflammasomes, microbial homeostasis and chronic diseases. We have shown that dysbiosis of the microbiota leads to IBD and Metabolic Syndrome, including Obesity, Fatty Liver disease and Type 2 diabetes. For the past ten years, we have been developing and improving an entirely novel humanized mouse model capable of supporting engrafted human hematopoietic stem cells enabling generation of both adaptive and innate immune cells.

1. Wlodarska M, Thaiss CA, Nowarski R, Henao-Mejia J, Zhang JP, Brown EM, Frankel G, Levy M, Katz MN, Philbrick WM, Elinav E, Finlay BB, **Flavell RA**. NLRP6 inflammasome orchestrates the colonic host-microbial interface by regulating goblet cell mucus secretion. *Cell*. 2014 Feb 27;156(5):1045-59. PMID: 24581500; PMCID: PMC4017640.
2. Palm NW, de Zoete MR, Cullen TW, Barry NA, Stefanowski J, Hao L, Degnan PH, Hu J, Peter I, Zhang W, Ruggiero E, Cho JH, Goodman AL, **Flavell RA**. Immunoglobulin A coating identifies colitogenic bacteria in inflammatory bowel disease. *Cell*. 2014 Aug 28;158(5):1000-10. PMID: 25171403; PMCID: PMC4174347.
3. Rongvaux A, Jackson R, Harman CC, Li T, West AP, de Zoete MR, Wu Y, Yordy B, Lakhani SA, Kuan CY, Taniguchi T, Shadel GS, Chen ZJ, Iwasaki A, **Flavell RA**. Apoptotic caspases prevent the induction of type I interferons by mitochondrial DNA. *Cell*. 2014 Dec 18;159(7):1563-77. PMID: 25525875; PMCID: PMC4272443.
4. Gagliani N, Vesely MC, Iseppon A, Brockmann L, Xu H, Palm NW, de Zoete MR, Licona-Limón P, Paiva RS, Ching T, Weaver C, Zi X, Pan X, Fan R, Garmire LX, Cotton MJ, Drier Y, Bernstein B, Geginat J, Stockinger B, Esplugues E, Huber S, **Flavell RA**. Th17 cells transdifferentiate into regulatory T cells during resolution of inflammation. *Nature*. 2015 Apr 29; PMID: 25924064.

B. Positions and Honors

Positions and Employment

1970 - 1972	Postdoctoral Fellow, University of Amsterdam
1972 - 1973	Postdoctoral Fellow, University of Zurich
1974 - 1979	Assistant Professor (equivalent), University of Amsterdam
1979 - 1982	Head, Lab of Gene Structure and Expression, NIMR, Mill Hill, London
1982 - 1988	President, Chief Scientific Officer; Director, Molecular Immunology, Biogen, Cambridge, MA
1988 -	Sterling Professor, Chairman, Yale University
1988 -	Investigator, Howard Hughes Medical Institute
1995 - 1995	Darwin Trust Visiting Professor, Department of Cellular and Molecular Biology, University of Edinburgh, Scotland
2009 -	Adjunct Professor, Scripps Research Institute, FL
2014 -	President, International Cytokine and Interferon Society (ICIS)

Other Experience and Professional Memberships

1978 -	Elected Member, EMBO
1981 - 1982	Member, EMBO Council
1984 -	Elected Fellow, The Royal Society
1989 - 2000	Member, Scientific Advisory Committee, Harold C. Simmons Arthritis Research Center, Dallas, TX
1990 - 1993	Member, Scientific Advisory Board, Zentrum fur Molekulare Biologie der Universitat, Heidelberg
1991 - 1993	Member, NIH Study Section, (NIAID) AIDS and Related Research Review Group
1993 -	Member, National Institutes of Health Reviewers Reserve
1994 - 2001	Member, Scientific Advisory Committee, Jane Coffin Childs Memorial Fund
1995 - 1998	Member, Scientific Advisory Committee, Gwen Knapp Center for Lupus and Immunology Research
1997 -	Member, Intl. Scientific Advisory Board, Netherlands Cancer Institute/Antoni van Leeuwenhoek Hosp.
1998 - 2001	Member, Chairman, International Scientific Advisory Board, Joint Infrastructure Fund, Wellcome Trust
2000 -	Member, Advisory Board, MRC, NIMR
2000 - 2002	Elected Fellow, American Association for the Advancement of Science
2001 - 2011	Member, Imperial Cancer Research Fund Scientific Advisory Committee
2002 -	Elected Member, The National Academy of Sciences
2006 -	Elected Member, Institute of Medicine
2007 -	Elected Member, The Henry Kunkel Society
2009 -	Member, European Research Institute for Integrated Cellular Pathology (ERI-ICP)
2010 -	Member, Yale Comprehensive Cancer Center, Yale University
2011 -	Founding Member, European Academy for Tumor Immunology (EATI)
2012 -	Member, Board of Honorary Advisors of the IUBMB
2013 -	Member, American Association for Cancer Research (AACR)
2013 -	Honorary Lifetime Member, The Scandinavian Society for Immunology
2014 -	Honorary Lifetime Member, British Society for Immunology

Honors

1980	Colworth Medal, The Biochemical Society
1980	FEBS Anniversary Prize, Federation of European Biochemical Societies
1995	Darwin Trust Prize, University of Edinburgh
2001	J.S. and H.R. Blumenthal Lectureship, Center for Immunology
2001	Distinguished Service Award, Miami Nature Biotechnology Winter Symposia
2007	Honorary Professor, NanKai University
2007	Honorary Professor, Wuhan University
2008	Rabbi Shai Shacknai Memorial Prize & Lectureship in Immunology and Cancer Research , Hebrew University-Hadassah Medical School, Jerusalem
2008	AAI-Invitrogen Meritorious Career Award, American Association of Immunologists
2010	Honorary Professor, Suzhou University, China
2011	Andrew Lazarovitz Award, Canadian Society of Transplantation, Quebec
2011	Honorary Professor, Division of Infection and Immunity, University College London
2011	The Gold Medal and Certificate of Honor for his outstanding contributions to understanding of the immune system using reverse genetics in the mouse, Cell Signaling Networks
2012	The William B. Coley Award for Distinguished Research in Basic and Tumor Immunology, Cancer Research Institute
2013	The Vilcek Prize in Biomedical Science, Vilcek Foundation
2013	Honorary Director of the International Immunology Center of the Biomedical Translational Research Institute, Jinan University, Guangzhou

2014 Star of Hope Award, JDRF Connecticut Chapter

C. Contribution to Science

1. I was the first to develop and employ reverse genetics, genotype to phenotype, as a postdoc with Weissmann. Before this study, all genetics were forward, i.e. phenotype to genotype. In my own laboratory, I continued in this field and this approach formed the basis of my subsequent studies until the present.
   a. **Flavell RA**, Sabo DL, Bandle EF, Weissmann C. Site-directed mutagenesis: generation of an extracistronic mutation in bacteriophage Q beta RNA. *J Mol Biol*. 1974 Oct 25;89(2):255-72. PMID: 4444051.
   b. **Flavell RA**, Sabo DL, Bandle EF, Weissmann C. Site-directed mutagenesis: effect of an extracistronic mutation on the in vitro propagation of bacteriophage Qbeta RNA. *Proc Natl Acad Sci U S A.* 1975 Jan;72(1):367-71. PMID: 47176; PMCID: PMC432306.

2. I am a co-discoverer of introns in cellular genes and I showed how DNA methylation correlates inversely with, and prevents gene expression.
   a. Jeffreys AJ, **Flavell RA**. The rabbit beta-globin gene contains a large large insert in the coding sequence. *Cell*. 1977 Dec;12(4):1097-108. PMID: 597859.
   b. Waalwijk C, **Flavell RA**. DNA methylation at a CCGG sequence in the large intron of the rabbit beta-globin gene: tissue-specific variations. *Nucleic Acids Res.* 1978 Dec;5(12):4631-4. PMID: 745990; PMCID: PMC342778.
   c. van der Ploeg LH, **Flavell RA**. DNA methylation in the human gamma delta beta-globin locus in erythroid and nonerythroid tissues. *Cell*. 1980 Apr;19(4):947-58. PMID: 6247075.
   d. Busslinger M, Hurst J, **Flavell RA**. DNA methylation and the regulation of globin gene expression. *Cell*. 1983 Aug;34(1):197-206. PMID: 6883509.

3. I have been instrumental in discovering the molecular basis of T-cell differentiation from precursor cells into differentiated subsets and provided the first example of gene regulation in trans via “kissing chromosomes”. We showed recently that when Th17 cells are tolerized, they transdifferentiate into regulatory T cells.
   a. Zheng W, **Flavell RA.** The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. *Cell*. 1997 May 16;89(4):587-96. PMID: 9160750.
   b. Spilianakis CG, Lalioti MD, Town T, Lee GR, **Flavell RA**. Interchromosomal associations between alternatively expressed loci. *Nature*. 2005 Jun 2;435(7042):637-45. PMID: 15880101.
   c. Esplugues E, Huber S, Gagliani N, Hauser AE, Town T, Wan YY, O'Connor W Jr, Rongvaux A, Van Rooijen N, Haberman AM, Iwakura Y, Kuchroo VK, Kolls JK, Bluestone JA, Herold KC, **Flavell RA**. Control of TH17 cells occurs in the small intestine. *Nature*. 2011 Jul 17;475(7357):514-8. PMID: 21765430; PMCID: PMC3148838.
   d. Gagliani N, Vesely MC, Iseppon A, Brockmann L, Xu H, Palm NW, de Zoete MR, Licona-Limón P, Paiva RS, Ching T, Weaver C, Zi X, Pan X, Fan R, Garmire LX, Cotton MJ, Drier Y, Bernstein B, Geginat J, Stockinger B, Esplugues E, Huber S, **Flavell RA**. Th17 cells transdifferentiate into regulatory T cells during resolution of inflammation. *Nature*. 2015 Apr 29; PMID: 25924064.

4. My laboratory has elucidated mechanisms of innate immune sensing to bacteria and viruses.
   a. Alexopoulou L, Holt AC, Medzhitov R, **Flavell RA**. Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. *Nature*. 2001 Oct 18;413(6857):732-8. PMID: 11607032.
   b. Kobayashi K, Inohara N, Hernandez LD, Galán JE, Núñez G, Janeway CA, Medzhitov R, **Flavell RA**. RICK/Rip2/CARDIAK mediates signaling for receptors of the innate and adaptive immune systems. *Nature*. 2002 Mar 14;416(6877):194-9. PMID: 11894098.
   c. Kobayashi K, Hernandez LD, Galán JE, Janeway CA Jr, Medzhitov R, **Flavell RA**. IRAK-M is a negative regulator of Toll-like receptor signaling. *Cell*. 2002 Jul 26;110(2):191-202. PMID: 12150927.
   d. Alexopoulou L, Thomas V, Schnare M, Lobet Y, Anguita J, Schoen RT, Medzhitov R, Fikrig E, **Flavell RA**. Hyporesponsiveness to vaccination with Borrelia burgdorferi OspA in humans and in TLR1- and TLR2-deficient mice. *Nat Med.* 2002 Aug;8(8):878-84. PMID: 12091878.

5. My lab has shown that the NLRP6 inflammasome maintains homeostasis with the gut microbiota through controlling the mucus layer. In the absence of this control, dysbiosis, and hence susceptibility to IBD, metabolic

syndrome and colon cancer results. We showed that dysbiotic microbes drive intestinal inflammation and elicit specific IgA responses that identified the organisms that appear to play a causal role in human Crohn's Disease and Ulcerative Colitis.

a. Elinav E, Strowig T, Kau AL, Henao-Mejia J, Thaiss CA, Booth CJ, Peaper DR, Bertin J, Eisenbarth SC, Gordon JI, **Flavell RA**. NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. *Cell*. 2011 May 27;145(5):745-57. PMID: 21565393; PMCID: PMC3140910.
b. Henao-Mejia J, Elinav E, Jin C, Hao L, Mehal WZ, Strowig T, Thaiss CA, Kau AL, Eisenbarth SC, Jurczak MJ, Camporez JP, Shulman GI, Gordon JI, Hoffman HM**, Flavell RA**. Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. *Nature*. 2012 Feb 1;482(7384):179-85. PMID: 22297845; PMCID: PMC3276682.
c. Wlodarska M, Thaiss CA, Nowarski R, Henao-Mejia J, Zhang JP, Brown EM, Frankel G, Levy M, Katz MN, Philbrick WM, Elinav E, Finlay BB, **Flavell RA**. NLRP6 inflammasome orchestrates the colonic host-microbial interface by regulating goblet cell mucus secretion. *Cell*. 2014 Feb 27;156(5):1045-59. PMID: 24581500; PMCID: PMC4017640.
d. Palm NW, de Zoete MR, Cullen TW, Barry NA, Stefanowski J, Hao L, Degnan PH, Hu J, Peter I, Zhang W, Ruggiero E, Cho JH, Goodman AL, **Flavell RA**. Immunoglobulin A coating identifies colitogenic bacteria in inflammatory bowel disease. *Cell*. 2014 Aug 28;158(5):1000-10. PMID: 25171403; PMCID: PMC4174347.

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/myncbi/browse/collection/40343835/?sort=date&direction=descending

D. Research Support

Ongoing Research Support

Abbvie-Yale Collaboration (Flavell) 07/10/14 – 07/09/18
YA007
Rerouting pathogenic T cells to regulatory T cells to cure autoimmune disease
1) Test the conversion of effector to regulatory T cells during an immune response. 2) To identify bioactive compounds that target the molecular pathways responsible for the conversion by unbiased HTS screening and by screening candidate inhibitors predicted by Aim1; and validate the target pathways involved by gene targeting. 3) To test the efficacy of selected compounds to block inflammatory diseases in reporter mice and our newly developed humanized mice.
Role: PI

Abbvie-Yale Collaboration (Flavell) 07/10/15 – 06/30/17
YAP-013-2015
Innate lymphoid cells as a target in psoriasis and autoimmune disease
The purpose of this grant is to 1. Develop a mouse reporter strain that will allow us to follow the fate of ILC2s and possible conversion to ILC3s. 2. Establish a suitable experimental model to test the conversion between ILCs in the psoriasis induced setting.
Role: PI

Blavatnik Family Foundation 03/01/13 – 02/28/18
(Flavell/Medzhitov)
Inflammation, Homeostasis, and Chronic Disease
To investigate key aspects of the relationship between inflammatory system and chronic diseases such as obesity, type 2 diabetes, metabolic disease, and inflammatory bowel disease to pave the way for the discovery of new prevention strategies.
Role: PI

N01-HHSN272201100019C (Fikrig / Montgomery) 06/21/11 – 06/20/16
NIH
Innate immune pathways in elderly and immunosuppressed populations

This is a contract to examine the mechanisms of impaired immune responses to agents of biodefense in elderly and immunosuppressed populations.
Role: KP

R21 AI110776-02 (Flavell) 04/01/14 – 03/31/16
NIH / NIAID
Identifying lincRNAs critical in asthma pathogenesis
This project will explore the potential role of the recently identified class of large intergenic non-coding RNAs (lincRNAs) in the development of asthma and will seek to identify novel targets for therapeutic intervention.
Role: PI

OPP1021571 (Flavell) 11/01/10 – 10/31/15
Bill & Melinda Gates Foundation
A Mouse Model for Human Malaria Infection
Goals: To generate a mouse model for plasmodium falciparum and vivax infection.
Role: PI

Completed Research Support (Last 3 years)
None

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Mark Gerstein

eRA COMMONS USER NAME (credential, e.g., agency login): MGERSTEIN

POSITION TITLE: Albert L. Williams Professor of Biomedical Informatics

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR	FIELD OF STUDY
Harvard College	AB	1989	Physics
Cambridge University	PhD	1993	Bioinformatics/Chemistry
Stanford University	post-doc	1993-1996	Bioinformatics

A. Personal Statement

This proposal involves work in computational genomics. Prof Gerstein is a leader in this field and thus well-suited to be part of this the proposal. He has many peer-reviewed publications (as of late 2014, >475 total with an H-index via Google scholar of >120). He has served as the "computational" lead on many previous NIH-funded genomics projects (e.g. ENCODE & 1000 Genomes). Most recently, he has developed quantitative approaches and practical tools for the processing of next-generation sequencing data, including those related to chIP-seq, RNA-seq and the detection of DNA structural variation. He has also developed approaches and tools to construct and analyze molecular networks and to perform integrative data mining on a wide variety of functional genomics datasets.

B. Positions and Honors

Positions and Employment

2006- AL Williams Prof. Biomedical Informatics, Yale
2002- co-director Yale Computational Biology and Bioinformatics Program
1999- Prof. of Computer Science, Yale (asst., '99-'01; assoc. '01-'06)
1997- Prof. Molecular Biophysics & Biochemistry, Yale (asst., '97-'01; assoc '01-'06)

Honors

1989-1993 Herchel-Smith Scholarship funding for PhD at Cambridge
1993-1996 Damon Runyon-Walter Winchell post-doctoral Fellowship
1997-2001 Young Investigator Awards from Navy & IBM, PhRMA, Donaghue, & Keck foundations
2009 AAAS Fellow
2015 ISCB Fellow

Other Experience and Professional Memberships

Editorial boards: *Genome Res*, *MSB*, *J Struc Func Gen*, *PLoS Comp Bio*, *GenomeBiology*
Analysis Working Group co-chair:

2007-14 modENCODE
2013- exRNA Consortium
2009- Brainspan Project
2012- 1000 Genomes Functional Interpretation Group
2014- PsychENCODE

C. Contribution to Science

1. Interpretation of Human Genetic Variation

The sheer number of DNA variations between people from re-sequencing of human genomes, both healthy and diseased, makes it a daunting task to look for the proverbial needle in the haystack that point to interesting biology. Identifying variants that are most likely to impact function is therefore crucial to understand their effects.

Toward this goal, our research focuses on tying together annotation, prediction methods and integration of data to understand the effects of genetic variants.

a. E Khurana, Y Fu, V Colonna, XJ Mu... (42 authors)... H Yu, MA Rubin, C Tyler-Smith, **M Gerstein** (2013). Integrative annotation of variants from 1092 humans: Application to cancer genomics. *Science* 342: 1235587 [PMC3947637].
b. E Khurana, Y Fu, J Chen, **M Gerstein** (2013). Interpretation of genomic variants using a unified biological network approach. *PLoS Comput Biol* 9: e1002886. [PMC3591262]
c. A Abyzov, R Iskow, O Gokcumen, DW Radke, S Balasubramanian, B Pei, L Habegger, The 1000 Genomes Project Consortium, C Lee, **M Gerstein** (2013). Analysis of variable retroduplications in human populations suggests coupling of retrotransposition to cell division. *Genome Res* 23: 2042 [PMC3847774]
d. Y Fu, Z Liu, S Lou, J Bedford, X Mu, KY Yip, E Khurana, **M Gerstein** (2014). FunSeq2: A framework for prioritizing noncoding regulatory variants in cancer. *Genome Biol* 15: 480. [PMC4203974]

2. Human Genome Annotation

Comprehensive annotation is the cornerstone for genomic analyses. A high-quality annotation set is essential for correct interpretation of genomics studies. We have developed tools to identify cis-regulatory elements, non-coding RNAs (ncRNAs), pseudogenes and regions of intergenic transcription.

a. **M Gerstein**, A Kundaje... (50 authors)... R Myers, S Weissman, M Snyder (2012). Architecture of the human regulatory network derived from ENCODE data. *Nature* 489: 91 [PMC4154057]
b. KY Yip, C Cheng, N Bhardwaj, JB Brown, J Leng, A Kundaje, J Rozowsky, E Birney, P Bickel, M Snyder, **M Gerstein** (2012). Classification of human genomic regions based on experimentally determined binding sites of more than 100 transcription-related factors. *Genome Biol* 13: R48. [PMC3491392]
c. A Harmanci, J Rozowsky, **M Gerstein** (2014). MUSIC: Identification of enriched regions in ChIP-Seq experiments using a mappability-corrected multiscale signal processing framework. *Genome Biol* 15: 474. [PMC4234855]
d. D Wang, KK Yan, C Sisu, C Cheng, J Rozowsky, W Meyerson, **M Gerstein** (2015). Loregic: a method to characterize the cooperative logic of regulatory factors. *PLoS Comput Biol* 11: e1004132. [PMC4401777]

3. Comparative Genomics

Capitalizing on the uniformly processed and matched experimental data obtained by mod/ENCODE consortia, we have performed a series of comparative studies across distant metazoan phyla. A comparative analysis of human, worm, and fly revealed remarkable conservation of general properties of regulation.

a. **M Gerstein**, ZJ Lu... (128 authors)... L Stein, JD Lieb, RH Waterston (2010). Integrative analysis of the Caenorhabditis elegans genome by the modENCODE project. *Science* 330: 1775. [PMC3142569].
b. **M Gerstein**, J Rozowsky, KK Yan, D Wang...(89 authors)... TR Gingeras, R Waterston (2014). Comparative analysis of the transcriptome across distant species. *Nature* 512: 445. [PMC4155737]
c. C Sisu, B Pei, J Leng, A Frankish, Y Zhang, S Balasubramanian, R Harte, D Wang, M Rutenberg-Schoenberg, W Clark, M Diekhans, J Rozowsky, T Hubbard, J Harrow, **M Gerstein** (2014). Comparative analysis of pseudogenes across three phyla. *PNAS* 111: 13361. [PMC4169933]
d. KK Yan, D Wang, J Rozowsky, H Zheng, C Cheng, **M Gerstein** (2014)."OrthoClust: an orthology-based network framework for clustering data across multiple species. *Genome Biology* 15:R100 [PMC4289247]

4. Analysis of Diverse Networks

Network representations can be applied consistently to many different types of biological data. We have developed tools to build and analyze regulatory networks, protein-protein interactions and metabolic pathways, identifying key nodes such as hubs and bottlenecks. Moreover, we have integrated networks with dynamic gene-expression data (identifying transient hubs), 3D-protein structures, and even satellite imagery.

a. PM Kim, LJ Lu, Y Xia, **M Gerstein** (2006). Relating three-dimensional structures to protein networks provides evolutionary insights. *Science* 314:1938-41. [PMID: 17185604]
b. PV Patel, TA Gianoulis, RD Bjornson, KY Yip, DM Engelman, **M Gerstein** (2010). Analysis of membrane proteins in metagenomics: networks of correlated environmental features and protein families. *Genome Res* 20:960-71. [PMC2892097]

c. KK Yan, G Fang, N Bhardwaj, RP Alexander, **M Gerstein** (2010). Comparing genomes to computer operating systems in terms of the topology and evolution of their regulatory control networks. *PNAS* 107:9186-91. [PMC2889091]
d. C Cheng, E Andrews, KK Yan, M Ung, D Wang, **M Gerstein** (2015). An approach for determining and measuring network hierarchy applied to comparing the phosphorylome and the regulome. *Genome Biol* 16: 63. [PMC4404648]

5. Data Science and Large-scale Computational Analyses

Large-scale computational analyses go hand in hand with advances in sequencing technology. We are developing high-throughput pipelines and tools and also developing approaches to data integration and data protection.

a. A Abyzov, AE Urban, M Snyder, **M Gerstein** (2011). CNVnator: an approach to discover, genotype, and characterize typical and atypical CNVs from family and population genome sequencing. *Genome Res* 21: 974-84. [PMC3106330]
b. D Greenbaum, A Sboner, X J Mu, **M Gerstein** (2011). Genomics and Privacy: Implications of the New Reality of Closed Data for the Field. *PLoS Comput Biol* 7: e1002278 [PMC3228779]
c. L Habegger, A Sboner, TA Gianoulis, J Rozowsky, A Agarwal, M Snyder, **M Gerstein** (2011). RSEQtools: a modular framework to analyze RNA-Seq data using compact, anonymized data summaries. *Bioinformatics* 27: 281. [PMC3018817]
d. J Rozowsky, A Abyzov, J Wang, P Alves, D Raha, A Harmanci, J Leng, R Bjornson, Y Kong, N Kitabayashi, N Bhardwaj, M Rubin, M Snyder, **M Gerstein** (2011). AlleleSeq: analysis of allele-specific expression and binding in a network framework. *Mol Syst Biol* 7: 522. [PMC3208341]

Complete List of Publications

http://www.ncbi.nlm.nih.gov/sites/myncbi/mark.gerstein.1/bibliography/44005333/public

D. Research Support

Ongoing Research Support

U01 MH103365-01 (Vaccarino, Gerstein, Weissman) 06/15/14 – 05/31/17
NIH
Gene Regulatory Elements and Transcriptome in iPSCs and Embryonic Human Cortex
This application is in response to FOA [MH-14-020 ("PsychENCODE")]. The major aims are to provide a comprehensive catalogue of all types of RNA and their regulatory elements in the cerebral cortex of the mid-gestational human fetal brain as compared to induced pluripotent stem cells (iPSCs) derived from the same fetuses. Role: PI

5 R01 DA030976-05 (Wilhelmsen) 09/30/10 – 05/31/16
NIH (University of North Carolina)
Deep Sequencing Studies for Cannabis and Stimulant Dependence
The major goals of this project are to determine structural variants in the genome from deep sequencing studies for cannabis and stimulant dependence. Role: Co-I

DE-AC02-98CH10886 (Maslov) 10/17/11 – 07/31/16
Brookhaven National Laboratory - DOE
Kbase: An Integrated Knowledgebase for Predictive Biology and Environmental Research
The major goal of this project is to assist in the construction of the DOE Knowledgebase. Our role is to provide support to the plant and microbial subcomponents. Role: Co-I

5 U54 HG006504-03 (Gerstein, Lifton, Gunel, Mane) 12/05/11 – 11/30/15
NIH
Yale Center for Mendelian Disorders
The major goal of this project is to develop informatics approaches to characterize rare variants in the framework of the Centers for Mendelian Genomics. Role: PI

U41 HG007000-03 (Weng) 09/21/12 – 07/31/16
NIH (University of Massachusetts)
EDAC: ENCODE Data Analysis Center
The major goal of this project is to perform global and integrative data analysis for the entire ENCODE project.
Role: Co-I

U41 HG007355-02 (Waterston) 09/20/13 – 07/31/17
NIH (University of Washington)
Creating Comprehensive Maps of Worm and Fly Transcription Factor Binding Sites
Our role on the project is the determination of binding sites for transcription factors in worm and the fly. We will analyze large-scale Chip-Seq experiments to identify regions in the genome that are bound by transcription factors. Role: Co-I

U43 DA036134-01 (Gerstein, Galas, Milosavljevic) 08/01/13 – 07/31/18
NIH (Baylor College of Medicine)
Data Management and Resource Repository for the exRNA Atlas
Our role on the project is administering DIAC (data integration and analysis center) for ex-RNA data. Role: PI

U41 HG007234-02 (Harrow) 04/01/13 – 03/31/17
Wellcome Trust / NIH
GENCODE: Comprehensive Gene Annotation for Human and Mouse
Our role in the project is to identify pseudogenes comprehensively in human and mouse genomes and provide a systematic annotation of them. Role: Co-I

SUB#HG007497-02/PO#204077 (Lee) 09/20/13 – 08/31/16
Jackson Laboratory / NIH
An Integrative Analysis of Structural Variation for the 1000 Genomes Project
Our role on the project is analyzing the 1000 genomes data set to determine structural variation on a large scale.
Role: Co-I

R01 GM108663-02 (Reinke) 02/01/14 – 01/31/18
NIH
Deciphering Mechanisms Governing Functional Partitioning of the C. elegans Genome
Our role on the project is assisting the PI with bioinformatic analyses related to the worm genome. Role: Co-I

R01 MH100914-01A1 (Vaccarino, Sestan, Gerstein) 01/01/14 – 12/31/18
NIH
Genomic Mosaicism in Developing Human Brain
Our role on the project is to analyze somatic variations in the human genome. Role: PI

U01 HL126495 (Freedman, Gerstein, Mukamal, O'Donnell) 08/01/14 – 04/30/19
NIH (Univ of Massachusetts)
Racial and Ethnic Diversity in Human Extracellular RNA
The primary goal of this proposal is the generation of exRNA profiles in healthy individuals in two large and well-defined cohorts, the Framingham Heart Study and the Multi-Ethnic Study of Atherosclerosis, to be used as a reference to facilitate disease diagnosis and discovery. Role: PI

P50 MH106934-01 (Sestan) 09/19/14 – 07/31/19
NIH
Functional Genomics of Human Brain Development
This grant will apply functional genomics to study human brain development. Role: Co-I

School of Engineering (Peccia / Jordan) 11/01/14 – 10/31/16
Harnessing Big Data Models and Networks to Genetically Engineer Cyanobacteria for Advanced Biofuel Production
The major goals of this project are to do data processing related to biofuel research. Role: Co-I

Completed Research Support in the Last Three Years

5U54 HG004558-05 (Snyder) 01/01/10 – 06/30/13
Production Center for Global Mapping of Regulatory Elements
The major goal of this project is to comprehensively probe transcription factor binding throughout the human genome. Role: Co-I

5U54 HG004555-04S1 (Hubbard) 09/27/07 – 03/31/13
NIH
Integrated Human Genome Annotation: Generation of a Reference gene Set
The major goal of this project is to construct a pseudogene annotation of the human genome. Role: Co-I

5U01 HG004267-05S1 (Snyder) 01/01/10 – 03/31/13
NIH
Global Identification of Transcription Factor Binding Sites in *C. elegans*
The major goal of this project is to build a genome-wide map of the binding sites for every *C. elegans* transcription factor. Role: Co-I

5U01 HG005718-02 (Zhao) 09/13/10 – 06/30/14
NIH
Loss-of-function Variants in the 1000 Genomes Data Set and Implications to GWAS
The goals of this project are to survey loss-of-function (LOF) variants in the 1000 genomes data set and make this analysis available to the community as a useful resource. Role: Co-I

5R01 CA152057-03 (Rubin) 08/01/11 – 07/31/15
NIH
Weill Medical College of Cornell
Comprehensive Prostate Cancer Characterization by Genomic and Transcriptomic Profiling
The major goal of this project is to identify biomarkers for prostate cancer through analysis of various types of omics data. Role: Co-I

DE-SC0004856 08/15/10 – 08/14/15
DOE
Tools and Models for Integrating Multiple Cellular Network
The major goals of this project are the development of tools for the analysis of network and pathways in micro-organisms for the Systems Biology Knowledgebase proposed by the DOE. Role: PI

BIOGRAPHICAL SKETCH

NAME: Jose L Gomez

eRA COMMONS USER NAME (credential, e.g., agency login): JGVILLALOBOS

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Pontificia Universidad Javeriana, Bogota, Colombia	M.D.	12/1999	Medicine
Yale University, New Haven, CT	M.S.	05/2012	Computational Biology and Bioinformatics

A. Personal Statement

My academic training, clinical career and research experience in genetics, genomics and computational biology lay the foundation for my contribution to the current proposal on the Asthma and Allergic Diseases Cooperative Research Center (ADCRC). I have expertise on the analysis of genetic and genomic datasets, and their integration with clinical and phenotypic information of patients with asthma. My motivation to complete advanced training in computational biology and bioinformatics is my focus on interdisciplinary research and their significant impact on scientific challenges. I am working on developing advanced skills on the design and interpretation of next-generation sequencing and single cell analysis. My long-term goal is to become a leading translational researcher in the field of pulmonary medicine, specifically, the identification of pathways associated with asthma severity and airway remodeling. My ultimate goal is to identify key determinants on the pathobiology of asthma and develop novel approaches to diagnosis and therapeutics based on the characterization of these unique determinants identified in this project.

B. Positions and Honors

Professional Experience

1996-1999 Teacher Assistant, Physiology Department, Pontificia Universidad Javeriana. Bogota, Colombia
2000-2001 Attending Physician, Emergency Department, Hospital Universitario. Neiva, Colombia
2001 Attending Physician, Emergency Department, Hospital Central Policia Nacional. Bogota, Colombia
2001-2002 Attending Physician, Primary Care Practice, Bogota, Colombia
2002-2004 Instructor, Department of Medicine, Fundacion Universitaria de Ciencias de la Salud. Bogota, Colombia
2004-2007 Resident, Hospital of St. Raphael. New Haven, CT
2007-2008 Attending Physician, Hospitalist Section, Midstate Medical Center. Meriden, CT
2008 -2012 Postdoctoral Fellow, Section of Pulmonary and Critical Care Medicine, Yale University School of Medicine. New Haven, CT
2010-2012 Master of Science, Computational Biology and Bioinformatics, Yale University. New Haven, CT
2012-2015 Instructor in Medicine, Pulmonary, Critical Care and Sleep Section, Yale University School of Medicine. New Haven, CT
2015- Assistant Professor in Medicine, Pulmonary, Critical Care and Sleep Section, Yale University School of Medicine. New Haven, CT

Grants and Honors

2005 Outstanding PGY-1 Resident, Hospital of Saint Raphael.
2010 Aerocrine US Fellows Program
2012- Young Scientist Award Fellow, Flight Attendant Medical Research Institute
2015- Mentored Research Scientist Development Award (K01), NIH

Professional Organizations

2004-	Member, American College of Physicians
2008-	Member, American Thoracic Society
2009-	Member, American College of Chest Physicians
2015-	Member, Assembly on Allergy, Inflammation, and Immunology, American Thoracic Society

C. Contribution to Science

1. **Characterization of the hemodynamic tolerance to hypercapnia in analbuminemia.** We investigated the interaction between hypoalbuminemia and analbuminemia in an experimental model of respiratory failure. We identified that despite differences in the acid-base compensation mechanisms between controls, hypoalbuminemic and analbuminemic rats. No substantial differences were present in their hemodynamic compensation in the setting of hypercapnia. These observations suggest that despite the acid-base impact associated with decreased serum albumin concentrations, animals were able to compensate a hypercarbic challenge, similar to that seen in hypercapnic respiratory failure in the intensive care unit.

a. **Gomez JL**, Gunnerson KJ, Song M, Li J, Kellum JA. Effects of hypercapnia on blood pressure in hypoalbuminemic and Nagase analbuminemic rats. *Chest* 2007; 131:1295-1300. PMID: 17494780.

2. **Identification of a genetic variant associated with a chitinase-like protein in human asthma, airway expression and asthma severity.** In collaboration with the Severe Asthma Research Program (SARP), we identified a single nucleotide polymorphism on the *CHI3L1* gene encoding for the YKL-40 protein. YKL-40 is a chitinase-like protein associated with severe asthma and airway remodeling. We identified the association between rs12141494 with serum and airway expression of YKL-40, and importantly we also identified an association with severe asthma. These functional genetic observations suggest the association with increased risk for severe asthma in individuals with the AA risk allele in rs12141494.

a. **Gomez JL,** Crisafi GM, Holm CT, He X, Cohn L, Meyers DA, Hawkins GA, Bleecker ER, Jarjour N for the SARP Investigators, Chupp GL. Genetic variation in *CHI3L1* Contributes to asthma severity and airway expression of YKL-40. *J Allergy Clin Immunol* 2015; 136(1):51-58.e10. PMID:25592985; PMCID:PMC4494869

3. **Non-invasive translational research on asthma heterogeneity.** As part of the Yale Center for Asthma and Airways Disease (YCAAD), I participate in a translational program aimed at the non-invasive characterization of asthma and the identification of pathobiologic determinants of asthma. Our landmark study using a novel pathway based unsupervised cluster analysis of induced sputum gene expression to discriminate subgroups of asthma, identified a 50 gene profile that can measured in the blood to determine an individual's subgroup and risk. We have collaborated with multiple investigators to provide samples from the YCAAD biorepository. My recently funded K01 application will investigate the role of microRNA 504 in severe asthma, as part of a larger longitudinal study for characterization of the Transcriptional Endotypes of Asthma (TEA clusters) identified in our original assessment of the YCAAD cohort.

a. Yan X, Chu J-H, **Gomez JL,** Koenigs M, Holm CT, He X, Zhao H, Mane S, Martinez FD, Ober C, Nicolae DL, Barnes KC, London SJ, Gilliland F, Weiss ST, Raby BA, Cohn L, Chupp GL. Non-invasive analysis of the airway transcriptome discriminates clinical phenotypes of asthma. *Am J Respir Crit Care Med.* 2015 May 15;191(10):1116-1125. PMID:25763605; PMCID:PMC4451618

Link to Dr. Gomez's Publications:
http://www.ncbi.nlm.nih.gov/sites/myncbi/1ZoT6XrWLkyAh/bibliography/42351900/public/?sort=date&direction=ascending

D. Research Support

Ongoing Research Support

1K01 HL125474-01 (Gomez) 07/01/15 – 06/30/20

NIH/NHLBI
Functional role of hsa-miR-504 in airway inflammation and remodeling in asthma
The main goal of this study is to characterize the biology and association between hsa-miR-504 and severe asthma in a longitudinal study of the mechanisms of asthma. Using an integrative functional genomics method to identify subgroups of patients, study will evaluate gene expression in sputum from asthmatics.
Role: PI

Supplement to R01 HL118346 (Chupp) 09/01/14 – 08/01/16
NIH/NHLBI
Longitudinal Analysis of Transcriptomic Endophenotypes in Asthma
This research project explores the role of microRNAs on the sputum of individuals with asthma and its effect on asthma heterogeneity.
Role: Mentee

R11552 (Gomez) 07/01/12 – 06/30/16
Flight Attendant Medical Research Institute, FAMRI
A Three-gene Signature in Smoking Exposure and Asthma
This project is evaluating the effect of second-hand smoke exposure in the inflammatory response on the airway of individuals with asthma focused on the characterization of three genes identified in a transcriptional signature of asthmatics and macrophages in smoking subjects.
Role: PI

Completed Research Support (last 3 years)
None

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Smita Krishnaswamy

eRA COMMONS USER NAME (agency login): SKRISHNASWAMY

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
University of Michigan, Ann Arbor, MI	BS	08/2002	Computer Science and Engineering
University of Michigan, Ann Arbor , MI	MS	06/2004	Computer Science and Engineering
University of Michigan, Ann Arbor, MI	PHD	12/2008	Computer Science and Engineering
Kalamazoo College, Kalamazoo, MI	BA	09/2010	Mathematics
Columbia University, New York, NY	Postdoctoral Fellow	08/2015	Computational Biology

A. Personal Statement

Emerging high-throughput single cell technologies are driving a big-data revolution in biology. In immunology, new technologies such as mass cytometry enable us to track dozens of protein markers in thousands of cells simultaneously. This data holds the key to gaining an unprecedented view into the workings of a cell, and cellular responses to various stimuli. However, there is currently a paucity of scalable computational methods that are able to analyze this data, which can be complex because cellular processes are rife with feedback, crosstalk, unmeasured environmental variables and noise. My research has been addressing this issue, by bridging the gap between computational techniques and biological understanding.

My postdoctoral work addresses both the processing and normalization of this data and inferring dynamic computational models of cellular signaling. My work on normalization proposes a time-varying bead based normalization to correct for machine decay and calibrate between runs of CyTOF. Further, I was also involved in developing an error-correcting barcoding scheme that simultaneously allows for multiplexing of experiments and for the detection of doublet events in data.

The main focus of my research is modeling the phenotypic space and interaction between signaling molecules in order to understanding information processing in cells. However, associations and influences between molecules in a signaling network can be difficult to determine in single cell data because of many confounding factors such as noise, and biased sampling of the cell states in the measurements. Therefore, many existing statistical methods fail to reveal significant influences between molecules in the data. In order to address this challenge, I developed a scalable method known as DREVI (conditional-Density Rescaled Visualization) to visualize and extract mathematical descriptions of signaling interactions between pairs of signaling proteins. DREVI is based on conditional density estimation between the independent and dependent variable, and reveals the functional shape of the dependency between molecules as well as the stochastic spread in the function along the full dynamic range of molecular operation. I also developed a mutual-information-based metric called DREMI (conditional-Density Resampled Estimate of Mutual Information) for scoring the strength of relationships based on the conditional probability.

Previously, signaling networks were primarily described qualitatively, as protein-interaction graphs. But with DREVI and DREMI, one can quantitatively determine the strength of information transfer and the functions computed by these networks. The quantitative, behavioral descriptions offered by DREVI and DREMI allow us tease out subtly altered signaling functionality in closely related cell types or between diseased and healthy cells. Such differences are important because related cell types often contain similarly wired circuits, which reuse the same molecules, but behave phenotypically differently. For example, my methods found differences in activation thresholds and shapes of response functions between the signaling networks of naïve and activated T cells. I also used my methods to track differences in signaling response between T cells from healthy mice and from non-obese diabetic (NOD) mice, which are prone to developing Type 1 diabetes. I showed that the signal transduction in NOD mice is slightly impaired very close to the location of antigen-engagement and that this

slight impairment can get amplified through the fanout and reconvergence of signals in the network, downstream of the surface receptor.

In the proposed project, I plan to utilize my computational background to develop methods of single-cell analysis for this project. I propose to contribute methods for both the analysis of CyTOF data and single-cell RNA-sequencing data. For CyTOF data, I plan to develop methods that:

(1) Determine cell subpopulations present in patients by unsupervised clustering methods;
(2) Match of subpopulations and quantify heterogeneity between patients using matching methods;
(3) Characterize of signaling responses by higher-dimensional DREVI with a fuzzy logic model for integration with RNA-sequencing data.

This work requires an extension of the DREMI framework to multiple dimensions in order to assess multi-molecule interactions in signaling data. We plan to use this to characterize the clusters of cells present in asthma sputum samples and differentiate this behavior from healthy as I did in the case of Type 1 diabetes.

Further, since single-cell RNA sequencing is a new technology, without established pipelines for correction of noise and error, I plan to combine previously existing techniques to develop a robust processing pipeline. The pipeline will 1) debarcode reads and corrects errors, 2) align read and maps UMI read-sets to genes, and 3) select genes that show meaningful biological variability rather than technical variability. Further, I propose to analyze single-cell RNA sequencing data with DREMI and DREVI analysis by first performing nonlinear dimensionality reduction and clustering to missing values in gene dimensions and then performing analysis on imputed gene-gene interactions. I believe that my extensive experience with both single-cell data and computational and algorithmic techniques will put me in good stead for this project.

a. Finck R, Simonds EF, Jager A, **Krishnaswamy S,** Sachs K, Fantl W, Pe'er D, Nolan GP, Bendall SC. Normalization of mass cytometry data with bead standards. *Cytometry A.* 2013 May;83(5):483-94. PMID: 23512433; PMCID: PMC3688049.

b. Amir el-AD, Davis KL, Tadmor MD, Simonds EF, Levine JH, Bendall SC, Shenfeld DK, **Krishnaswamy S,** Nolan GP, Pe'er D. viSNE enables visualization of high dimensional single-cell data and reveals phenotypic heterogeneity of leukemia. *Nat Biotechnol.* 2013 Jun;31(6):545-52. PMID: 23685480; PMCID: PMC4076922.

c. Mingueneau M, **Krishnaswamy S,** Spitzer MH, Bendall SC, Stone EL, Hedrick SM, Pe'er D, Mathis D, Nolan GP, Benoist C. Single-cell mass cytometry of TCR signaling: amplification of small initial differences results in low ERK activation in NOD mice. *Proc Natl Acad Sci U S A.* 2014 Nov 18;111(46):16466-71. PMID: 25362052; PMCID: PMC4246343.

d. **Krishnaswamy S,** Spitzer MH, Mingueneau M, Bendall SC, Litvin O, Stone E, Pe'er D, Nolan GP. Systems biology. Conditional density-based analysis of T cell signaling in single-cell data. *Science*. 2014 Nov 28;346(6213):1250689. PMID: 25342659; PMCID: PMC4334155.

B. Positions and Honors

Positions and Employment

2008 - 2010	Research Staff, IBM TJ Watson Research Center, New York, NY
2015 -	Assistant Professor, Yale Medical School, Dept. of Genetics, New Haven, CT

Other Experience and Professional Memberships

2001 -	Member, Eta Kappa Nu Honor Society
2008 -	Member, ACM
2008 -	Member, IEEE
2008 - 2010	Member, IEEE Council on Electronic Design Automation
2008 - 2010	Social Network Liason, ACM Special Interest Group on Design Automation
2010 -	Co-Chair of Post-CMOS VLSI Track, TPC member, Great Lakes Symposium on VLSI
2011 -	Technical Program Committee Chair, International Workshop on Biological Design Automation
2013 -	Co-Organizer, Modeling of Biological Systems Workshop
2013 - 2014	Technical Program Committee Member, Cancer Panomics Conference

2013 - 2015	Technical Program Committee Member, International Conference on Computer-Aided Design
2014 - 2015	Technical Program Committee , Design Automation Conference

Honors

2001	Futures Fellowship, Motorola
2005	Best Paper Award, Design Automation and Test in Europe
2007	Second Place, IWLS Programming Challenge, IEEE
2007	ACES Engineering Academic Scholar, University of Michigan, College of Engineering
2009	Outstanding Dissertation Award, European Design Automation Association
2009	First Invention Award, IBM Research
2010	Research Division Accomplishment, IBM

C. Contribution to Science

1. Modern computer chips are essentially massive networks of interconnected computational components (logic gates) through which input signals are integrated, mapped into outputs, and fed back to form memory. Traditional methods of designing and optimizing such networks use deterministic representations such as netlists of Boolean gates. However as technology scales with Moore's law and beyond, the physical realization of such chips becomes smaller and smaller, naturally leading to stochastic effects and vulnerability to soft errors. Further, new device technologies can operate in the quantum realm or in a biological substrate with inherent stochasticity. In order to deal with these effects, I was one of the first researchers to propose the fundamental paradigm shift from deterministic to probabilistic modeling and to develop algorithms for the scalable verification, design and testing of circuits subject to stochastic behavior. My paper in the DATE conference 2005 proposed the probabilistic transfer matrix framework for representing gate operations, and developed algorithms for compressing and computing with these types of matrices to facilitate exact probabilistic verification of logic blocks. This paper won the best paper award in the test category at DATE 2005. I published several additional papers that used monte carlo methods for approximate verification and design of probabilistic logic circuits. In order to avoid the addition of massive redundancy, as had been done in the past, my algorithms harnessed subtle informational redundancies within logic circuits in order to boost reliability. My testing techniques, derived optimized vectors through an adapted set-cover formulation, that were orders of magnitude faster for assessing circuit vulnerability to soft error. My Ph.D. dissertation which encompassed probabilistic design, analysis and test of logic circuits was recognized with an outstanding dissertation award by the Electronic Design Automation Association in 2009, and published a book by Springer in 2012.

    a. **Krishnaswamy S,** Viamontes GF, Markov IL, Hayes JP. Accurate reliability evaluation and enhancement via probabilistic transfer matrices. IEEE Proceedings on Design Automation and Test in Europe. 2005; 282-287.
    b. **Krishnaswamy S,** Markov IL, Hayes JP. Tracking uncertainty with probabilistic logic circuit testing. IEEE Design & Test of Computers. 2007; 24(1):312-321.
    c. **Krishnaswamy S,** Plaza SM, Markov IL, Hayes JP. Signature-based SER analysis and design of logic circuits. IEEE Transactions on Computer-Aided Design of Integrated Circuits and Systems. 2009; 28(1):74-86.
    d. **Krishnaswamy S,** Markov IL, Hayes JP. Design, analysis and test of logic circuits under uncertainty. Dordrecht, Netherlands: Springer Science & Business Media; 2012.

2. The design of modern computer chips is a lengthy process that starts with the high level specification of by chip architects. This followed by a myriad of steps, automated to various degrees, of high level synthesis into large logic modules, decomposition and synthesis into gates, and physical design which includes technology mapping (to physical components), network placement and wire routing. Errors in logic and timing can accumulate any of the steps either due to human error or bugs in the employed algorithms that are used to design 80% of high-performance chips today. There is however, a large gap between what is designed and what can be verified and corrected. Complete verification of a chip is #P-hard and requires enumeration of exponentially many testvectors. At IBM's TJ Watson Research Center, I contributed to several problems that detect and correct logic errors and optimize timing in late stages of design, so as not to invalidate design efforts and costs. My methodology, named DeltaSyn, proposes a novel approach to match parts of logic from

the input and output ends, using recursive Boolean matching and satisfiability solving, to hone in on a small region of error that is then automatically resynthesized. This method was recognized as a division accomplishment of the year and gave rise to two patents. I also developed SPIRE, a global retiming transformation system to enable timing closure in late design stages by efficiently encoding a large series of modifications into an integer-linear program that can be solved efficiently. I further proposed a generalized SAT-sweeping method to optimize logic circuits that have already been physically mapped to technology. These methods have subsequently been deployed in the design of IBM's P and Z series high-performance chips, as well as in the chips that form the BlueGene supercomputer.

a. **Krishnaswamy S,** Ren H, Modi N, Puri R. DeltaSyn: an efficient logic difference optimizer for ECO synthesis. ACM proceedings International Conference on Computer-Aided Design. 2009; 789-796.
b. Papa DA, **Krishnaswamy S,** Markov IL. SPIRE: a retiming-based physical synthesis transformation system. IEEE Proceedings of the International Conference on Computer-Aided Design. 2010; 373-380.
c. **Krishnaswamy S,** Ren H, Modi N, Puri R. In: Advanced Techniques in Logic Synthesis, Optimizations and Applications. Khatri SP, Gulati K, editors. New York, NY: Springer Science & Business Media; 2011. Chapter 12, Logic Difference Optimization for Incremental Synthesis; p.203-224. 203-225p.
d. Welp T, **Krishnaswamy S,** Kuehlmann A. Generalized SAT-Sweeping for Post-Mapping Optimization. IEEE/ACM Proceedings of the Design Automation Conference. 2012; 814-819.

3. CyTOF is a new technology that utilizes rare metal-ions to tag antibodies bound to surface and internal proteins within a cell, and enables the measurement of dozens of proteins simultaneously at the single-cell resolution. However, CyTOF data can be difficult to process and analyze because of 1) technical noise in measurements, potentially due to protein accessibility, 2) decay in machine sensitivity during the course of a run, 3) lack of calibration between runs, 4) inherent biological noise from unmeasured (or uncontrolled) variables. My research enables the analysis of CyTOF data by proposing methods for data normalization using bead standards. These bead standards can help calibrate between runs and correct for signal decay. Additionally, I've also helped develop barcoding schemes for multiplexing experiments and eliminating cell-cell doublets from the data. However, my main interest in CyTOF data is in inferring dynamic signaling relationships from measurements of internal signaling proteins downstream of surface receptors.

   Associations between molecules in a signaling relationship can be difficult to determine in single cell data due to noise, biased sampling of cell states, and hidden influences that induce background correlations. Therefore many statistical methods cannot distinguish between significant and insignificant influences. To address this, in my recent Science paper, I developed DREVI which visualizes and extracts mathematical descriptions of signaling relationships by considering the entire dynamic range of molecular interaction through conditional density estimation. I also developed a mutual-information based metric called DREMI for scoring the strengths of relationships based on the conditional density. The reliable quantitative and behavioral descriptions offered by DREMI and DREVI enable the quantitative characterization of signaling relationships and comparisons between signaling interactions closely related cell types or between healthy and diseased cells. My methods reveal subtle differences between signaling in naive and effector/memory T cells, and also how subtle differences in signaling can be amplified to disastrous effects under diseases such as Type 1 Diabetes.

   a. Finck R, Simonds EF, Jager A, **Krishnaswamy S,** Sachs K, Fantl W, Pe'er D, Nolan GP, Bendall SC. Normalization of mass cytometry data with bead standards. *Cytometry A.* 2013 May;83(5):483-94. PbMed PMID: 23512433; PMCID: PMC3688049.
   b. Mingueneau M, **Krishnaswamy S,** Spitzer MH, Bendall SC, Stone EL, Hedrick SM, Pe'er D, Mathis D, Nolan GP, Benoist C. Single-cell mass cytometry of TCR signaling: amplification of small initial differences results in low ERK activation in NOD mice. *Proc Natl Acad Sci U S A.* 2014 Nov 18;111(46):16466-71. PMID: 25362052; PMCID: PMC4246343.
   c. **Krishnaswamy S,** Spitzer MH, Mingueneau M, Bendall SC, Litvin O, Stone E, Pe'er D, Nolan GP. Systems biology. Conditional density-based analysis of T cell signaling in single-cell data. *Science*. 2014 Nov 28;346(6213):1250689. PMID: 25342659; PMCID: PMC4334155.

d. Zunder ER, Finck R, Behbehani GK, Amir el-AD, **Krishnaswamy S,** Gonzalez VD, Lorang CG, Bjornson Z, Spitzer MH, Bodenmiller B, Fantl WJ, Pe'er D, Nolan GP. Palladium-based mass tag cell barcoding with a doublet-filtering scheme and single-cell deconvolution algorithm. *Nat Protoc*. 2015 Feb;10(2):316-33. PMID: 25612231; PMCID: PMC4347881.

http://www.ncbi.nlm.nih.gov/sites/myncbi/1B1TvlyNqe6kh/bibliography/48010002/public/?sort=date&direction= descending

D. Research Support

Current Research Support
None

Completed Research Support (last 3 years)
None

BIOGRAPHICAL SKETCH

NAME: Shirkant Mane

eRA COMMONS USER NAME: (credential, e.g., agency login): SMMANE

POSITION TITLE: Senior Research Scientist

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	YEAR	FIELD OF STUDY
Shivaji University, India	BS	1977	Zoology
University of Bombay, India	MS	1979	Animal Physiology
University of Bombay, India	PhD	1984	Cancer Biology
The Johns Hopkins University School of Medicine, Baltimore MD	Postdoc	1985-1988	Biochemistry & Molecular Biology

A. Personal Statement

Dr. Mane brings expertise for genomic analysis using both microarray and high-throughput DNA sequencing technologies. He received his Ph.D. in Cancer Biology in 1985 and has published more than 25 articles related to cancer. He is the Director of both The Yale Center for Genome Analysis (YCGA) and The Keck Biotechnology Resource Labortory at Yale. He has published more than 100 articles, holds 2 patents, and has amassed over 25 years of research experience in both academic and private industry. He has attracted significant funding from NIH and other sources to maintain cutting edge genomic technologies at Yale. Currently, he is one of four PIs of the Yale Center for Mendelian Genomics established in 2012 through an $11.2 million dollar grant from NHGRI. Besides directing the YCGA, he pursues research in the field of neuroscience. Dr. Mane has a demonstrated record of establishing a successful and productive genomic facility that has provided over 58,000 sequence analyses (library prep, sequencing and analyses) to 225 Yale and 124 non-Yale principal investigators from 72 national and 16 international institutions.

a. Choi M, Scholl UI, Yue P, Björklund P, Zhao B, Nelson-Williams C, Ji W, Cho Y, Patel A, Men C, Lolis E, Wisgerhof MV, Geller DS, **Mane S,** Hellman P, Westin G, Åkerström G, Wang W, Carling T, Lifton RP. K^+ channel mutations in adrenal aldosterone-producing adenomas and hereditary hypertension. *Science*, 2011, 331, 768-72. PMID:21311022; PMCID:PMC3371087

b. Krauthammer M, Kong Y, Ha BH, Evans P, Bacchiocchi A, McCusker JP, Cheng E, Davis MJ, Goh G, Choi M, Ariyan S, Narayan D, Dutton-Regester K, Capatana A, Holman EC, Bosenberg M, Sznol M, Kluger HM, Brash DE, Stern DF, Materin MA, Lo RS, **Mane S**, Ma S, Kidd KK, Hayward NK, Lifton RP, Schlessinger J, Boggon TJ, Halaban R. Exome sequencing identifies recurrent somatic RAC1 mutations in melanoma. N*at Genet,* 2012, 44 (9):1006-1014. PMID:22842228; PMCID:PMC3432702

c. Clark VE, Erson-Omay EZ, Serin A, Yin J, Cotney J, Ozduman K, Avsar T, Li J, Murray PB, Henegariu O, Yilmaz S, Günel JM, Carrión-Grant G, Yilmaz B, Grady C, Tanrikulu B, Bakircioglu M, Kaymakçalan H, Caglayan AO, Sencar L, Ceyhun E, Atik AF, Bayri Y, Bai H, Kolb LE, Hebert R, Omay SB, Mishra-Gorur K, Choi M, Overton JD, Holland EC, **Mane S,** State MW, Bilgüvar K, Baehring JM, Gutin PH, Piepmeier JM, Vortmeyer A, Brennan CW, Pamir MN, Kiliç T, Lifton RP, Noonan JP, Yasuno K, Günel M. Genomic Analysis of Non-NF2 Meningiomas Reveals Mutations in TRAF7, KLF4, AKT1, and SMO. *Science,* 2013, 339(6123):1077-80. PMID:23348505

B. Positions and Honors

1972 – 1976 National Scholarship for undergraduate studies
1976 – 1978 Daxina Fellowship for Master's degree
1979 – 1984 Junior Research Fellowship for graduate studies

1984 – 1985	Senior Research Fellowship, Indian Council of Medical Research
1988 – 1990	Research Associate, University of Maryland, Baltimore, Maryland
1990 – 1993	Instructor, The Johns Hopkins Uni. Sch. of Medicine, Baltimore, Maryland
1993 – 1997	Senior Scientist, Cellco Inc., Germantown, Maryland
1997 – 1999	Sr. Vascular Biologist, W.L. Gore & Associates, Inc., Flagstaff, Arizona
1999 – 2001	Staff Scientist, Microarray Core, Moffitt Cancer Center, Tampa, Florida
2001 – present	Director, Microarray, DNA sequencing and Oligonucleotide synthesis Resources, Keck Laboratory, Yale University, New Haven, Connecticut
2005 – present	Director, Yale/NIH Microarray Center for Research on the Nervous System
2007 – 2009	Senior Deputy Director, Keck Biotechnology Resource Laboratory, Yale University
2009 – present	Director, Keck Biotechnology Resource Laboratory, Yale University
2009 – present	Director, Yale Center for Genome Analysis, Yale University

Editorial Tasks: Journals, Ad hoc reviewer
Proceedings of National Academy of Sciences, USA
Biotechniques
Physiological Genomics

C. Contribution to Science

1. My early publications directly related to cancer biology. During my Ph.D. thesis I studied the role of growth factors in tumor development and progression. This study led to the identification of a polypeptide that enhances cellular binding of epidermal growth factor. During my post-doctoral work I continued working on Ras gene activation in acure Myelogenous Leukemia. I served as the primary investigator in these studies.

a. Deo MG, Mulherkar R, **Mane SM.** Isolation of a polypeptide that enhances cellular binding of epidermal growth factor. Indian *J Biochem Biophys,* 1983 Aug;20(4):228-31. PMID:6323306

b. **Mane SM,** Meltzer SJ, Gutheil J, Kapil V, Schiffer CA, Needleman SW: Ras gene activation in acute Myelogenous leukemia: Analysis by in vitro amplification and DNA base sequence determination. *Genes Chromosomes Cancer,* 1990, 2:71-77. PMID:2278967

c. **Mane SM,** Marzella L, Baiton DF, Holt V, Hildreth J, August JT: Purification and characterization of human Lysosomal membrane glycoproteins and their presence in the plasma membrane of myelomonocytic leukemia cells. *Arch Biochem Biophys*, 1990, 268:360-378. PMID:2912382

2. After assuming the role of the founding Director of the Yale Center for Genome Analysis I have demonstrated the leadership in development and adoption of cutting-edge genomic technologies that are essential for carrying out genomic studies to answer various biological questions. During the past few years, I have attracted significant funding from NIH and other sources, to maintain the cutting-edge in genomic technologies at Yale. The Affymetrix microarray platform was established though $150,000 in funding from HHMI. The high-throughput Illumina BeadArray platform was established in 2007 through the support provided by NIH Administrative supplement ($375,000; 3 U24 NS051869-02S1, PI: S. Mane). The Tecan robotics and associated acessories requeired for the establishment of high throughput workflow for the genotyping assay were purchaed throught the combined support (~ $ 300,000) provided by my NIH Neuroscience Microarray Center grant (5 U24 NS051869) and Yale School of Medicine (YSM). I also established custom genotyping platforms: Sequenom (NIH-CTSA; YSM) and ABI 7900 Sequence Detection System (HHMI). Finally, in 2008, I established next-generation sequencing technology by acquiring two Illumina Genome Analyzers utilizing $1.1 million funds provided through NIH/NCRR high end instrumentation grant (1S10RR023004; PI: S. Mane).

Along with the acquisition of cutting-edge genomic technologies, in 2008 we developed a robust and scalable exome sequencing platform in collaboration with Nimblegen and in 2009 published the utility of the platform, along with an original analysis pipeline we wrote to annotate exome data. This paper included the first demonstration of the utility of exome sequencing, reporting the first clinical diagnosis made by a genome-level sequencing approach. After this initial success we have published more than 60 articles (including more than 30 articles in high profile journals such as Science, Nature, Cell, New England Journal

of Medicine and Nature Genetics) describing disease causing variants in several human disorders using exome sequencing.

a. Bilgüvar K, Oztürk AK, Louvi A, Kwan KY, Choi M, Tatlı B, Yalnızoğlu D, Tüysüz B, Cağlayan AO, Gökben S, Kaymakçalan H, Barak T, Bakırcıoğlu M, Yasuno K, Ho W, Sanders S, Zhu Y, Yılmaz S, Dinçer A, Johnson MH, Bronen RA, Koçer N, Per H, **Mane S,** Pamir MN, Yalçınkaya C, Kumandaş S, Topçu M, Ozmen M, Sestan N, Lifton RP, State MW, Günel M. Whole-exome sequencing identifies recessive WDR62 mutations in severe brain malformations. *Nature*, 2010, 467: 207-210. PMID:20729831; PMCID:PMC3129007

b. Boyden LM, Choi M, Choate KA, Nelson-Williams CJ, Farhi A, Toka HR, Tikhonova IR, Bjornson R, **Mane SM,** Colussi G, Lebel M, Gordon RD, Semmekrot BA, Poujol A, Valimaki MJ, De Ferrari ME, Sanjad SA, Gutkin M, Karet FE, Tucci JR, Stockigt JR, Keppler-Noreuil KM, Porter CC, Anand SK, Whiteford ML, Davis ID, Dewar SB, Bettinelli A, Fadrowski JJ, Belsha CW, Hunley TE, Nelson RD, Trachtman H, Cole TRP, Pinsk M, Bockenhauer D, Shenoy M, Vaidyanathan P, Foreman JW, Rasoulpour M, Thameem F, Al-Shahrouri HZ, Radhakrishnan J, Gharavi AG, Goilav B and Lifton RP. Mutations in kelch-like 3 and cullin 3 causes hypertension and electrolyte abnormalities. *Nature*, 2012, 482:98-102. PMID:22266938; PMCID:PMC3278668

c. Sanders SJ, Murtha MT, Gupta AR, Murdoch J, Raubeson MJ, Willsey J, Sencicek G-E, Parikshak NN, Stein JL, DiLullo NM, Walker M, Terran N, Song Y, El-Fishawy P, Murtha R, Choi M, Overton J, Bjornson R, Carrierio N, Meyer K, Bilguvar K, **Mane S,** Sestan N, Lifton RP, Gunel M, Roeder K, Geschwind D[§], Devlin B[§], and State MW *De novo* point mutations, revealed by whole-exome sequencing, are strongly associated with Autism Spectrum Disorders. *Nature*, 2012, 485(7397):237-41; PMID:22495306; PMCID:PMC3667984

d. Zaidi S, Choi M, Wakimoto H, Ma L, Jiang J, Overton JD, Romano-Adesman A, Bjornson RD, Breitbart RE, Brown KK, Carriero NJ, Cheung YH, Deanfield J, DePalma S, Fakhro KA, Glessner J, Hakonarson H, Italia MJ, Kaltman JR, Kaski J, Kim R, Kline JK, Lee T, Leipzig J, Lopez A, **Mane SM,** Mitchell LE, Newburger JW, Parfenov M, Pe'er I, Porter G, Roberts AE, Sachidanandam R, Sanders SJ, Seiden HS, State MW, Subramanian S, Tikhonova IR, Wang W, Warburton D, White PS, Williams IA, Zhao H, Seidman JG, Brueckner M, Chung WK, Gelb BD, Goldmuntz E, Seidman CE, Lifton RP. De novo mutations in histone-modifying genes in congenital heart disease. *Nature*, 2013, 498(7453):220-3. PMID:23665959; PMCID:PMC3706629

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/1Dw-NM4w3sNkZ/bibliography/46703160/public/?sort=date&direction=ascending

D. Research Support

Ongoing Research Support

U54 HG006504-01 (Mane, Lifton, Gerstein, Gunel) 12/05/11 – 11/30/15
NIH
Yale Center for Mendelian Disorders
The major goal is to sequence exomes to identify new Mendelian trait genes.
Role: PI

1S10 OD018034-01 (Mane) 06/01/14 – 05/31/19
6500 QTrap Mass Spectrometer for Yale University
The major goal of this proposal is to obtain a state-of-the-art QTrap Mass Spectrometer to support the proteomic research goals of the Yale investigators
Role: PI

1 S10 OD018521-01 (Mane) 07/16/15 – 06/30/19
NIH

High Performance Computing Instrumentation for the Yale Center for Genome Analysis
This instrumentation will increase computational capacity to match growing demand, will greatly improving data storage and networking, and reduce the power and cooling requirements.
Role: PI

5 R01 NS 057756 (Gunel) 03/15/12 – 02/28/17
NIH/NINDS
Molecular Genetic Pathogenesis of Intracranial Aneurysms
The major goal of the proposal is to identify genetics factors associated with Intracranial Aneurysm using state of the art genomic technologies.
Role: Co-I

5 P30 CA 016359-34 (Lynch) 08/01/13 – 07/31/18
NIH/NCI
Yale Comprehensive Cancer Center Support Grant
The goal of the Yale Center for Genome Analysis (YCGA) is to rapidly and cost-effectively bring state-of-the-art genomic technologies within reach of largest possible number of cancer investigators.
Role: Co-I

1R01 HL118346-01 (Chupp) 08/15/13 – 05/31/17
NIH / NHLBI
Longitudinal Analysis of Transcriptomic Endophenotypes in Asthma
Using an integrative functional genomics method to identify subgroups of patients, we will evaluate gene expression in sputum from asthmatics. These studies will improve our understanding of the molecular diversity of asthma and will identify patients with similar gene networks.
Role: Co-I

5UL1TR000142-10 (Sherwin) 09/30/06 – 06/30/16
NIH/NCATS: CTSA
Yale Center for Clinical and Translational Research.
This is a University resource for clinical and translational investigation.
Role: Co-I

P30 DK079310 (Aronson) 09/20/13 – 07/31/18
NIH
Yale Center for Kidney Disease Research
This is a Core Center aimed at providing physiology, mouse and human genetic resource support to facilitate translational and clinical research projects that will advance the prevention and treatment of kidney diseases.
Role: Co-I

1 R01 MH102342-01A1 (Gunel) 05/08/15 – 02/28/19
NIH
Integrating the Genomics of Autism Spectrum Disorders(ASD) in Consanguineous and "Idiopathic" Families
This proposal is aimed at advancing the understanding of the genetics and biology of ASD in the interests of identifying novel approaches to diagnosis, and therapeutic development.
Role: Co-I

Completed Research Support (Last 3 Years)
R01 GM099149 (Khokha) 08/01/11 – 04/30/15
NIH
Transcriptome Profiling and Targeted Genic Improvement of the X.tropicalis Genome
The goal of this application is to perform targeted sequencing to improve and annotate the genic portion of the X. tropicalis genome.
Role: Co-I

VA (Concato) 03/01/14 – 02/28/15
VA JPA
Genetics of Functional Disability in Schizophrenia and Bipolar Illness
The aim of this study is to carry out genome wide association to identify genetic factors associated with schizophrenia and bipolar illness.
Role: Co-I

U01 MH 081896-01 (Sestan) 09/01/09 – 10/31/14
NIH/NIMH
Development of Organization of the Human Frontal Cortex
The goal of this grant is the detailed cellular expression mapping of the developing human frontal cortex using whole-genome exon arrays.
Role: Co-I

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Ruth R. Montgomery

eRA COMMONS USER NAME (credential, e.g., agency login): rmontgomery

POSITION TITLE: Associate Professor of Medicine and Pathology, Associate Dean for Scientific Affairs

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Pennsylvania, Philadelphia, PA	B.A. cum laude	1981	Biology
The Rockefeller University, New York, NY	Ph.D.	1987	Cell Biology
Yale University School of Medicine	Postdoc Fellowship	1988-1990	Cell Biology

A. Personal Statement

As Associate Dean for Scientific Affairs, I work to facilitate access for Yale investigators to the exceptional resources of our institution including instrumentation, collaborating investigators, and a diverse pool of human subjects for translational studies. The CTSA is central to these efforts. My lab's research focus is the function of innate immune cells and use of novel technology to advance translational studies. I have expertise in infectious models including Lyme disease and West Nile virus, as well as in effects of autoimmunity and aging on innate immune responses and pathways. My lab employs primary human cells to profile individual variations in immunity that result in divergent immune responses. Our recent publications define age-related deficiencies in toll-like receptor pathways and immune responses of multiple cell types (macrophages, neutrophils, and dendritic cells). We have conducted 'Systems immunology' studies of markers of individual susceptibility to viral infections with West Nile virus. I oversee studies of differential innate immune responsiveness in human cohorts with successful enrollment of >1800 healthy individuals, >150 immunosuppressed subjects and >150 subjects with West Nile or hepatitis C virus infections. I am co-PI with Dr. Erol Fikrig of an NIH Biodefense contract HHS N272201100019C "Innate Immune Pathways in Elderly and Immunosuppressed Populations" and am Co-Project leader on a U19 award with Drs. Fikrig and David Hafler to investigate susceptibility and resistance to infection with West Nile virus. I have been certified for BL3 work since 2004. My lab regularly collaborates with other Yale investigators on studies of autoimmunity and diverse infections. I have served as mentor for numerous trainees in my lab (undergraduate and graduate fellows) and provide expertise to many colleagues for translational studies involving imaging, flow cytometry, techniques to reduce variation, and single cell mass cytometry (CyTOF). I managed the confocal microscopy facility for the Department of Internal Medicine for 10 years and now I am Director of the Yale CyTOF facility. I am senior author on Yale's first CyTOF publication in which we demonstrate efficient detection of cell subsets by CyTOF starting with as few as 10,000 cells.

B. Positions and Honors

Positions and Employment

1980	Cambridge University Veterinary School, England
1981-1982	University of Pennsylvania, Biology Department, Research Assistant
1982-1987	Rockefeller Univ., Lab. of Cell. Physiol. & Immunol. (Z.A. Cohn & C.F. Nathan)
1988-1990	Post-doc. Fellow, Yale Univ. School of Med., Dept. of Cell Biology (Dr. Ira Mellman)
1991- 1997	Assoc. Res. Scientist, Dept. of Int. Med./Sect. of Rheum., Yale Univ. School of Medicine
1997- 2002	Res. Scientist, Dept. of Int. Med./Sect. of Rheum., Yale Univ. School of Medicine
2002-2012	Senior Res. Scientist, Dept. of Int. Med./Sect. of Rheum., Yale Univ. School of Medicine
2012-	Associate Professor, Dept. of Int. Med./Sect. of Rheum., Yale Univ. School of Medicine
2015-	Associate Dean for Scientific Affairs, Yale Univ. School of Medicine

Honors

1976	Philadelphia Science Council Award
1977	National Merit Finalist
1977-81	Benjamin Franklin Scholar, Univ. of Pennsylvania
1990	Invited Speaker, Gordon Conference on Lysosomes
1991, 1992	Invited Speaker, American College of Rheumatology
1992, 94, 96, 99	Invited Speaker/Presenter, International Conference on Lyme Disease
1996, 2004, 2008	Invited Speaker, Gordon Conference on Spirochetes
2000	Fellow of Saybrook College, Yale University
2001	Distinguished Young Alumna Award of The Baldwin School
2002	Invited Speaker, International Conference on Lyme Disease
2004	Advisory Council, Peabody Museum Science Education Partnership Award
2004-2013	Director of Confocal Microscopy, Dept of Internal Medicine
2012-	Grant Reviewer, CDC
2013-	Grant Reviewer, MRC, NIH Special Emphasis Panel
2013-	Director of CyTOF facility
2013	Intl Conf on Lyme Borreliosis, Abstract Selection Committee (Immunopathogenesis)
2014	Guest Editor, *Current Opinion Immunology*, Immune Senescence (Vol. 29)
2014	Session Chair, Annual Meeting of Society of Leukocyte Biology (Immune Ontogeny)
2015	Invited Speaker, 4th Eur. Congress of Immunol, Immunosenescence Satellite symposium

C. Contribution to Science

1. Novel methodologies in investigation of primary cells. Traditional studies of immune responses through imaging of primary cells and surveying cell markers by FACS or immunoblot have advanced our understanding significantly. However, these studies are generally limited technically by the small sample size available from patients and the inability to conduct complex laboratory techniques on multiple human samples. My lab has published detailed methods using primary human cells with several technical advances such as ImageStream technology, which combines quantitative flow cytometry with simultaneous high-resolution digital imaging. We used this to demonstrate impaired cellular mechanisms of TLR5 pathways in aging. Recently, we showed efficient detection of immune cells from very small samples of blood using Mass cytometry or CyTOF (Cytometry by Time-Of-Flight). CyTOF is a new technology for multiparameter single cell analysis that overcomes many limitations of fluorescence-based flow cytometry and can routinely detect as many as 40 markers per sample. This technology provides tremendous detail for cellular analysis of multiple cell populations simultaneously and is a powerful technique for translational investigations. I led the study showing reproducible detection of immune cell subsets starting with as few as 10,000 cells and from biopsies of human skin. Our study provides methods to employ CyTOF for small samples, which is especially relevant for investigation of limited patient biopsies in translational and clinical research. Recently, we have employed CyTOF to define alterations in Natural Killer cell repertoire that increase following infection with either HIV or West Nile virus.

a. Qian F, and **Montgomery RR.** 2012. Quantitative imaging of lineage specific Toll-like receptor mediated signaling in monocytes and dendritic cells from small samples of human blood. *JoVE* 62: e3741. PMCID: PMC3466655
b. Yao Y, Liu R, Shin MS, Trentalange M, Allore H, Nassar A, Kang I, Pober J, and **Montgomery RR.** 2014. CyTOF supports efficient detection of immune cell subsets from small samples. *J Immunol Methods* 415, 1-5. PMCID: PMC4269324
c. Strauss-Albee, D. M., Fukuyama, J., Liang, E. C., Yao, Y., Jarrell, J. A., Drake, A. L., Kinuthia, J., **Montgomery, R. R.**, John-Stewart, G., Holmes, S., Blish, C. A. 2015. NK cell repertoire diversity reflects immune experience and predicts viral susceptibility. Sci Trans Med 7:297ra115.

2. Effects of aging and autoimmunity on innate Immunity. I co-direct the research team that carried out the most comprehensive studies to date on alterations in the expression and function of Toll-like receptors (TLRs) on immune cells from populations of immunosuppressed and older adults. We were the first to show a reduction in surface expression and functional activity of TLRs in monocytes, dendritic cells, and neutrophils. The reduced or dysregulated TLR pathways contribute to decreased influenza vaccine responsiveness in aged humans and may contribute to increased susceptibility to selected infections including West Nile virus. In contrast to other

TLRs, my lab showed elevated levels of TLR5 and TLR5-induced cytokines from cells of older donors, and demonstrated an age-related increase in nuclear localization of NFκβ. The importance of this finding is a cellular mechanism underlying a potentially powerful strategy to enhance vaccination through targeting TLR5 to address the decreased response to vaccines in the elderly. This therapeutic advance can now be understood from the foundation of basic cell mechanisms from our translational study. In addition, I directed studies in PMN that demonstrate a reduction in TLR1 expression and TLR1-mediated responses with aging, and reduced efficiency of bioenergetics in PMN that likely contributes to reduced PMN efficiency through multiple aspects of PMN function. These studies also identify potential therapeutic opportunities to improve PMN function through addressing glucose utilization.

a. Kong KF, Delroux K, Wang X, Qian F, Arjona A, Malawista SE, Fikrig E, and **Montgomery RR**. 2008. Dysregulation of TLR3 impairs the innate immune response to West Nile virus in the elderly. *J Virol* 82:7613-7623. PMCID: PMC2493309
b. Qian F, Wang X, Zhang l, Lin A, Zhao H, Fikrig E, and **Montgomery RR**. 2011. Impaired interferon signaling in dendritic cells from older donors infected in vitro with West Nile virus. *J Infect Dis* 203:1415-1424. PMCID: PMC3080893
c. Qian F, Wang X, Zhang L, Chen S, Piecychna M, Allore H, Bockenstedt LK, Malawista SE, Bucala R, Shaw A, Fikrig E, and **Montgomery RR**. 2012. Age-associated elevation in TLR5 leads to increased inflammatory responses in the elderly. *Aging Cell* 11:104-110. PMCID: PMC3257374.
d. Qian F, Guo X, Wang X, Yuan X, Chen S, Malawista SE, Bockenstedt LK, Allore HG, and **Montgomery RR.** 2014. Reduced bioenergetics and toll-like receptor 1 function in human polymorphonuclear leukocytes in aging. *Aging* 6: 131-139. PMCID: PMC3969281

3. Individual variation in response to infections with hepatitis C virus, West Nile virus (WNV). In my lab's work in the pathogenesis of infection with WNV, we have examined the interaction of macrophages, dendritic cells, and polymorphonuclear leukocytes (PMN) with WNV. We have conducted an integrated systems-level transcriptional and functional analysis from stratified cohorts of subjects with a history of WNV infection. We employ cutting-edge techniques despite the technical challenges of working with primary cells and the inherent variation in human samples. We have demonstrated that PMNs have a biphasic response to WNV infection, serving as a reservoir for replication and dissemination in early infection and later contributing to viral clearance. We have shown an age-dependent dysregulation of TLR3 in macrophages that results in higher production of cytokines, which may contribute to the increased severity of WNV infection in older individuals. We have demonstrated impaired anti-WNV interferon signaling in dendritic cells from older donors based on defective positive-feedback regulation of type I IFN production and elevated expression of the inhibitory TAM (Tyro3, Axl and Mer) family of receptor tyrosine kinases. Recently, we have defined specific markers that reproducibly distinguish susceptibility to WNV in humans and offers insights into potential therapeutic strategies.

a. Bai F, Kong KF, Dai J, Qian F, Zhang L, Brown CR, Fikrig E, and **Montgomery RR.** 2010. A paradoxical role for neutrophils in the pathogenesis of West Nile virus. *J Infect Dis* 202:1804-1812. PMCID: PMC3053000
b. Qian F, Bolen CR, Wang X, Jing C, Fikrig E, Bruce RD, Kleinstein SH, and **Montgomery RR.** 2013. Impaired TLR3-mediated interferon responses from macrophages of patients chronically infected with Hepatitis C virus *Clin Vaccine Immunol* 20:146-155. PMCID: PMC3571267.
c. Qian F, Thakar J, Yuan X, Nolan M, Murray KO, Lee WT, Wong SJ, Meng H, Fikrig E, Kleinstein SH, and **Montgomery RR.** 2014. Immune markers associated with host susceptibility to infection with West Nile virus. *Viral Immunol* 27: 39-47. PMCID: PMC3949440
d. Qian F, Goel G, Meng H, Wang X,You F, Devine L, Raddassi K, Garcia MN, Murray KO, Bolen CR, Gaujoux R, Shen-Orr SS, Hafler D, Fikrig E, Xavier RJ, Kleinstein SH, and **Montgomery RR.** 2015. Systems immunology reveals markers of susceptibility to West Nile virus infection. *Clin Vaccine Immunol* 22: 6-16. PMCID: PMC4278927

4. Immunophenotyping in translational investigation. In collaborative studies I have worked with several colleagues to employ rigorous quantitative assays in primary human cells from diverse disease models. In these studies I contribute functional assays for human immune cells and in depth analytic methods.

a. Dunne DW, Shaw AC, Bockenstedt LK, Allore H, Chen S, Malawista SE, Leng L, Mizue Y, Piecychna M, Zhang L, Towle V, Bucala R, **Montgomery RR**, and Fikrig E. 2010. Increased TLR4 expression and downstream cytokine production in immunosuppressed adults compared to non-immunosuppressed adults. *PLoS ONE* 5:e11343. PMCID: PMC2893205

b. Reis EAG, Hagan JE, Ribeiro GS, Carvalho AT, Martins-Filho OA, **Montgomery RR**, Shaw AC, Ko AI and Reis MG. 2013. Cytokine response signatures in disease progression and development of severe clinical outcomes for Leptospirosis. *PLOS Negl Trop Dis,* 7:e2457. PMCID: PMC3777885
c. You F, Wang P, Yang L, Yang G, Zhao YO, Qian F, Walker W, Sutton RE, **Montgomery RR**, Lin R, Iwasaki A, and Fikrig E. 2013. ELF4 is critical for induction of type I interferon and the host antiviral response. *Nat Immunol,* 14: 1237-1246. PMCID: PMC3939855
d. Zhou Y, Peng H, Sun H, Tang C, Gan Y, Peng X, Peng H, Chen X, Mathur A, Hu B, **Montgomery RR**, Shaw AC, Homer RJ, Lee CG, Elias JA and Herzog EL. 2014. Chitinase 3-like 1 is stimulated in patients with idiopathic pulmonary fibrosis and has distinct roles in pulmonary injury and fibroproliferative repair. *Sci Trans Med* 6, 240ra76. PMCID:PMC4340473

5. Interaction of spirochetes and phagocytes in the pathogenesis of Lyme disease. My studies in the 1990s and early 2000s identified the uptake and intracellular fate of *Borrelia burgdorferi* (*Bb*), the agent of Lyme disease, in monocytes, macrophages, and neutrophils using biochemical methods, quantitative imaging *in vitro*, and *in vivo* models. These studies reveal the responses to spirochetes before the development of antibodies and secondary immunity. In particular, I conducted studies showing efficient phagocytosis of *Bb* by macrophages in the absence of antibody, which is quite different from previous studies of *Treponema pallidum*, the spirochetal agent of syphilis. I led studies finding that numerous PMN granule components efficiently kill spirochetes and that the abundant PMN cytosolic protein, calprotectin, is a potent bacteriostatic agent from a cell primarily recognized for its oxidative and granular antibacterial mechanisms. We investigated aspects of vector ticks in the pathogenesis of Lyme disease and showed that two proteins from the saliva of Ixodid ticks reduce PMN efficiency in the uptake and killing of spirochetes via down-regulation of β2 integrins – findings relevant to the initial survival of spirochetes introduced into the host.

a. **Montgomery RR,** Nathanson MH and Malawista S. 1993. The fate of B. burgdorferi, the agent for Lyme disease, in mouse macrophages: Destruction, survival, recovery. *J Immunol* 150:909-15. PMID:8423346
b. **Montgomery RR**, Lusitani DL, Chevance A, Malawista SE. 2002. Human phagocytic cells in the early innate immune response to *Borrelia burgdorferi*. *J Infect Dis* 185:1773-1779. PMID: 12085324
c. Lusitani DL, Malawista SE, and **Montgomery RR**. 2003. Calprotectin, an abundant cytosolic protein from human PMN, inhibits the growth of *B. burgdorferi*. *Infect Immun* 71:4711-4716. PMCID: PMC166021
d. **Montgomery RR**, Lusitani D, de Boisfleury Chevance A, and Malawista SE. 2004. Tick saliva reduces adherence and area of human neutrophils. *Infect Immun* 72:2989-2994. PMCID: PMC387908

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/ruth.montgomery.1/bibliography/41467269/public/?sort=date&direction=ascending.

D. Research Support

Ongoing Research Support

N01-HHSN272201100019C (Fikrig & Montgomery) 06/21/11 – 03/31/16
NIH/NIAID
Innate Immune Pathways in Elderly and Immunosuppressed Populations
This contract examines host responses to agents of biodefense in the elderly and immunosuppressed.
Role: PI

U19 AI 089992 (Hafler & Fikrig) 07/12/10 – 6/30/16
NIH
Defining signatures for immune responsiveness by functional systems biology
The goal of this proposal is to employ high-resolution profiling to identify the molecular signatures defining individual immune responses to infection and vaccination.
Role: Co-Project Leader

U19 AI 089992 Pilot (Montgomery & Kumar) 07/01/13 – 06/30/16

NIH/NIAID
Immune signatures of responses to infection with Dengue virus
This pilot project funded jointly by the US-India Bilateral Collaborative Research Grants on Human Immune Phenotyping and Infectious Disease to examine immune responses to infection with Dengue virus (DENV) in India employs recent advances in immune profiling methods developed through the HIPC program to define critical elements that contribute to DENV resistance and susceptibility.
Role: Pilot PI

U19 AI 089992 Pilot (Montgomery) 09/01/14 – 06/30/16
NIH/NIAID
Novel technologies to define functional attributes of T cells in West Nile virus
This pilot project funded by the Human Immune Phenotyping Consortium is to conduct a coordinated examination of T cell responses from a stratified cohort of subjects with West Nile virus (WNV) to establish the molecular profiles of WNV-specific T cells and identify key functional differences in T cell functions between severe and asymptomatic subjects.
Role: Pilot PI

Completed Research Support (last 3 years)
U19 AI 089992 Pilot (Montgomery) 11/01/13 – 06/30/15
NIH/NIAID
Deciphering the role of Natural Killer cells in resistance to infection with West Nile virus
This pilot project funded by the Human Immune Phenotyping Consortium is to use CyTOF to evaluate NK cell phenotype and function in susceptibility or resistance to infection with WNV.
Role: Pilot PI

U19 AI 089992 Pilot (Montgomery) 01/01/14 – 06/30/15
NIH/NIAID
Influenza Tetramers and CyTOF
This pilot project funded by the Human Immune Phenotyping Consortium is to employ a novel single cell mass cytometry, or CyTOF, to test an influenza CyTOF panel and participate in cross-site tetramer testing with Stanford to obtain highly detailed, epitope-specific characterization of T cell responses to flu vaccination.
Role: Pilot PI

U19 AI 089859 (Montgomery) 01/01/12 – 12/31/13
NIH/NIAID
High-throughput immunophenotypic analyses of humoral responses to West Nile virus
This pilot project examines B cell repertoire in a stratified cohort of patients with WNV
Role: Pilot PI (Yale site)

UO1 AI 070343 (Fikrig) 08/15/06 – 07/31/12
NIH
Immunotherapeutics for Treatment of Flavivirus Infection
This project is to develop human monoclonal antibodies for the treatment of lethal WNV infection.
Role: Investigator

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Xiting Yan

eRA COMMONS USER NAME (credential, e.g., agency login): XITINGYAN

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Peking University, Beijing, China	B.Sc.	07/2001	Probability & Statistics
Peking University, Beijing, China	Ph.D.	07/2006	Applied Statistics
University of Southern California, Los Angeles, CA	Ph.D.	06/2009	Computational Biology and Bioinformatics

A. Personal Statement

I have the expertise, training and motivation necessary to successfully conduct biostatistical analyses for the Yale Asthma U19 cooperative agreement. I am a longstanding member of the YCAAD research team with expertise in statistics and computational biology. I am a Yale Center for Clinical Investigation (YCCI) Scholar and Director of the Precision Pulmonary Medicine Data Analysis hub. My research focus has been on defining endotypes of lung disease to improve and "personalize" the approach to management of chronic lung diseases. I received my first PhD in applied statistics from Peking University in China in 2006, during which I was introduced to all the challenges of biostatistics and recognized that to be at the cutting edge of translational research in disease pathogenesis. To augment this, I pursued a second PhD in computational biology at the University of Southern California. My training in statistics has provided me with extensive skills to both carry out standard data analysis and design novel computational models, which can be shown from my publications, most recently on transcriptional endotypes in asthma. In addition, my training in Computational Biology and Bioinformatics has enabled me to understand the basic biology and has strengthened my ability to tailor my data analysis towards the existing biological questions or issues. As a postdoctoral associate and research scientist, I successfully collaborated with different biologists and medical researchers doing various types of biomedical research. During this process, I gained extensive experience in working and communicating within a multidisciplinary research team. I became interested in analyzing microarray gene expression data during my training for my first PhD and moved onto analyzing gene expression data measured by RNA sequencing technology during my second PhD training. As a result of these previous experiences, I am aware of possible technical issues in the two types of data and have the skills to render solutions for them.

B. Positions and Honors

Positions and Employment

2009 – 2010 Postdoctoral Associate, Biostatistics Resource, Keck Laboratory, Yale School of Medicine
2010 – 2014 Associate Research Scientist, Biostatistics Resource, Keck Laboratory, Yale School of Medicine
2014 – present Assistant Professor, Section of Pulmonary, Critical Care and Sleep Medicine, Yale School of Medicine

Other Experience and Professional Memberships

2012 – 2014 Associate Director, Biostatistics Resource, Keck Laboratory, Yale School of Medicine

2014 – present Director, Data Analysis and Bioinformatics Hub, Center for Pulmonary Personalized Medicine (P2MED), Section of Pulmonary, Critical Care and Sleep Medicine, Yale School of Medicine

C. Contribution to Science

1. My early publications focused on improving computational methods in identifying phenotype associated genes and gene sets using gene expression data. Traditional methods for identifying phenotype associated genes performs well when the distribution of the gene expression values across subjects follows a uni mode distribution, like Gaussian distribution. However, more and more studies showed that subgroups of subjects with different gene expression profiles exist in many data sets. These subgroups will make the gene expression value distribution have multiple modes. In this case, the traditional methods will not perform well. I designed a method that uses the relative entropy as test statistics to identify phenotype associated genes and showed that the method performs better than the traditional methods, especially when subgroups of subjects exist in the data. To identify gene sets enriched in phenotype associated genes, most methods use all genes in predefined set to calculate the test statistics, while it may be true that only part of genes inside a pathway are perturbed. We designed a new gene set enrichment analysis method which calculates the test statistics based on subsets of genes that are more likely to be phenotype associated. I served as key personnel in these studies.

    a. **Yan X,** Deng M, Fung WK & Qian M (2005). Detecting differentially expressed genes by relative entropy. *J Theoretical Biol*, 234(3), 395-402. PMID 15784273
    b. Cheng C, Ma X, **Yan X,** Sun F & Li LM (2006). MARD: a new method to detect differential gene expression in treatment-control time courses. *Bioinformatics*, 22(21), 2650-2657. PMID:16928738
    c. Cheng C, **Yan X,** Sun F, & Li LM (2007). Inferring activity changes of transcription factors by binding association with sorted expression profiles. *BMC Bioinformatics,* 8, 452. PMID:18021409; PMCID:PMC2194743
    d. **Yan X** & Sun F (2008). Testing gene set enrichment for subset of genes: Sub-GSE. *BMC Bioinformatics*, 9, 362. PMID:18764941; PMCID:PMC2543030

2. In addition to the contributions described above, I designed a novel computational method to identify asthma heterogeneity from the induced sputum gene expression data. Traditional unsupervised clustering methods were applied but did not provide clearly separated and clinically meaningful subclusters of asthma patients. The computational method that I developed cluster patients based on pathways instead of individual genes, which was shown to be more robust to noise and has a better chance to include functional genes in the analysis. By using this method, we identified 3 subtypes of asthma that correlate with clinical characteristics of severe disease including a history of hospitalization and near fatal asthma attack. Biomarker genes for these subtypes in whole blood have also been found which can potentially be developed into a clinical test for the asthma subtypes we found. I served as key personnel in this study.

    a. **Yan X,** Chu JH, Gomez J, Koenigs M, Holm C, He X, Perez MF, Zhao H, Mane S, Martinez FD, Ober C, Nicolae DL, Barnes KC, London SJ, Gilliland F, Weiss ST, Raby BA, Cohn L, Chupp GL (2015). Non-invasive analysis of the sputum transcriptome Discriminate clinical phenotypes of asthma. *Am J Respir Crit Care Med*, 2015; 191(10):1116-25. PMID:25763605

A complete list of my publications can be accessed at:
http://www.ncbi.nlm.nih.gov/sites/myncbi/xiting.yan.1/bibliography/47895204/public/?sort=date&direction=ascending.

D. Research Support

Ongoing Research Support

Yale Center for Clinical Investigation
UL1 TR000142 (Yan) 09/15/14 – 09/14/16
Computational Analysis of Longitudinal Blood and Airway Gene Expression Data to Define Endophenotypes of Asthma

The goal of this study is to identify time invariant subtypes of asthma from longitudinal gene expression data from asthma patients.
Role: PI

NIH / NHLBI
R01 HL116235 (Kazmierczak) 09/26/12 – 06/30/16
Microbiome Acquisition and the Progression of Inflammation and Airway Disease in Infants with Cystic Fibrosis
The goal of this study is to determine whether the commensal microflora of the gastrointestinal tract and the respiratory tract influences the clinical manifestations and progression of Cystic Fibrosis (CF).
Role: Biostatistician

Completed Research Support (last 3 years)
NIH / NHLBI
R01 HL118346 (Chupp) 08/15/13 – 08/31/14
Longitudinal Analysis of Transcriptomic Endophenotypes in Asthma
The goal of this study is to identify subgroups of asthma patients from the gene expression in sputum using an integrative functional genomics method.
Role: Biostatistician

NIH / NHLBI
R01 HL095390 (Chupp) 09/24/08 – 07/31/12
Gene Expression Profiling in Asthma Severity: CHI3L1 Genotypes and Serum YKL-40
The goal of this study is to characterize the genome wide expression profile associated with asthma severity and YKL 40, CHI3L1 genotypes and phenotypes. By correlating these profiles in the lung and peripheral blood, we aim to identify novel pathways associated with asthma severity and chitinases and develop gene profiles that will have practical use in the clinical management of asthma.
Role: Biostatistician

HHSN272201100019C (Fikrig) 06/21/11 – 08/31/14
Innate Immune Pathways in Elderly and Immunosuppressed Populations
The goal of this study is to examine the host response to agents of biodefense in the elderly and immunosuppressed. These studies are aimed at elucidating alterations in innate immune responses, including Toll-like receptor signaling, in individuals who are elderly or taking immunosuppressive medications
Role: Biostatistician

NIH
U01 AI088752 (Ko) 08/10/10 – 07/31/15
Disease Determinants for Urban Leptospirosis
The goal of this study is to apply state-of-the art molecular approaches to field investigations in Brazil for the purpose of characterizing the disease determinants of leptospirosis and identifying novel strategies for intervention, such as improved diagnostics and vaccines.
Role: Biostatistician

OMB Number: 4040-0010
Expiration Date: 06/30/2016

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	YALE UNIVERSITY
Duns Number:	0432075620000
Street1*:	300 Cedar St
Street2:	TAC S440
City*:	NEW HAVEN
County:	
State*:	CT: Connecticut
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	065208237
Project/Performance Site Congressional District*:	CT-003

File Name

Additional Location(s)

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ● **Yes** ❍ **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ❍ Yes ● No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6

If NO, is the IRB review Pending? ● **Yes** ❍ **No**

IRB Approval Date:

Human Subject Assurance Number FWA0000257

2. Are Vertebrate Animals Used?* ❍ **Yes** ● **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ❍ Yes ❍ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. Is proprietary/privileged information included in the application?* ❍ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ❍ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ❍ Yes ❍ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ❍ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ❍ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Overall_Project_Summary_Asthma_U19.pdf
8. Project Narrative*	Overall_Project_Narrative_Asthma_U19.pdf
9. Bibliography & References Cited	Overall_References_Cited.pdf
10.Facilities & Other Resources	
11.Equipment	

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

Project Summary

Asthma research efforts have moved to defining subgroups of asthmatics that have similar clinical and physiologic manifestations of disease that are likely driven by novel biologic mechanisms. Examples include modulation of airway inflammation by adipose tissue, immunogenic airway microbiota, or relative differences in the expression of known pathways such as those driven by IL-13 and IL-5, or CHI3L1/YKL-40. Dissecting these endotypes of disease will enable pathogenesis research, therapeutic development, and clinical management to focus on distinct subsets of asthma and their associated phenotypes, leading to a precise, personalized approach to disease management, and ultimately a cure. With the emergence of single cell technologies, we have the capacity to take minute amounts of biologic sample non-invasively from the airway of individuals with asthma and study in depth individual cell responses using single cell profiling by CyTOF and single cell RNA sequencing. To accomplish this ambitious task, we have assembled a multidisciplinary team of scientists in asthma, immunology, and computational medicine to leverage these technologies. We have developed methods to capture single cells from the sputum and sequence their individual transcriptomes, profiling their cell lineage and functional state by CyTOF. In addition, we have adapted a ground-breaking technique of IgA-binding and sequencing, for use in sputum samples to define microbiota that reside in the airway. This novel technique will help us identify disease driving microbiota and how these organisms contribute to asthma heterogeneity. The overall goal for the Yale Asthma and Allergic Diseases Cooperative Research Center (AADCRC) is to generate a Systems-level understanding of immune mechanisms that define asthma phenotypes including cell profiling, transcriptional phenotypes, biomarkers of disease, novel cell populations, molecular pathway regulation, and microbial drivers of disease. The Yale Asthma U19 program is composed of 2 service Cores and 3 Projects. In Project 1, our multidisciplinary team will determine the single cell signatures and cell populations present in the airway that are associated with YKL-40 endotypes TEA clusters, and integrated endotypes in samples collected from a well-defined cohort of individuals with asthma. Associations between YKL-40 endotypes and T follicular helper (Tfh) cells, (Dickkopf) Dkk-1 levels, and IgA-seq results from Project 2 will be identified. In Project 2, we will determine the relationship between novel cell populations (Tfh cells), IgE responses (Dkk-1), and immunogenic microbial populations in the airway with asthma endotypes and phenotypes. In Project 3, RNA sequencing and CyTOF data will be used to develop an integrated model of asthma to better understand key aspects of its heterogeneity. RNA sequencing pipelines will be developed for processing of bulk-cell and single-cell RNA-Seq and CyTOF data. Data will be modeled to define pathobiologically meaningful endotypes of disease and datasets will be unified to a publicly accessible, searchable, integrated asthma MAP website.

Project Narrative

The goal of the Yale Asthma and Allergic Diseases Cooperative Research Center is based on the hypothesis that asthma heterogeneity can be resolved through integrated analysis of proteins, molecular pathways, cell populations, and gene networks that are pathobiologically relevant to the disease. We have designed an integrated and interactive set of Projects and Cores to study the heterogeneity of asthma using methods our team has developed for sputum samples to capture single cells and sequence their individual transcriptomes, profile cell lineage and functional state by CyTOF, IgA-binding and sequencing to define immunogenic microbiota that reside in the airway, and integrated computational modeling of clinical features and molecular phenomena.

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: GEOFFREY Middle Name L Last Name*: CHUPP Suffix:

Position/Title*: Associate Professor
Organization Name*: YALE UNIVERSITY
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Street2: PO Box 208057
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County:
State*: CT: Connecticut
Province:
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Credential, e.g., agency login: GCHUPP

Project Role*: PD/PI Other Project Role Category:

Degree Type: Degree Year:

File Name

Attach Biographical Sketch*:

Attach Current & Pending Support:

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

1. Project Director / Principal Investigator (PD/PI)

Prefix:
First Name*: GEOFFREY
Middle Name: L
Last Name*: CHUPP
Suffix:

2. Human Subjects

Clinical Trial? ● No ○ Yes
Agency-Defined Phase III Clinical Trial?* ○ No ○ Yes

3. Permission Statement*

If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?

○ Yes ● No

4. Program Income*

Is program income anticipated during the periods for which the grant support is requested? ○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

Budget Period*	Anticipated Amount ($)*	Source(s)*

PHS 398 Cover Page Supplement

5. Human Embryonic Stem Cells

Does the proposed project involve human embryonic stem cells?* ● No ○ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s): ❑ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

6. Inventions and Patents (For renewal applications only)

Inventions and Patents*: ○ Yes ○ No

If the answer is "Yes" then please answer the following:

Previously Reported*: ○ Yes ○ No

7. Change of Investigator / Change of Institution Questions

❑ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:

First Name*:

Middle Name:

Last Name*:

Suffix:

❑ Change of Grantee Institution

Name of former institution*:

PHS 398 Research Plan

Please attach applicable sections of the research plan, below.

OMB Number: 0925-0001

1. Introduction to Application (for RESUBMISSION or REVISION only)	
2. Specific Aims	Overall_Specific_Aims.pdf
3. Research Strategy*	Overall_Research_Strategy.pdf
4. Progress Report Publication List	
Human Subjects Sections	
5. Protection of Human Subjects	Protection-HumanSubjects_Asthma_U19.pdf
6. Inclusion of Women and Minorities	Inclusion-Women_Minorities_Asthma_U19.pdf
7. Inclusion of Children	Inclusion-Children_Asthma_U19.pdf
Other Research Plan Sections	
8. Vertebrate Animals	
9. Select Agent Research	
10. Multiple PD/PI Leadership Plan	
11. Consortium/Contractual Arrangements	
12. Letters of Support	SupportLetter_DeanSlayman.pdf
13. Resource Sharing Plan(s)	
Appendix (if applicable)	
14. Appendix	APPX_1_YCAAD_Study_HIC_0102012268.pdf APPX_2_Adenotonsillectomy_Study_HIC_1009007345.pdf Appx_3_Submitted_manuscript_Bothwell_Immunity.pdf

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

Program Goals and Specific Aims

Asthma is a chronic inflammatory disease of the airways that will likely afflict over 10% of the U.S. population by the end of this decade(1). Differences in genetic susceptibility, environmental exposures, and medication compliance are known to contribute to the heterogeneous clinical manifestations of disease. However, it is increasingly evident that the molecular alterations are also heterogeneous and that differences in the expression of many biologic pathways underlie differences in the phenotypic expressions of the disease. (2-4) Therefore, asthma could be considered a collection of airway diseases, each driven by a different set of biologic networks with unique, but overlapping, genomic, cellular, transcriptomic, inflammatory, physiologic, and clinical features of disease (5, 6). In keeping with this paradigm shift, asthma research efforts have moved to defining subgroups of asthmatics that have similar clinical and physiologic manifestations of disease that may be driven by novel biologic mechanisms such as adipose tissue in obese individuals with asthma, the airway microbiota, or relative differences in the expression of known pathways such as those driven by IL-13 and IL-5, or IL-21 (7, 8). Dissecting these endotypes of disease will enable pathogenesis research, therapeutic development, and clinical management to focus on distinct subsets of asthma and their associated phenotypes, leading to a precise, personalized approach to disease management, and ultimately a cure (9).

To date, most efforts to define asthma subgroups have relied on clustering individuals by clinical features of severity. These studies, including the Severe Asthma Research Program (SARP), have generated novel insights but are driven by analytical approaches that utilize clinical parameters that may be distal to many molecular perturbations that drive the disease (10, 11). In contrast to these clinically biased approaches, integrative functional transcriptomics has the potential to discriminate asthma subtypes at a level that is reflective of patterns in gene expression and pathobiology that cause common clinical features of disease, but will likely require vastly different therapies. Now, with the emergence of single cell technologies, we have the capacity to take minute amounts of biologic sample non-invasively from the airway of individuals with asthma and study in depth individual cell responses using single cell profiling by CyTOF and single cell RNA sequencing. This will eliminate the averaging of signals that results from the analysis of bulk samples or statistical comparison of means. To accomplish this ambitious task, we have assembled a multidisciplinary team of scientists in asthma, immunology, and computational medicine to leverage these technologies, making best use of the established infrastructure and resources of the Yale Center for Asthma and Airway Disease (YCAAD) for the study of asthma heterogeneity. We have developed methods to capture single cells from the sputum and sequence their individual transcriptomes, profiling their cell lineage and functional state by CyTOF. In addition, we have adapted a ground-breaking technique of IgA-binding and sequencing, used in samples from the gut to identify immunogenic microbiota, for use in sputum samples to define microbiota that reside in the airway. This novel technique will help us identify disease driving microbiota and how these organisms contribute to asthma heterogeneity. The overall goal for the Yale Asthma and Allergic Diseases Cooperative Research Center (AADCRC) is to generate a **Systems-level understanding** of immune mechanisms that define asthma phenotypes including cell profiling, transcriptional phenotypes, biomarkers of disease, novel cell populations and molecular pathway regulation, and microbial drivers of disease. We will contribute these datasets to a publicly accessible, searchable, integrated ASTHMAMAP website. Our project goals are as follows:

Aim1/Project 1: Hypothesis: There are unique cell populations, transcriptomes, and functional responses that are associated with YKL-40 endotypes and TEA clusters of asthma. Our multidisciplinary team will determine the single cell signatures and cell populations present in the airway that are associated with YKL-40 endotypes TEA clusters, and integrated endotypes (identified in Project 3) in samples collected from a well-defined cohort of individuals with asthma. Associations between YKL-40 endotypes and T follicular helper (Tfh) cells, (Dickkopf) Dkk-1 levels, and IgA-seq results from Project 2 will be identified.

Aim 2/Project 2: Hypothesis: Tfh cells, Dkk-1 and airway microbiota contribute to the heterogeneity of asthma. In these studies, we will determine the relationship between Tfh cells, Dkk-1, and immunogenic microbiota that are present in the airway with asthma endotypes and phenotypes associated with these novel cell populations, IgE responses, and microbial populations in the airway. The immunophenotype of cell populations associated with these immunologic phenomena will be integrated with functional profiles of airway cells.

Aim 3/Project 3: Hypothesis: Endotype clusters from a combination of transcriptional and protein profiling can discriminate asthma heterogeneity and mechanisms of disease. For these studies RNA sequencing and CyTOF data will be used to develop an integrated model of asthma to better understand key aspects of its heterogeneity. RNA sequencing pipelines will be developed for processing of bulk-cell and single-cell RNA-Seq and CyTOF data. Data will be modeled to define pathobiologically meaningful endotypes of disease and datasets will be unified to a publicly accessible, searchable, integrated asthma MAP website.

Background and Significance

Asthma epidemiology. Asthma afflicts over 300 million people worldwide, ~30 million in the U.S. population, and 280,000 in Connecticut. For reasons that are largely unknown, the prevalence of asthma has risen to epidemic proportions over the past five decades (12). In the United States this translates into more than 15 billion dollars in health expenditures (13). While we have advanced our understanding of disease pathogenesis and added therapeutic options to the clinic, the morbidity related to asthma remains high, accounting for 10 million school absences each year and limitations to physical activity reported by approximately half of asthma patients (14). Why we are not doing better in managing asthma is multi-factorial and an issue of intense debate (12). Reasons cited include barriers to healthcare access, lack of adherence to asthma guidelines by physicians, non-compliance, and underestimation of disease severity by both patients and physicians. Efforts in all these areas including billions spent by the pharmaceutical industry and governmental health agencies have failed to change outcomes significantly (15). A major reason for this and the motivation for the Yale Asthma U19 program is that asthma is a biologically heterogeneous disease and its heterogeneity remains poorly understood. To achieve better outcomes for our patients, we must understand this heterogeneity in humans and embrace the fact that this requires that we model the complex interactions of genes, pathways, and networks to elucidate this heterogeneity.

Shifting paradigms of asthma pathogenesis: the age of inflammation. Decades ago asthma was considered to be a disease of smooth muscle and was treated exclusively with bronchodilators. With the 1980s came the realization that asthma is a disease of chronic inflammation. This period witnessed a paradigm shift in focus of research and therapeutic development from bronchospasm to the concept that airway inflammation causes variable airflow obstruction. The prevailing paradigm of airway inflammation in asthma suggests that there is an imbalance in T-helper (Th) lymphocytes characterized by increased Th-2 lymphocytes and decreased Th-1 lymphocytes (16-18). Th-2 lymphocytes generate cytokines such as interleukin-4 (IL-4), IL-5 and IL-13 that result in eosinophil and mononuclear cell proliferation and recruitment to the lung, mucus production and allergen specific IgE production (19). In addition, there is increasing appreciation for the role that resident lung cells, such as bronchial epithelium, dendritic cells, and cells in regional lymph nodes such as T follicular helper (Tfh) cells, play in both orchestrating and perpetuating allergic inflammation. Tfh cells regulate the humoral response by providing helper signals to cognate B cells in the follicle and germinal centers of lymph nodes. The canonical Tfh cytokine IL-21 supports proliferation and differentiation of germinal center B cells (20, 21). In type 2 infections such as those caused by helminthes, or in allergic illnesses, Tfh cells, like their Th2 counterparts in the tissue, produce IL-4. This cytokine in Tfh cells directs class-switching to isotypes appropriate to the invading pathogen or allergen (*i.e.*, IgE and IgG4), and it maintains a robust germinal center B cell phenotype (22). By contrast to Tfh cells, tissue Th2 cells also produce IL-5 and IL-13 that support, among other features, eosinophil function, and production of mucus and IgE (23). B-cell production of IgE requires Tfh-dependent soluble and contact-dependent signals. The role of IL-21 in IgE switching is more controversial. In human B cells, IL-21 signals via STAT3 to enhance IgE secretion by naive and memory B cells stimulated with anti-CD40, acting synergistically with IL-4 (24, 25). While past studies have suggested that IL-21 suppresses IgE switching in murine B cells (26-28), more recent data reveals IgE production is ultimately reliant upon a Tfh-cell dependent germinal center pathway (29, 30), although the independent, and potentially synergistic, roles of IL-4 and IL-21 in its production remain unclear. In Project 2 we will evaluate this process in Tfh cells isolated from the blood and tonsil and determine how this critical cell population is associated with asthma heterogeneity. Specifically, Tfh cells will be correlated with sputum cell immunophenotype determined by CyTOF and the single cell transcriptome as described in Project 2.

Systems picture of airway pathology. Epithelial injury and repair paradigms are also emerging as critical pathways that contribute to pathobiology in asthma. In addition to evaluating endotypes of disease associated with novel T helper cells, we will also be examining novel moieties that suggest injury-repair paradigms are drivers of Th2 inflammation. In this model, the primary goal of the airway immune response is to eliminate the main trigger of inflammation and contribute to the structural and functional integrity of the airway. During the process, circulating blood leukocytes migrate to sites of infection or injury and develop polarized immune responses (31). The canonical Wnt signaling pathway that induces cell proliferation is involved in these processes which if inhibited could delay tissue repair and contribute to chronic inflammation. In Project 2, we will examine how the quintessential Wnt inhibitor, Dickkopf-1 (Dkk-1), contributes to asthma heterogeneity. Dkk-1 was originally discovered for regulating head formation of *Xenopus Laevis* (32, 33). Project 2 Co-Lead Bothwell has discovered that Dkk-1 enhances Th2 inflammation, possibly, through a mechanism that includes increased injury and delayed repair.

Microbial drivers of asthma heterogeneity. The role of the airway microbiome in the development of disease is being increasingly appreciated. Commensal microbiota are now appreciated to be critical regulators of host immune system homeostasis. This is an emerging area for interdisciplinary research. Disturbances in the composition of commensal bacteria can result in imbalanced immune responses and affect an individual's susceptibility to various diseases, including inflammatory (IBD and colon cancer), autoimmune (e.g., celiac disease, arthritis), allergic (e.g., asthma and atopy) and metabolic (e.g., diabetes, obesity, metabolic syndrome). Investigation of the microbiota in the lower respiratory tract is a relatively new field in comparison to the extensive work on the intestinal tract. In fact, the lung, once considered a sterile organ, was excluded from the original Human Microbiome Project (34). A limited number of reports have investigated the changes in the lung microbiota between healthy, non-smoking and smoking individuals as well as in patients suffering from Cystic Fibrosis (CF), Chronic Obstructive Pulmonary Disease (COPD) or Asthma (35-39). Despite emerging data on airway microbiota, little is known about the role of the lung microbiome in modulating pulmonary mucosal immune responses. The lung microbiota in humans consists of hundreds of bacterial species per person and exhibits exceptional inter-individual diversity (40). It may have dramatic effects on host physiology in health and disease through its interactions with and effects on the immune system. Recent advances in sequencing have led to an explosion of phylogenetic, taxonomic, and metagenomic studies of the microbiota in health and disease (41, 42). Such studies have revealed the diversity and complexity of the human microbiota (43). However, they have largely failed to identify individual bacterial species in humans that shape the immune system and contribute to disease (44). To overcome this problem, the Flavell Laboratory (Project 2) developed a new technology that uses the host Immunoglobulin A (IgA) response to the microbiota as a guide to identify specific bacterial species that selectively influence immune responses. In their seminal studies, they showed that IgA responses to bacteria identify species that contribute to immune responses and development of colitis in mice. Now, in collaboration with YCAAD, the Flavell laboratory has adapted this ground-breaking methodology for the most in depth to date identification of immunogenic microbiota in sputum samples, showing in Project 2 the identification of bacteria contributing to adaptive immune response that have previously been associated with severe asthma (45).

The unmet need of asthma heterogeneity. In parallel to changes in paradigms of asthma pathogenesis, has been the appreciation that the clinical disease of asthma is highly heterogeneous. In most individuals, symptoms are easily controlled by treatment with bronchodilators and low doses of inhaled corticosteroids. However, a significant proportion of asthmatics have poorly controlled disease and persistent symptoms despite receiving high doses of inhaled corticosteroids. Compared to controlled asthmatics, these "refractory" asthmatics frequently require hospitalization and have medical costs that are 6- to 7-fold higher than individuals with controlled asthma (46). Studies over the last two decades have defined several molecular targets in the Th2 inflammatory pathway that contribute to severe asthma, leading multiple pharmaceutical companies to develop therapeutic monoclonal antibodies that are currently in clinical trials. Thus far, these agents target Th2 cytokines including IL-5, IL-13, and their receptors (2, 47, 48). While the phase II and III clinical trials are promising, it is also clear to those of us who are conducting clinical trials, that the response to each of these biologics is heterogeneous and that, ultimately, none of these agents will achieve "remission" level control in all refractory patients. In addition, since as many as 30% of severe asthmatics are not allergic and have no evidence of eosinophilia, it is clear that we have a poor understanding of the pathobiology of a large percentage of individuals with asthma (4). This latter group often has severe airway obstruction that may not be reversible (airway remodeling) and is at significant risk of intubation. Taken together, these data highlight the heterogeneous nature of immunologic responses in asthma and reveal a major unmet need to understand the biologic mechanisms that underlie asthma heterogeneity. Only then will we be able to achieve true precision medicine and give "the right therapy to the right patient, at the right time" (9). Doing this requires that we move from defining phenotypes of disease (subgroups of disease based on clinical features of disease) to defining endotypes of disease (Figure 1). Instead of identifying a group of patients with similar clinical characteristics and then studying their biology, the Yale Asthma U19 model for

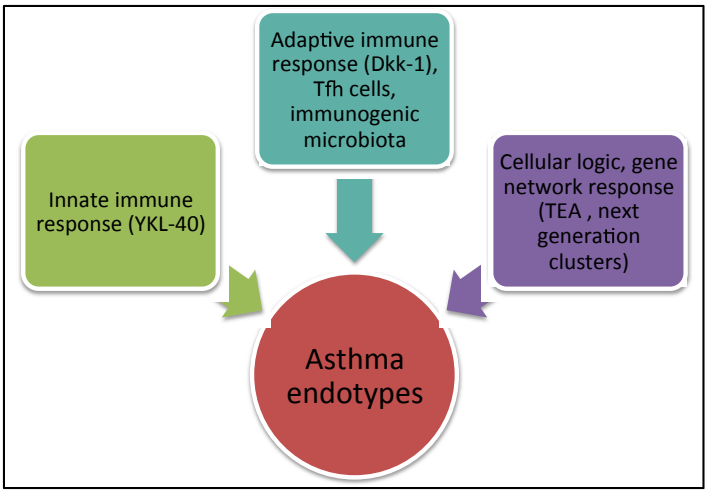

Figure 1: Several biologic phenomena contribute to asthma heterogeneity, driving specific endotypes of disease.

this proposal is to define the molecule(s), molecular pathway(s), and or gene network(s) that drive inflammation and/or demonstrate a common pattern of expression among a subgroup of individuals with asthma and then use this cluster definition to dichotomize asthma cohorts for further study. For the last 10 years, cutting edge technologies have allowed our group to pursue this approach including functional genomics and transcriptomics, leveraging it to discriminate subgroups of disease at the gene expression level that is mechanistically important to the pathogenesis of asthma (5). In this application, we will leverage single cell technologies to enhance our understanding of these endotypes of asthma defined by our multidisciplinary team of researchers (YKL-40 and TEA clusters in Project 1) and their associated cellular transcriptome and immunophenotype. In addition, we will characterize the endotypes that are associated with novel immune phenomena (Tfh cells, Dkk-1, immunogenic microbiota identified by IgA-seq in Project 2), and discover new endotypes based on integrated analysis of single cell CyTOF and RNA-seq data (integrated clusters in Project 3) and how these different endotypes, representing different biologic phenomena (innate immunity, humoral immunity, and cellular logic) inter-relate (Figure 2).

Summary. The clustering efforts to date, of clinical variables and the sputum transcriptome, have generated clear evidence that machine learning improves our resolution of asthma heterogeneity and indicates that more asthma specific computational tools are needed to define the full breadth of endotypes that are related to this complex disease. This approach provides us an opportunity to redefine how to sub-classify individuals with asthma. Only then will we be able to truly achieve precision medicine for this disease and achieve novel, potentially curative treatments. The evolution of high throughput sequencing, mass spectrometry analytical devices, and single cell capture technologies provide an opportunity **to define individual responses at unprecedented levels.** This offers tremendous specificity over averaging across cell populations and individuals. By examining cellular expression and responses to stimuli at the single cell level, we have the capacity to clearly define the cell populations driving the clinical phenotypes that we currently consider as severity. We are truly on the precipice of a paradigm shift in how we will look at and study asthma, from a single disease defined by variable airflow obstruction and chronic symptoms to a complex physio-immunologic inflammatory disease juxtaposed between the environment and the genome consisting of numerous endotypes. In this proposal, we will leverage the combined expertise of our multidisciplinary collaborative team of scientists to characterize the pathophysiologic features and cellular functional responses of the asthma subgroups identified in each of the driving projects, ultimately conducting integrative clustering. Using the iterative approach, we will develop customized experiments and, using samples collected from follow-up visits from the NextGen study, functional studies will be conducted to define the inflammatory response of the integrated clusters. All of these clusters and analyses will be provided to the community on a publicly accessible, searchable, integrated asthma MAP website that will be dedicated to this effort.

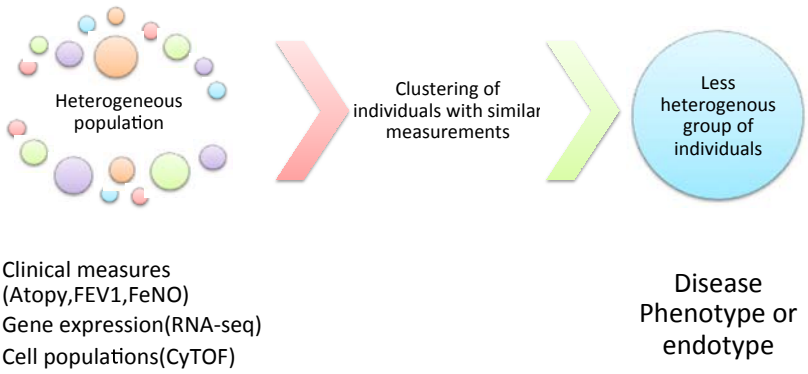

Figure 1: Conceptualization of clustering from heterogeneous collection of points to a more homogeneous group of individuals with similar characteristics. The goal of Yale Asthma U19 program is to use these methods using molecular characteristics to identify meaningful endotypes of disease.

Program Theme/Research Plan.

The overall theme of this cooperative program is based on the hypothesis that asthma heterogeneity can be resolved through integrated analysis of proteins, molecular pathways, cell populations, and gene networks that are pathobiologically relevant to the disease. Our U19 is built around a highly integrated and interactive set of Projects and Cores (Figure 3) designed to identify endotypes of asthma that are clinically meaningful and pathobiologically relevant. To accomplish this ambitious goal, we have assembled an interdisciplinary team of investigators in translational airway disease research, immunobiology, and computational medicine. This combination of expertise creates a natural synergy among the Yale Asthma U19 team and with the scientific goals of the projects. The combination of expertise between the cores and driving projects highlights the concept of “team science” that is fostered by the leadership of Yale University (see letter of support from Deputy Dean Slayman) and the translational research program central to the mission of YCAAD. Dr. Chupp’s expertise in genomics, translational asthma research and mission to pursue transformative research in populations with asthma formed the foundation for this team to begin collaborating. Drs. Craft, Bothwell, and

Flavell, with laboratories in the Howard Hughes Institute of Immunobiology one floor above Drs. Chupp, Cohn and Montgomery, have made many discoveries over several decades on the primary mechanisms driving adaptive and innate immune responses that will be central to our goal to define asthma endotypes. Dr. Gerstein's leadership at an international level in computational biology and the design and deployment of analytical tools to analyze the multidimensional datasets we are constructing brings tremendous expertise and opportunity. It not only provides an essential interaction and synergy for all the projects that is often lacking in translational research proposals where high dimensional datasets are being generated, but will create a powerful shared resource for the field. Our U19 program is both scientifically and functionally multidimensional and synergistic. This will enable the maximal use of the single cell data that will be generated for these projects, providing tools for the larger research community to ultimately develop a web-based user interface for public use. Dr. Krishnaswamy, a rising star in computational biology, leads the way in single cell RNA-seq and CyTOF and theory of cellular logic that is essential to next generation analysis of asthma endotypes. Drs. Montgomery, Cohn, Gomez, and Yan provide expertise in immunology and the biology of airway inflammation. Integrated multi-disciplinary lung research teams like ours are emerging at many institutions and represent the next generation of scientists with the triple threat of biology, medicine, and computational skillsets that are required to achieve transformative discovery.

Another critical feature of the Yale Asthma U19 team is the unique combination of the YCAAD phenotyping protocol and the Center for Precision Pulmonary Medicine (P^2MED) which provides an innovative opportunity to conduct the human studies that are required to understand the pathogenesis of endotypes of

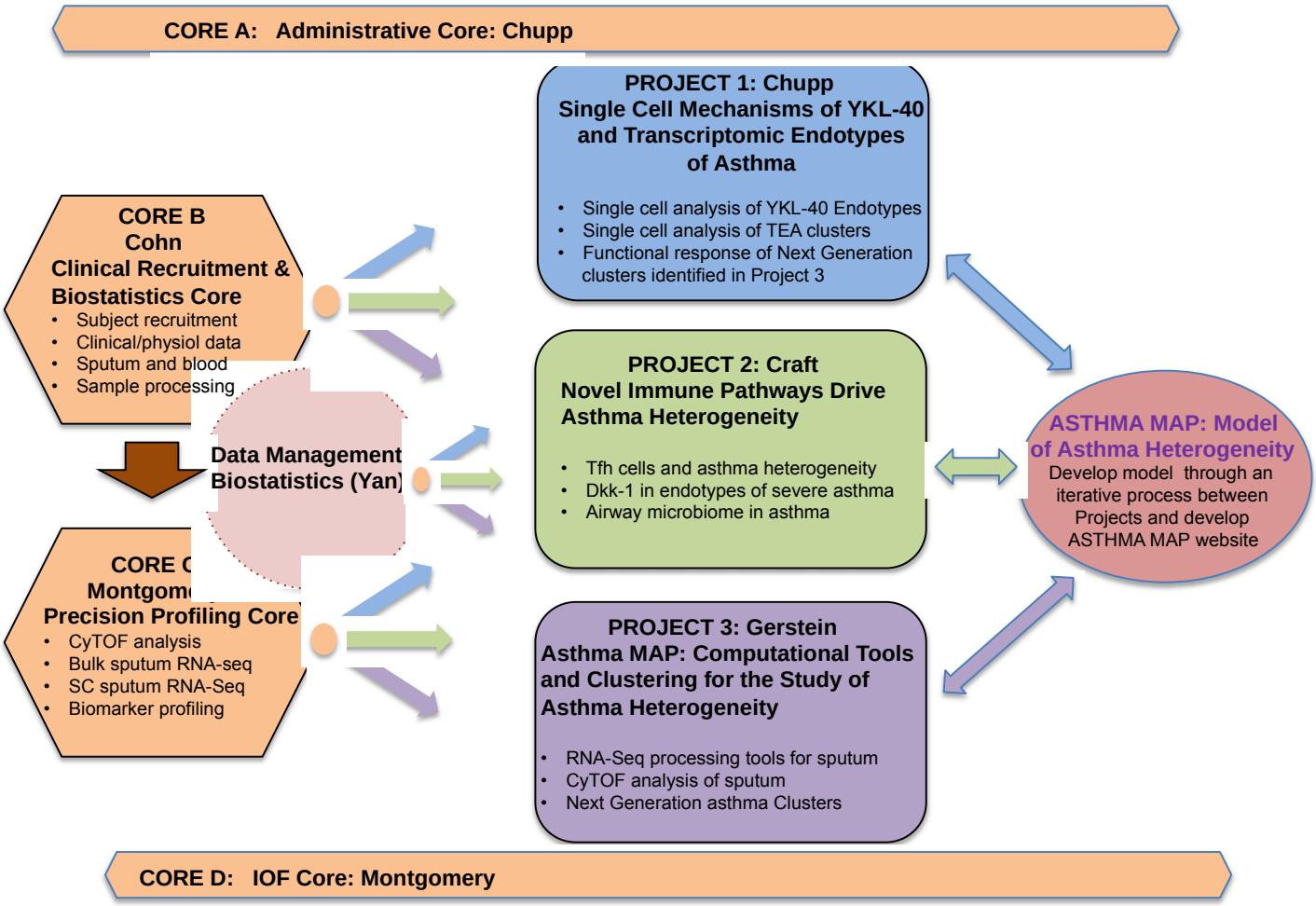

Figure 2: Structure of the Yale Asthma U19 program: **Next Generation Endotyping of asthma Heterogeneity**. Two service cores B and C generate human samples and multidimensional data for three driving projects. Through an iterative process between the projects, integrated modeling of disease will generate an **Asthma MAP** website for public access with menu driven interrogation of the high dimensional data generated from these studies available for public use.

asthma. This has been the vision and investment of the Center's Director, Naftali Kaminski, and Co-director, Geoffrey Chupp, PI of this U19 proposal and has made single cell technologies available for the studies outlined herein. Housed within the Pulmonary section and YCAAD's laboratories, these technologies include

the Fluidigm C1 single cell sorter and MSD protein quantitation platform, part of the Precision Profiling Core C, and the Nanostring Ncounter to quantitatively assess gene signatures that will be used to determine an asthmatic individual's TEA cluster in Project 1. These tools will be essential to dissect asthma endotypes for all the projects. Along with the CyTOF facility directed by Dr. Montgomery on the same hallway as the YCAAD investigators and laboratories, P2MED has provided these opportunities for the innovative studies highlighted in this proposal. This is coupled with YCAAD's robust phenotyping protocol with the capacity to enroll at least 100 new subjects a year for longitudinal collection of biologic specimens. This ongoing protocol routinely studies 6-10 subjects per week, and we expect no problem handling the volume of patient visits required for the longitudinal study outlined in this proposal. The YCAAD biorepository is growing at a rate of ~120 patients per year and has banked samples from over 800 patients with airway disease as well as gene expression data from hundreds of patients with asthma, sarcoidosis, and COPD, available for collaborative studies. Our research team is committed to building this repository and gene expression library for lung disease research as outlined in Project 3 and the Resource Sharing section of this proposal. Compared to other multicenter trials on asthma heterogeneity, such as the Severe Asthma Research Program (SARP), where data can be difficult for scientists to access, our vision is to make our integrated model of asthma heterogeneity publicly accessible through a user friendly interface that will enhance research efforts for all asthma researchers (**Asthma MAP)**.

Driving project summaries

Project 1: *Project Lead and overall PI: Geoffrey Chupp, MD, Professor of Medicine, Division of Pulmonary and Critical Care and Sleep Medicine, Director Yale Center for Asthma and Airway Disease.*

This project seeks to characterize the molecular and cellular phenomena associated with novel endotypes of disease. Most efforts to identify asthma subgroups have used clustering algorithms of clinical variables, but a true understanding of disease endotypes requires the examination of the molecular perturbations associated with the disease and/or clusters already established. We have identified two endotypes of disease by: 1) the identification of a molecule that is associated with subgroups of patients with refractory asthma, Chitinase-3-Like-1 (CHI3L1)/YKL-40; and 2) unsupervised cluster analysis of sputum transcriptomes that identified transcriptomic endotypes of asthma (TEA) clusters associated with severe disease (5). The first discovery was that circulating levels of CHI3L1/YKL-40 are elevated in a subgroup of patients with severe asthma and correlates with airway remodeling, genetic polymorphisms in *CHI3L1*, and the levels of YKL-40 in the airway. The second discovery was made with a novel pathway-based, unsupervised cluster analysis of sputum gene expression that identified 3 "transcriptional endotypes" of asthma (TEA clusters): one subgroup with a history of near fatal attacks, one with a high rate of hospitalizations for asthma, and a subgroup with milder disease. We hypothesize the existence of unique cell populations, transcriptomes, and functional responses that are associated with YKL-40 endotypes and TEA clusters of asthma. Our multidisciplinary team will determine the single cell signatures and populations present in a heterogeneous cohort of individuals with asthma enrolled in the NextGen study (outlined below in Core B) that are associated with the YKL-40 endotypes, TEA clusters and integrated endotypes to be identified in Project 3. In these studies we will determine the immunophenotype of the cell populations that produce YKL-40 in the airway using single cell CyTOF analysis of sputum cells. Single cell signatures associated with YKL-40 endotypes and TEA clusters of asthma will be defined, and the functional responses of the cell populations associated with these endotypes and their relation to Tfh cells, Dkk-1, and immunogenic airway microbiota will be determined (Project 2). The discoveries resulting from this project will significantly enhance our understanding of the YKL-40 endotypes and TEA clusters and their relationship to novel immune mechanisms of airway inflammation.

Research Project 2: *Project Lead: Joseph Craft, MD, Paul B. Beeson Professor of Medicine and Professor of Immunobiology.*

Each of the Aims, or sub-projects in Project 2 begins with an environmental stimulus that activates an inflammatory pathway that impacts asthma. Effective humoral immune responses are essential for protection from infection, especially at mucosal surfaces like the respiratory tract. These responses must be tightly regulated, as the development of antibodies against common environmental stimuli or self results in allergy or autoimmunity. This project focuses on the role of three pathways that are associated with dysregulated humoral immune responses. The first aim addresses allergen-induced IgE and a novel Tfh cell subset that has been defined by the Craft team. Studies in animals determined that this novel T cell is essential cell to IgE class-switching and plasma cell development. Understanding these cells and their impact on subgroups of asthma in humans will validate a novel mechanism that drives adaptive type 2 inflammatory responses possibly related to IL-21. In the second aim, Dr. Bothwell and his team, expert in immune response to injury

and repair, have identified a novel molecule Dickkopf (Dkk-1), an inhibitor of the Wnt signaling pathway that is upregulated upon exposure to inhaled house dust mite antigen, and in synergy with TSLP, a well-known epithelial derived cytokine, stimulates Th2 development and Th2 activation. Importantly, Dkk-1 and TSLP are both significantly upregulated in the sputum of individuals that belong to TEA cluster 1 (as described in Project 1 and 2), so we hypothesize that upon exposure to inhaled antigen and epithelial damage, Dkk-1 augments asthmatic inflammatory pathways. Interestingly, Dkk1 is known to be released primarily from platelets, so its expression in the sputum was not expected. In this project we will define the cellular subpopulations in the airway the express Dkk-1, define the clinical features associated with the Dkk-1 endotypes, and determined how Dkk-1 driven pathways influence asthma. In Aim 3, Dr. Richard Flavell's team will explore the role of immunogenic airway microbiota in asthma pathogenesis and how commensal organisms contribute to the heterogeneity of asthma. Using a novel technique published by his laboratory, termed IgA-seq (Palm et al, Cell 2014) immunogenic bacteria are identified by their coating with IgA, which is copiously produced in the respiratory tract as one of its protective mechanisms (49). The bacterial species in the airway that stimulate an inflammatory response are likely to be associated with asthma endotypes. To our knowledge, these studies have never been done on sputum or the lung, so we pursued studies where preliminary data demonstrates the identification of an IgA response to *leptotrichia*, a commensal bacteria that has been associated with corticosteroid resistant asthma (50). Taken together, these studies will validate the relationship of these novel pathways in humans and determine the molecular and cellular phenomena associated with these endotypes.

Research Project 3. *Project lead: Mark Gerstein, Ph.D., Albert L. Williams Professor of Biomedical Informatics; Co-Director, Yale Computational Biology and Bioinformatics Program.*

Project 3 brings together Yale's outstanding computational program to synergize with other Yale Asthma U19 team members to harness the multidimensional single cell data from CyTOF and single cell RNA-Seq. The goal is to derive clinically informative clusters of genes, cells, and patients that define asthma phenotypes in terms of novel cell populations and molecular pathway regulation. The hypothesis is that clusters from a novel combination of bulk-cell and single-cell transcription (RNA-seq) and protein profiling (CyTOF) data from a well-characterized, well-defined cohort of heterogeneous asthmatic patients can differentiate patients in a way that identifies the mechanisms of asthma and its heterogeneity. In Aim 1, a comprehensive suite of human RNA-Seq tools to generate pipelines for the uniform processing of bulk-cell RNA-seq data isolated from sputum cell populations will be developed for all the projects. This will build on a considerable body of preliminary results that the Gerstein group has in developing human RNA-Seq pipelines, both for long and short RNA species. Workflow will be created to quantify transcript abundances, determine the degree to which they have been spliced and modified, observe the extent to which the transcripts correspond to annotated portions of the genome, as well as identify novel non-coding RNAs and transcribed pseudogenes and how these relate to endotypes of disease. In Aim 2, single-cell measurements of protein and mRNA abundances generated using mass cytometry (CyTOF) and RNA-Seq will be utilized to deeply characterize populations of cells produced in the airways from individuals with asthma. We will identify cell-signatures from multidimensional CyTOF measurements of signaling and surface proteins based on an unsupervised community detection method. These cell-signatures will be used to generate protein and cell specific clusters to stratify cell types and signaling pathways. In Aim 3, data from Aims 1 and 2 of this project, results from Projects 1 and 2, along with clinical stratifiers, the datasets generated by Core C, and external datasets will be used to make integrative clusters by patients, cells and genes. Each of these clusters, networks and models will be evaluated in the context of established clinical measurements (e.g. FEV1 and FeNO) to identify effective measures to stratify patients and how they might give insight to the mechanisms of asthma disease and heterogeneity. To effectively disseminate the data, pipelines and analyses generated by this and the other driving projects in this cooperative proposal, and an **Asthma MAP** website will be created for public access to all of the data, tools, pipelines and analyses described here (51).

Supporting Cores:

Core A. Administrative Core. *Core Lead and PI: Geoffrey Chupp, MD, Professor of Medicine, Director of YCAAD.*

The U19 project will be directed by an Administrative Core to provide oversight and coordination for all activities within the program, so goals are achieved and any problems are expeditiously addressed. This Core will also provide fiscal management to ensure cost-effective utilization of center resources and to promote communication and dissemination of research findings and technology. This core will be led by PI Chupp, who has significant experience with collaborative studies and has established, productive partnerships with the current U19 team.

Core B. Clinical Recruitment and Biostatistics Core. *Core Lead: Lauren Cohn, MD, Associate Professor of Medicine, Co-Director YCAAD*

This core will serve this Program and contribute to each of the Projects though acquisition of clinical data and human samples, and provide database and analytic support. The Yale Center for Asthma and Airway Diseases (YCAAD) supports the infrastructure of a well established, finely tuned clinical research center designed by Core Lead Cohn and PI Chupp to obtain human data and biospecimens for airway diseases research. YCAAD currently enrolls up to 6-10 patients per week and has a repository of 800 biospecimen in place. The clinical research tools developed over a decade in YCAAD include rapid recruitment from a large, urban population of asthmatic subjects with a range of disease activity and severity, and a study visit that delivers high quality clinical and physiological data, and biospecimens including sputum and blood. Through this mechanism we have conducted human-based research which led to the discovery of CHI3L1/YKL-40 and other immune pathways associated with asthma and developed novel methods to characterize disease heterogeneity using transcriptomic data and define novel endotypes of asthma (5, 52). The Clinical Recruitment and Biostatistics Core is the starting point of the 3 driving Projects in this U19 Program. Each Project will harness the strengths of this Core to obtain essential human data and biospecimens to conduct the proposed studies. The Biostatistical Component of the Core will support a custom built, web-based, HIPAA compliant database for data entry, biospecimen management and a portal for data retrieval and analysis, as well as extensive biostatistical support for analyses of the complex datasets generated in this proposal.

Core C. Precision Profiling Core. *Core Lead: Ruth R Montgomery, PhD., Associate Professor of Medicine, Director, CyTOF Facility*

The Precision Profiling Core will conduct standardized, quantitative, in depth phenotypic and functional cell profiling for samples provided by Projects 1 and 2, and will provide the generated data to investigators in Projects 3 for integration of data for computational modeling. Key platforms include Mass cytometry or CyTOF (Cytometry by Time-Of-Flight), multiplex Meso Scale protein quantitation (MSD), and bulk and single cell RNA-Seq. CyTOF is a new technology for multiparameter single cell analysis for high-dimensional analysis of cellular samples including detection of ~ 40 labels in a sample that provides functional data from multiple cell lineages simultaneously. CyTOF technological advances provide functional data from multiple airway or blood immune cell types simultaneously and are a powerful assessment of cell function. The aim of this core is to conduct multiparameter immune and airway cell markers using shared platforms to define cellular functions relevant to the heterogeneity of asthma. Samples collected from Projects 1 and 2 will be processed and analyzed by CyTOF using a set of panels of antibodies to stimulate different populations and analyze relevant surface and intracellular markers for the Projects. Core Lead Montgomery directs the CyTOF facility at Yale and her lab is actively involved in CyTOF studies such as defining the lower limits of immune cell detection and the functional significance of Natural Killer cell diversity in viral infection (53, 54). Samples collected from Projects 1 and 2 will be processed and analyzed for bulk and single cell RNA-Seq with guidance from Dr. Mane, Director of the Yale Center for Genome Analysis.

Core D. IOF Infrastructure and Opportunities Fund (IOF) Management Core. Core Lead: Montgomery. If awarded to the Yale Asthma U19 Program, the IOF Core will administer innovative, early phase research in human asthma and allergic diseases. Core Lead Dr. Montgomery has successfully led several Pilot Projects for the U19 grant in the Human Immunology Project Consortium (HIPC). She will be in regular communication with the AADCRC Steering Committee and NIAID staff regarding disbursement and tracking of IOF funds, as well as ensuring regulatory guidance and resource sharing.

Leadership plan

The investigators in this proposal have established collaborations, regular interactions, and a track record of shared success. As detailed in the Administrative Core, PI Chupp will conduct monthly meetings and frequent face-to-face meetings for the duration of this award to include regular oversight of current progress, and to refine research directives as results are generated. Investigators will meet monthly with all research personnel to provide a forum for updates and discussions on the status of each project and to tackle any pitfalls that arise. Dr. Chupp will provide oversight on the development and implementation of the entire program. However, given the highly interactive nature of the Yale U19 program and experience of the investigators, a team approach will be employed. The experimental aspects of Projects 1, 2, and 3 and Service Cores C and B will be the responsibility of the Project and Core Leads who will report progress to Dr. Chupp at monthly

meetings as described in the Administrative Core A. Ms. Susan Ardito, the U19 administrative assistant, will report to PI Chupp. The iterative nature of this application dictates close interaction and communication between all the Projects and Core Leads with the PI. A considerable strength for our proposal is that almost all the investigators are located in the same building within a floor of each other and communication is established, efficient, and frequent. Dr. Chupp has a strong track record of collaborating, leading projects, mentoring postdocs and students and publishing with other investigators. Project and Core Leads will have weekly meetings with their laboratories to review details of the project. There will be at least weekly lab/operational meetings for the Projects and Cores open for attendance by all Yale Asthma U19 members. At these meetings where intellectual dialogue is encouraged, project teams will present preliminary data and discuss future directions. We will use these collaborative meetings as a platform to present, comment and exchange ideas on working progress of each project's aims. Lab members working on the project will actively participate in these meetings. Dr. Chupp will serve as contact PI and be responsible for financial oversight, regulatory compliance, submission of progress reports to NIH, and all communications.

E.1. Qualifications and Interactions of Project Leads and Co-Leads

Summary of expertise and Interactions:

Project 1:
Geoffrey Chupp, MD, Professor of Medicine; Section of Pulmonary, Critical Care & Sleep Medicine; Director, Yale Center for Asthma and Airways Disease. Dr. Chupp's translational asthma research program is focused on understanding the pathogenesis of asthma and asthma heterogeneity through the study of genotype-phenotype-endotype relationships in populations of asthmatics. As Director of the Yale Center for Asthma and Airways Disease (YCAAD), he has developed a translational research program integrated into an active clinical program that is focused on management of patients with asthma. Studies from his laboratory discovered the relevance of the YKL-40 pathway in inflammation and remodeling in individuals with asthma and demonstrated the capacity to identify endotypes of disease in complex inflammatory diseases of the lung, such as asthma, by measuring biomarkers in the circulation. These discoveries also laid the groundwork for the development of an anti-YKL-40 biologic therapy (Yaklizumab) as an NIH sponsored Center for Advanced Diagnostics and Experimental Therapeutics (CADET), of which he is Principal Investigator. Dr. Chupp's team also conducted an analysis of the airway transcriptome using genome-wide gene expression microarrays and identified three Transcriptomic Endotypes of Asthma (TEA) clusters that correlate with near fatal asthma and hospitalizations for asthma, again demonstrating the capacity to identify clinically meaningful endotypes of disease through the evaluation of cellular transcriptomes and molecular pathways.

Jose Gomez-Villalobos, MD, MS, Assistant Professor of Medicine, Section of Pulmonary, Critical Care & Sleep Medicine. Dr. Gomez is a physician scientist in YCAAD and has formal training in computational biology from the Yale Computational Biology and Bioinformatics Program directed by Dr. Gerstein. Dr. Gomez is an active member of the YCAAD clinical and research program and developed the YKL-40 endotype clustering algorithm that will be used in Aim 1 of Project 1. Dr. Gomez's research focus is on the characterization of asthma endotypes through the analysis and integration of high-throughput data generated in YCAAD laboratories and publicly accessible data to understand the biology of airways disease and the fundamental molecular processes present in asthma.

Xiting Yan, PhD, Assistant Professor of Medicine, Section of Pulmonary, Critical Care & Sleep Medicine, Director of Precision Pulmonary Medicine Data Analysis Hub. Dr. Yan has been an integral member of the YCAAD computational program for the last 6 years. Dr. Yan has PhDs in both biostatistics and computational biology, providing the perfect skillset to analyze the data that will be generated from humans samples in the course of these studies. During her time with YCAAD, Dr. Yan has also developed a keen understanding of airway biology and asthma, essential for her efforts to define endotypes of disease and their relationship to disease pathogenesis at the molecular level. Evidence of this expertise is Dr. Yan's recent description of the TEA clusters (5). This study is direct evidence that expression patterns of molecular pathways are linked to clinical phenotypes of asthma. This concept is a key theme of the Yale Asthma U19 proposal as well as Dr. Yan's Yale Center for Clinical Investigation Scholar Award for faculty career development.

Project 2:
Joseph Craft, MD, Paul B. Beeson Professor of Medicine (Rheumatology) and Professor of Immunobiology, Section Chief, Rheumatology, Program Director, Investigative Medicine. Dr. Craft has a longstanding interest in dissecting the pathogenesis of systemic autoimmunity, focusing upon the activation and differentiation of

CD4 T effector cell subsets in lupus. His lab characterized CD4 T cells that help B cells in immune responses, with the idea that information gleaned from the latter studies could be applied to our understanding of asthma. In recent work, they have begun to define the transcription factors critical for the development of these cells, Tfh cells. His group aims to further investigate how these cells contribute to asthma heterogeneity in the studies outlined in Project 2.

Alfred Bothwell, PhD, PhM, Professor of Immunobiology; Director of Graduate Studies Immunobiology. Dr. Bothwell's laboratory has been focused on characterizing how immune cells develop and evolve to migrate into vascular sites and drive inflammation. Dr. Bothwell's laboratory examines immune responses *in vitro* and *in vivo* which includes the development and use of humanized mice. In addition, his group has significant experience with synthetic microvessels and is working on a translational project to revascularize islets to treat type I diabetes. These efforts have resulted in the discovery of Dkk-1 as a driver of Type 2 inflammation through a novel mechanism that has implications for endotypes of asthma.

Richard Flavell, PhD, FRS, Sterling Professor of Immunobiology; Investigator, Howard Hughes Medical Institute; Department Chair, Immunobiology. Dr. Flavell's qualifications for the proposed studies and contributions to science are profound and too numerous to include in this short description. Dr. Flavell is co-discoverer of introns in cellular genes: he showed DNA methylation correlates inversely with, and prevents, gene expression. As a postdoc with Weissmann, he was the first to develop reverse genetics and his laboratory studies the molecular and cellular basis of the immune response. He has been instrumental in discovering the molecular basis of T-cell differentiation from precursor cells into differentiated subsets. This work led to the discovery that GATA3 is a critical regulator of the Th2 response and the first example of such a molecule in Th cell differentiation. His laboratory has elucidated the mechanisms of immunoregulation which prevent autoimmunity and overaggressive responses to pathogens. Specifically, Dr. Flavell's laboratory has elucidated the role of TGF-β in the regulation of immune responses. Dr. Flavell's laboratory has discovered the role of several receptor families in the innate immune response, including the role of several Toll-like receptors and intracellular Nod-like receptor families (NLRs). This recently led to the elucidation of function of Nod2 in inflammatory bowel diseases and Nlrp proteins in the production of IL-1. His group also established a powerful connection between inflammasomes, microbial homeostasis and chronic diseases. He showed that inflammasome dysfunction causes dysbiosis of the microbiota which, in conjunction with a susceptible diet, leads to inflammatory bowel disease and Metabolic Syndrome, including Obesity, Fatty Liver disease and Type 2 diabetes. As is shown in Aim 3 of Project 2, the Flavell lab has now adapted these efforts to asthma and will characterize not only the airway microbiome, but importantly, the microbiota that are driving immunes responses in the airway and are likely contributing to disease heterogeneity and the development of specific asthma endotypes. (49).

Project 3:

Mark Gerstein, PhD, Albert L. Williams Professor of Biomedical Informatics Co-Director, Yale Computational Biology and Bioinformatics Program. Dr. Gerstein's "dry bench" laboratory in computational biology does research in bioinformatics, applying computational approaches to problems in molecular biology. Broadly, his group is interested in large-scale analyses of genome sequences, macromolecular structures, annotating the human genome, data mining and machine learning, molecular simulation, functional-genomics datasets and database design. His group's scientific goal is to tackle broad statistical questions about modeling the biologic behavior of macromolecules by relating their physical properties, cellular function, interactions, and phylogenetic distribution. This research involves a number of quantitative techniques central to the Yale Asthma U19 Projects including database design, systematic data mining and machine learning, visualization of high-dimensional data, and molecular simulation. In keeping with "next generation science," Dr. Gerstein will not only develop asthma and sputum specific tools and pipelines for these projects and for public use in collaboration with the U19 team, his group will also develop a web-based **Asthma MAP** application with a menu driven interface for evaluating the molecular basis of endotypes to be characterized in this proposal.

Smita Krishnaswamy, PhD., Assistant Professor; Assistant Professor of Genetics; Assistant Professor of Computer Science. Dr. Krishnaswamy is new member of the Yale Computational Biology Program and the YCAAD team, bringing expertise in the analysis of CyTOF data. Her work on the verification of large, and often probabilistic Boolean networks, such as those in modern computer chips, led her to study electronic circuits and now biological circuits. Her laboratory's research centers around the idea that cells are actually complex computational networks. Cells contain biological circuitry for sensing the external environment and process these external signals through networks of interacting components, producing chemical output as well as regulating gene expression and state reconfiguration. Dr. Krishnaswamy's laboratory is especially

interested in the power of single-cell technologies such as mass cytometry and single-cell RNA-sequencing in learning predictive computational models of various biological phenomenon such as differentiation, response to antigen, and drug treatment. This expertise will be essential to characterizing the cellular networks present in the sputum (see Krishnaswamy et al. Science 2014) (55).

Cores within the Yale Asthma U19 Program

Core A: Administrative, Core Lead: Chupp. (See above)

Core B: Clinical Recruitment and Biostatistics, Core Lead: Cohn, Associate Professor of Medicine, Section of Pulmonary, Critical Care & Sleep Medicine. Dr. Cohn is an experienced translational investigator with a clinical and research focus in asthma. With PI Chupp, she is Co-Director of the Yale Center for Asthma and Airway Diseases (YCAAD). Dr. Cohn has a bench-based research program focused on allergic airway inflammation and immunology. This particular focus on lung immunology, mucus and the airway epithelium provides a critical basis for understanding pathways identified in our human studies. She has been active for the past ten years in subject recruitment for the YCAAD phenotyping protocol. Dr. Cohn manages personnel in the YCAAD laboratories and oversees processing of samples. She has been a key investigator leading to publications generated from YCAAD data as outlined below. She has an active clinical practice in asthma in YCAAD and will be fundamental to recruiting subjects for these studies.

Core C: Precision Profiling, Core Lead and Co-Lead: Montgomery and Mane.

Ruth Montgomery, **PhD**, Associate Professor of Internal Medicine, Associate Dean for Scientific Affairs, is a cell biologist and experienced translational investigator with expertise in innate immunity, West Nile virus, Lyme disease, and the immune response in aging. She is the Director of the Yale CyTOF Facility, a key platform in this core, and has worked closely with PI Chupp in development of multidimensional investigations of asthma for the current U19 award. She has long-standing collaborations with the other project leaders on this award including studies directly related to this core such as immune responsiveness in infection with Project 2 Co-Lead Flavell, asthma with PI Chupp, and RNA-Seq analysis with Project 3 leader Gerstein of immune cells in response to infection West Nile virus. As co-PI with Dr. Erol Fikrig of an NIH Biodefense contract HHS N272201100019C “Innate Immune Pathways in Elderly and Immunosuppressed Populations,” she has overseen successful enrollment of >1500 healthy individuals for studies of differential innate immune responsiveness in human cohorts and is experienced in directing multicomponent translational investigations.

Shrikant Mane, PhD, is the Director of The Yale Center for Genome Analysis (YCGA) and The Keck Biotechnology Resource Laboratory at Yale. He has published more than 100 articles, holds 2 patents, and has amassed over 25 years’ of research experience in both academic and private industry on the development of sequencing applications. Dr. Mane has a demonstrated record of establishing a successful and productive genomic facility and works closely with PI Chupp in developing single cell RNA-Seq protocols for analysis of sputum and the studies outlined in this proposal.

Core D: Infrastructure Opportunities Fund, IOF Pilot Core Lead: Montgomery (see above)

Interactions:

This is a highly synergistic team of investigators that has been collaborating for over 10 years, crisscrossing scientific interests and backgrounds. Evidence of these interactions and scientific diversity are the more than 15 publications co-authored by this team of investigators over the last decade (5, 56-68). In addition, we have broadened the team of investigators to enhance the feasibility of achieving our goal to develop a better understanding of asthma heterogeneity with 3 junior investigators with great promise, Drs. Krishnaswamy, Gomez, and Yan, who are already making a significant scientific impact.

Environment

This collaborative U19 takes advantage of enormous strengths that exist across our institution and campuses as well as the infrastructure that has been established for exactly the kind of research that is proposed in this multi-project U19 program. Yale provides unique opportunities for the successful achievements of the proposed work on defining asthma endotypes using next generation technologies. The academic office and laboratory of the PI, Dr. Chupp, are located on the fourth floor of the Anlyan Center (TAC) on the Yale School of Medicine campus – a new center designed to facilitate transdisciplinary collaboration for the advancement of

science. Almost all of the Investigators on the Yale Asthma U19 team are located in TAC or nearby on the Yale Campus, further enabling the collaborations that led to the transcriptomic and phenotyping studies outlined in this proposal. This center is connected to the Yale New Haven Hospital by indoor, over-street walkways, so within a 5 minute walk technicians, scientists, and research coordinators can be in the YCAAD clinical offices or TAC research laboratories. This is a significant advantage for the high throughput phenotyping protocol that is central to the YCAAD research program. In addition, Dr. Gerstein's laboratory is part of the Molecular Biophysics and Biochemistry Department with other inspirational scientists such as Thomas Steitz (awarded the Noble Prize in 2009 for his work on rRNA structure and function). This program is the intellectual hub of transcriptomics at Yale. Also, under the leadership of the current Dean, Robert Alpern and President Richard Levin, Yale acquired the Bayer Pharmaceutical property in West Haven in 2007, now a 14 building campus with conference center and multiple core facilities to support translational science. Two of these cores are central to the YCAAD program and the goals of this U19 program including the Yale Center for Genome Analysis, a full service facility dedicated to providing RNA expression profiling and DNA sequencing. This Center is led by Dr. Mane, Co-Lead of the Precision Profiling Core C and the High Performance Computing cluster that will support storage of the high dimensional data to be generated in the course of these studies.

Integrated laboratory and meeting space.

The Anlyan Center (TAC), constructed at a cost of $176 million, is part of Yale's $1 billion investment for new and reconstructed biomedical research facilities. Within TAC reside several academic medicine divisions including, Endocrinology; Nephrology; Gastroenterology; Pulmonary, Critical Care, and Sleep Medicine (where PI Chupp, Core Lead Cohn, Yan, and Gomez all have offices); Allergy and Immunology and Rheumatology (where Core Lead Montgomery has an office). One floor above YCAAD is the Department of Immunobiology where Drs. Flavell, Bothwell and Craft have offices and laboratories, providing Yale Immunology with a unique concentration of superb laboratories and investigators dedicated to the study of the immune system. On every floor of the TAC building, there are 2 large conference rooms and 2 large meeting areas that are available to all scientists and programs via an online scheduling system. These spaces are intended to enhance robust collaboration and interaction between scientists. All these rooms are equipped with advanced technologies for presentations and many are equipped for video conferencing. Lastly, there are several large conference rooms in TAC and a large auditorium on the first floor. There is no question that the intention for TAC to foster interdisciplinary research was realized with the collaborations developed amongst the Yale Asthma U19 team.

Data Sharing

Data generated in these studies will be made available to members of the research community. This includes cell function data and clinical phenotyping and any other resources developed in the course of this funding. Per NIH policy, this data will be uploaded into ImmPort or the Gene Expression Omnibus (GEO) website according to the data reporting plan created with the NIH officers. We will comply with the NIH Data Sharing Plan, which is designed to enable the widest dissemination of data, while also protecting the privacy of study participants and the utility of the data, by de-identifying and masking potentially sensitive data elements, consistent with HIPAA considerations. This approach is fully compliant with the NIH public data sharing policy (http://grants.nih.gov/grants/policy/data_sharing). Completed data sets will remain private for up to 3 years or until the online publication of the first manuscript. When the earlier of these two conditions is met, the data set(s) supporting the publication will be made available through the NIAID ImmPort data repository, along with relevant public databases (e.g., Gene Expression Omnibus (GEO) and the Sequence Read Archive (SRA)). In addition, as detailed in Project 3, the overall aim of this U19 proposal is to define endotypes of asthma that are associated with novel immunologic pathways, molecules, and microbiota at the single cell level and deliver asthma specific tools and pipelines for public use via a publicly accessible, user friendly, menu driven, interactive website: ASTHMA MAP.

Timeline and Milestones of Program (See Table 1). Our seasoned team of investigators and robust translational program has the capacity to conduct the ambitious studies that are outlined in this U19 proposal. As is evident in the Timeline below, all Cores and Projects will be active from the moment the program is initiated until the last year of the program. In addition, this is a highly interactive and synergistic program, where all Service Cores and Projects will interact.

Table 1: Yale Asthma U19 Program timeline	Year 1	Year 2	Year 3	Year 4	Year 5
Core A: Administrative Core A					
Monthly U19 laboratory meetings	XXXX	XXXX	XXXX	XXXX	XXXX
U19 Executive Committee meetings	XXXX	XXXX	XXXX	XXXX	XXXX
Preparation of Progress reports	XXXX	XXXX	XXXX	XXXX	XXXX
Core B: Clinical Recruitment and Biostatistics					
1. Enrollment and f/u NextGen study visits	XXXX	XXXX	XXXX	XXXX	
2. Processing and distribution of biologic samples and phenotype data	XXXX	XXXX	XXXX	XXXX	XXXX
3. Biostatistical and data management support for projects	XXXX	XXXX	XXXX	XXXX	XXXX
Deliverable/milestone	Enroll 200 subjects in NextGen study Process samples for Projects and Core C Conduct follow-up visits to acquire follow-up samples Provide clinical phenotype data for Projects Biostatistical support				
Core C: Precision Profiling Core					
1. Baseline CyTOF and bulk and SC RNA-seq studies	XXXX	XXXX	XXXX		
2. Protein profiling assays	XXX	XXX	XXXX	XXXX	XXXX
3. Customized CyTOF and SC RNAseq Stimulation studies	XXXX	XXXX	XXXX	XXXX	XXXX
Deliverable/milestone		Baseline CyTOF studies Bulk RNA-seq, single cell RNA-seq Cytokine profiles Customized CyTOF panels			
Project 1. Mechanisms of YKL-40 and Transcriptomic Endotypes of Asthma					
1. CyTOF, RNA-seq studies of YKL-40 endotype	XXXX	XXXX	XXXX	XXXX	XXXX
2. CyTOF, RNA-seq studies of TEA clusters	XXXX	XXXX	XXXX	XXXX	XXXX
3. Functional studies of integrated clusters	XXXX	XXXX	XXXX	XXXX	XXXX
Deliverables/milestone	Determination of the strongest YKL-40 endotype biomarker Further characterization cell populations and pathways that underlie TEA clusters Characterization of functional response of next generation clusters by CyTOF and SC RNA-seq				
Project 2: Novel Immune Pathways Drive Asthma Heterogeneity					
1. Characterization of Tfh cells in the blood and tonsil and their relationship to asthma heterogeneity	XXXX	XXXX	XXXX	XXXX	XXXX
2. Evaluation of Dkk-1 in the blood and sputum	XXXX	XXXX	XXXX	XXXX	XXXX
3. Identification of immunogenic microbiota and associated endotypes of asthma	XXXX	XXXX	XXXX	XXXX	XXXX
Deliverables/milestones					
Project 3: Single Cell and Clustering					
1. Generation of analytical tools and pipeline for bulk and single cell RNA-seq	XXXX	XXXX	XXXX	XXXX	XXXX
2. Identification of Next Generation Clusters using bulk and single cell RNA-seq, CyTOF data	XXXX	XXXX	XXXX	XXXX	XXXX
3. Development of ASTHMA MAP website	XXXX	XXXX	XXXX	XXXX	XXXX
Deliverables/milestones	Generation of asthma specific tools and pipelines for projects and public use Characterization of single cell and CyTOF asthma Clusters Identification of integrated novel clusters Deployment of tools and ASTHMAMAP				
DISSEMINATION Documentation preparation, presentation at national meetings, manuscript submission. Development of Asthma MAP	XXXX	XXXX	XXXX	XXXX	XXXX

Protection of Human Subjects

Risks to Human Subjects

a. Human Subjects Involvement and Characteristics. Over the course of this project, we plan to recruit 200 asthmatic and 50 non-asthmatic subjects through the Yale Center for Asthma and Airway Diseases (YCAAD). Each subject will return approximately every 9 months and complete 3 visits over the 5-year grant period. At each of the 3 visits the subject will be administered a questionnaire and perform physiological testing, and blood and sputum samples will be obtained. Asthmatic and non-asthmatic subjects recruited for these studies will be 12 years old or greater. Through a distinct HIC protocol, for these studies we will also recruit asthmatic and non-asthmatic children (age 7-17 years old) undergoing adenotonsillectomy, to donate tonsillar tissue, blood samples, and nasal brushings to the YCAAD biorepository.

Drs. Cohn and Chupp have recruited subjects for the past 15 years through the *Mechanisms and Mediators of Asthma and Chronic Obstructive Pulmonary Disease (COPD) human investigation* protocol (Yale HIC 0102012268, PI Chupp, Co-I Cohn). Over 800 subjects have been recruited to date. Tonsillar tissue will be obtained as part of the *Correlation of Gene Expression Profile with Clinical Response to Adenotonsillectomy* protocol (Yale HIC 1009007345, PI Karas, Co-I Chupp). This is a repository is to investigate the relationship between adenotonsillar hypertrophy and asthma and airway diseases, and identify genes, molecules and pathways that are associated with disease development, progression, and severity.

YCAAD Study Protocol: Exclusions will be based on health considerations. We will exclude any patient with a contraindication to any of the required testing including sputum induction, spirometry, or phlebotomy. These exclusions include severe cardiovascular, renal, or liver disease, bleeding diathesis, or advanced neuromuscular disease. A centerpiece of the YCAAD translational research program and this proposal is the airways disease phenotyping protocol developed to study human asthma. This protocol has been developed to acquire the full complement of clinical information, physiologic measurements and biologic samples to perform genotype-phenotype studies. Following informed consent, subjects are administered a questionnaire by the study coordinator. This includes a detailed asthma and medical history, asthma activity questionnaire (Asthma Control Test), Asthma Quality of Life Questionnaire, and details about medications, histories of smoking, occupational and environmental exposures, history of allergy, sinus disease, gastroesophageal reflux, obesity, and medication compliance. All data is uploaded into a HIPAA compliant web-based database developed by YCAAD. Blood is drawn and processed for the isolation of DNA, RNA, serum and plasma. Samples are aliquoted and banked for future studies. Blood is sent to the clinical laboratory for CBC and differential, serum IgE level, ImmunoCap testing for 22 common aeroallergens and routine chemistries. Following phlebotomy, patients undergo FeNO testing and spirometry (in adherence with ATS guidelines) before and after short acting bronchodilator administration. Sputum induction is done using hypertonic saline and immediately processed. Mucus plugs are removed using a dissecting microscope to remove squamous cell contamination, the cellular and aqueous compartments are separated, cell counts and differentials are quantified, and aliquots of supernatants, cell protein, and cellular RNA are stored for later analysis. The protocol is practical, efficient, and enrolls large numbers of subjects in a timely fashion. We will phenotype up to 5 subjects weekly. Subjects will be scheduled to return for repeat sampling as described, which is important for determination of the stability of their disease and their biological samples.

Asthma Inclusion criteria. Recruitment through YCAAD is designed to enroll a wide-range of asthmatic subjects in terms of age, disease severity and ongoing treatment. Inclusion criteria for asthmatic subjects are: (1) ≥ 12 years of age; (2) < 10 pack years of tobacco, and have not smoked for ≥ 1 year. Asthmatic subjects must have a diagnosis of asthma defined by the following criteria (based on the National Asthma and Education and Prevention Panel Guidelines): (1) a history of chronic symptoms (>2 years) compatible with asthma (determined by a study investigator/pulmonary physician specializing in asthma); and (2) historical evidence of variable airflow obstruction determine by: (a) > 12% improvement in FEV_1 after an inhaled $beta_2$ agonist, inhaled corticosteroids, or an oral prednisone burst; (b) methacholine reactivity with a provocative concentration causing a 20% decrease in FEV_1(PC20) of <8 mg/ml; or (c) 20% diurnal variation of peak expiratory flow rates on 2 days over a 2-3 week period. Patients will be excluded if they cannot safely undergo the studies required for participation or have other chronic lung disease or asthma variant disease (ABPA, Churg-Strauss Syndrome, vocal cord dysfunction, etc.). Normal subjects must have no history of any chronic lung disease. Non-asthmatic control subjects will be excluded if they have a diagnosis of any ongoing acute or chronic lung disease, have smoked ≥10 pack-years or have abnormal spirometry.

Adenotonsillectomy Study Protocol. Parents and patients identified by providers as potential subjects will be

invited to participate in the research study after they have been consented independently for their planned procedure of adenotonsillectomy. The parent and child will complete a YCAAD pediatric questionnaire and an asthma activity score, and spirometry will be done for those children aged six years or older who are capable of completing the test. Intraoperatively, a venous blood sample (5-10ml) and nasal swab sample will be obtained. After a portion of the tissue is sent to surgical pathology, as is standard for adenotonsillectomy, a portion of the removed tonsils and/or adenoid tissue will be taken for study purposes. An additional specimen will be held in the pathology department, flash frozen in OCT in the event more tissue is needed for analysis. Samples will be processed and banked with the assigned de-identified study subject number. A second study visit will take place six months following surgery and includes an interim history using the follow-up questionnaire focused on stability of upper airway and asthma symptoms. Repeat PFT testing will be performed in children aged six years or older, if possible. An additional venous blood sample will be obtained for the repository. Subjects may decline any of the testing at any of these visits.

b. Sources of Materials. In this proposal, the following materials will be collected from human subjects: clinical data (questionnaire, history and physical examination data, lung function data and relevant laboratory test results), blood, sputum, nasal brushings and tonsils/adenoids. Clinical data will be identified with the study subject's name. This information will be maintained in a locked file and these records are kept separate from other clinical records to assure confidentiality. Only Drs. Cohn, Chupp, Niu, and study coordinators will have access to these confidential records. All research specimens, including blood, and sputum specimens are identified by a patient code for correlation purposes. All samples and datasets will be de-identified and only known to study investigators involved in consent and sample acquisition. Investigators and staff will receive only de-identified data and samples for their studies and analyses. All data sets uploaded and samples delivered to Core C and the Yale Center for Genomic Analysis will be de-identified.

The studies in this proposal will adhere to the U.S. Department of Health and Human Services Office for the Protection from Research Risk (OPRR). All information relating to participating subjects will be de-identified. Specimens may only be used in accordance with the conditions stipulated in the Yale Human Investigation Committee (HIC) (see approval letters in Appendix).

c. Potential Risks. Risks associated with the YCAAD study (HIC 0102012268) including pulmonary function tests, blood draw and sputum induction are minimal (adults) and greater than minimal (children 12-17 years old) and are explained to subjects in detail at the time of consent (see consent forms in Appendix). Bronchospasm is a risk of sputum induction, but the safety of sputum induction in asthmatic subjects, even those with severe asthma has been well documented in several studies (ref). Spirometry is repeatedly tested during the procedure to monitor for bronchospasm, and if wheezing or a significant drop in FEV1 occurs, subjects are given bronchodilators. Wheezing, if it occurs, is usually transient and treatable with bronchodilators. A Data and Safety Monitoring (DSM) Plan and DSM Committee are in place to assess adverse events (see Appendix). To date, we have had no serious adverse events in our laboratory, even in severe asthma subjects undergoing sputum induction. Clinical Recruitment and Biostatistics Core Lead (Dr. Cohn) or a research coordinator will monitor all procedures performed. After any procedure performed in YCAAD, subjects are instructed to contact the YCAAD on-call physician if any problem occurs. YCAAD is covered 24 x 7 by pulmonary physicians who are familiar with the management of acute bronchospasm in asthmatic patients. Risks associated with the adenotonsillectomy study (Yale HIC 1009007345) are minimal for the blood draw and there is no added risk of apportioning adenoid and tonsil tissue to the biorepository. Emergency equipment and personnel are immediately available in the event of an adverse reaction at all times during these study visits.

Adequacy of Protection Against Risks

a. Recruitment and Informed Consent. Subjects will be recruited from YCAAD and from the pediatric ENT offices of Dr. Karas. Once a subject is deemed eligible for this study, informed consent will be obtained. The study procedures, risks and benefits will be clearly explained to each subject. All questions will be answered, and time will be allowed for the subject to make an informed decision before signing the consent form. If the subject agrees to enroll, a consent form (see Appendix) will be reviewed and explained, and the subject or consenting adult will be asked to sign it.

b. Protections Against Risk. Risks will be minimized by appropriate subject exclusions, close medical supervision throughout the protocol, and adaptation of testing to identify patients who might be at higher risk. Subjects experiencing adverse events will have access to full inpatient medical facilities of the Yale-New

Haven Hospital if more intensive treatment is necessary to reverse an asthma attack or complication during the study. All patients will be under full-time coordinator supervision throughout every phase of the YCAAD procedures. In the adenotonsillectomy protocol, at least one physician and/or nurse will be present throughout the study visits and procedures. Adverse events will be reported to the Yale IRB (Human Investigation Committee) and the DSMC.

Potential Benefits of the Proposed Research to Human Subjects and Importance of the Knowledge to be Gained

These studies are not designed to provide direct benefit to the participants. However, the knowledge gained from this work will enhance information about the pathogenesis of asthma and may lead to improved diagnosis, treatment and prevention of asthma in the future. All subjects receive asthma education and copies of blood work and lung function tests as part of their visit, and have full access to a YCAAD physician, if desired. If any major test abnormalities are encountered, YCAAD physicians counsel the subjects and provide referrals to appropriate specialists as needed.

Inclusion of Women and Minorities

Subjects will be recruited through the Clinical Recruitment and Biostatistics Core (Core B) and through the office of Dr. Karas for adenotonsillectomy without any bias of race or gender. The YCAAD asthma and control populations and the children undergoing adenotonsillectomy are racially diverse and reflect the population in New Haven and surrounding communities. All minority groups already take part in these research programs and we will continue to actively recruit women and minorities as these studies go forward. Based on demographics from past studies, the asthmatic population in YCAAD is approximately 71% female and 31% Black and Hispanic. The demographics of the adenotonsillectomy population is approximately 37% female, 14% Black and 36% Hispanic.

Inclusion of Children

The proposal will recruit children between the ages of 2 and 21 years (consent and assent form are included in Appendix 1 and 2). The investigative team for Yale #HIC0102012268 will request that the participants (12-21 years old) undergo phlebotomy, sputum induction, lung function testing and complete an asthma questionnaire with the subject and his/her parent/legal guardian. Venipuncture and pulmonary function testing and questioning are part of the routine care of children with asthma. Sputum induction is a low-risk procedure and is outlined in the Appendix. All interviews and procedures will be performed in the Yale Center for Asthma and Airways Disease. Overall, the study poses more than a minimal risk to minor subjects, but its benefits outweigh these risks. The investigative team for Yale # HIC1009007345 will request that the participant (2-18 years old) who was previously scheduled to undergo adenotonsillectomy, consent to have blood drawn intraoperatively, perform spirometry before surgery, if capable, and agree to donate excess tonsil tissue to the biorepository for analyses. These procedures are assessed to be of minimal risk to child subjects. The investigative team has experience caring for children should any adverse events arise during sample acquisition.

Inclusion of children in this study is important since we are interested in the relationship of transcriptomic endophenotypes across the spectrum of asthma. Our studies thus far suggest that there are stable Transcriptional Endophenotypes of Asthma (TEA) in children and adults with severe asthma and, in particular, near fatal asthma. Endotypes seen in adult asthmatics may be evident in young subjects, and this may help us predict the course of disease as they age. Further, we may be able to discern which patients will benefit from more aggressive care and elucidate molecular pathways involved in the pathogenesis of severe asthma. We will follow subjects longitudinally and will define how transcriptomic signatures relate with progression of this disease.

References

Recent collaborative work by Program team of Investigators is indicated by *

1. Moorman JE, Zahran H, Truman BI, Molla MT, Centers for Disease C, Prevention. Current asthma prevalence - United States, 2006-2008. Morbidity and mortality weekly report Surveillance summaries. 2011;60 Suppl:84-6. Epub 2011/03/25. PubMed PMID: 21430629.
2. Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. Nat Med. 2012;18(5):716-25. doi: 10.1038/nm.2678. PubMed PMID: WOS:000303763500039.
3. Xie M, Wenzel SE. A global perspective in asthma: from phenotype to endotype. Chinese medical journal. 2013;126(1):166-74. Epub 2013/01/05. PubMed PMID: 23286496.
4. Fahy JV. Type 2 inflammation in asthma - present in most, absent in many (vol 15, pg 57, 2015). Nat Rev Immunol. 2015;15(2):130-. doi: 10.1038/nri3807. PubMed PMID: WOS:000348642400011.
*5. Yan X, Chu JH, Gomez J, Koenigs M, Holm C, He X, Perez MF, Zhao H, Mane S, Martinez FD, Ober C, Nicolae DL, Barnes KC, London SJ, Gilliland F, Weiss ST, Raby BA, Cohn L, Chupp GL. Noninvasive analysis of the sputum transcriptome discriminates clinical phenotypes of asthma. Am J Respir Crit Care Med. 2015;191(10):1116-25. Epub 2015/03/13. doi: 10.1164/rccm.201408-1440OC. PubMed PMID: 25763605; PubMed Central PMCID: PMC4451618.
6. Steiling K, Christenson SA. Targeting 'types: Precision Medicine in Pulmonary Disease. Am J Respir Crit Care Med. 2015;191(10):1093-4. Epub 2015/05/16. doi: 10.1164/rccm.201503-0613ED. PubMed PMID: 25978565.
7. Woodruff PG, Modrek B, Choy DF, Jia GQ, Abbas AR, Ellwanger A, Koth LL, Arron JR, Fahy JV. T-helper Type 2-driven Inflammation Defines Major Subphenotypes of Asthma (vol 180, pg 388, 2009). Am J Resp Crit Care. 2009;180(8):796-. PubMed PMID: WOS:000270743100018.
8. Ortega H, Chupp G, Bardin P, Bourdin A, Garcia G, Hartley B, Yancey S, Humbert M. The role of mepolizumab in atopic and nonatopic severe asthma with persistent eosinophilia. The European respiratory journal. 2014;44(1):239-41. Epub 2014/03/25. doi: 10.1183/09031936.00220413. PubMed PMID: 24659543.
9. Collins FS, Varmus H. A new initiative on precision medicine. The New England journal of medicine. 2015;372(9):793-5. Epub 2015/01/31. doi: 10.1056/NEJMp1500523. PubMed PMID: 25635347.
10. Fitzpatrick AM, Teague WG, Meyers DA, Peters SP, Li X, Li H, Wenzel SE, Aujla S, Castro M, Bacharier LB, Gaston BM, Bleecker ER, Moore WC, National Institutes of Health/National Heart L, Blood Institute Severe Asthma Research P. Heterogeneity of severe asthma in childhood: confirmation by cluster analysis of children in the National Institutes of Health/National Heart, Lung, and Blood Institute Severe Asthma Research Program. The Journal of allergy and clinical immunology. 2011;127(2):382-9 e1-13. Epub 2011/01/05. doi: 10.1016/j.jaci.2010.11.015. PubMed PMID: 21195471; PubMed Central PMCID: PMC3060668.
11. Moore WC, Meyers DA, Wenzel SE, Teague WG, Li H, Li X, D'Agostino R, Jr., Castro M, Curran-Everett D, Fitzpatrick AM, Gaston B, Jarjour NN, Sorkness R, Calhoun WJ, Chung KF, Comhair SA, Dweik RA, Israel E, Peters SP, Busse WW, Erzurum SC, Bleecker ER, National Heart L, Blood Institute's Severe Asthma Research P. Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. Am J Respir Crit Care Med. 2010;181(4):315-23. Epub 2009/11/07. doi: 10.1164/rccm.200906-0896OC. PubMed PMID: 19892860; PubMed Central PMCID: PMC2822971.
12. Masoli M, Fabian D, Holt S, Beasley R, Global Initiative for Asthma P. The global burden of asthma: executive summary of the GINA Dissemination Committee report. Allergy. 2004;59(5):469-78. Epub 2004/04/15. doi: 10.1111/j.1398-9995.2004.00526.x. PubMed PMID: 15080825.
13. Lugogo NL, Kraft M. Epidemiology of asthma. Clinics in chest medicine. 2006;27(1):1-15, v. Epub 2006/03/18. doi: 10.1016/j.ccm.2005.10.006. PubMed PMID: 16543048.
14. Bousquet J, Bousquet PJ, Godard P, Daures JP. The public health implications of asthma. Bulletin of the World Health Organization. 2005;83(7):548-54. Epub 2005/09/24. PubMed PMID: 16175830; PubMed Central PMCID: PMC2626301.
15. Nathan RA, Meltzer EO, Blaiss MS, Murphy KR, Doherty DE, Stoloff SW. Comparison of the Asthma in America and Asthma Insight and Management surveys: did asthma burden and care improve in the United States between 1998 and 2009? Allergy and asthma proceedings : the official journal of regional and state allergy societies. 2012;33(1):65-76. Epub 2012/02/09. doi: 10.2500/aap.2011.32.3521. PubMed PMID: 22309828.
16. Neurath MF, Finotto S, Glimcher LH. The role of Th1/Th2 polarization in mucosal immunity. Nat Med. 2002;8(6):567-73. Epub 2002/06/04. doi: 10.1038/nm0602-567. PubMed PMID: 12042806.

17. Mosmann TR, Coffman RL. Heterogeneity of cytokine secretion patterns and functions of helper T cells. Advances in immunology. 1989;46:111-47. Epub 1989/01/01. PubMed PMID: 2528896.
18. Mosmann TR, Coffman RL. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu Rev Immunol. 1989;7:145-73. Epub 1989/01/01. doi: 10.1146/annurev.iy.07.040189.001045. PubMed PMID: 2523712.
19. Colavita AM, Reinach AJ, Peters SP. Contributing factors to the pathobiology of asthma. The Th1/Th2 paradigm. Clinics in chest medicine. 2000;21(2):263-77, viii. Epub 2000/07/25. PubMed PMID: 10907587.
20. Linterman MA, Beaton L, Yu D, Ramiscal RR, Srivastava M, Hogan JJ, Verma NK, Smyth MJ, Rigby RJ, Vinuesa CG. IL-21 acts directly on B cells to regulate Bcl-6 expression and germinal center responses. J Exp Med. 2010;207(2):353-63. doi: 10.1084/jem.20091738. PubMed PMID: 20142429; PubMed Central PMCID: PMC2822609.
21. Zotos D, Coquet JM, Zhang Y, Light A, D'Costa K, Kallies A, Corcoran LM, Godfrey DI, Toellner KM, Smyth MJ, Nutt SL, Tarlinton DM. IL-21 regulates germinal center B cell differentiation and proliferation through a B cell-intrinsic mechanism. J Exp Med. 2010;207(2):365-78. doi: 10.1084/jem.20091777. PubMed PMID: 20142430; PubMed Central PMCID: PMC2822601.
22. King IL, Mohrs M. IL-4-producing CD4+ T cells in reactive lymph nodes during helminth infection are T follicular helper cells. J Exp Med. 2009;206(5):1001-7. doi: 10.1084/jem.20090313. PubMed PMID: 19380638; PubMed Central PMCID: PMC2715031.
23. Liang HE, Reinhardt RL, Bando JK, Sullivan BM, Ho IC, Locksley RM. Divergent expression patterns of IL-4 and IL-13 define unique functions in allergic immunity. Nat Immunol. 2012;13(1):58-66. doi: 10.1038/ni.2182. PubMed PMID: 22138715; PubMed Central PMCID: PMC3242938.
24. Avery DT, Ma CS, Bryant VL, Santner-Nanan B, Nanan R, Wong M, Fulcher DA, Cook MC, Tangye SG. STAT3 is required for IL-21-induced secretion of IgE from human naive B cells. Blood. 2008;112(5):1784-93. doi: 10.1182/blood-2008-02-142745. PubMed PMID: 18579794.
25. Kobayashi S, Haruo N, Sugane K, Ochs HD, Agematsu K. Interleukin-21 stimulates B-cell immunoglobulin E synthesis in human beings concomitantly with activation-induced cytidine deaminase expression and differentiation into plasma cells. Hum Immunol. 2009;70(1):35-40. doi: 10.1016/j.humimm.2008.10.021. PubMed PMID: 19026702.
26. Hiromura Y, Kishida T, Nakano H, Hama T, Imanishi J, Hisa Y, Mazda O. IL-21 administration into the nostril alleviates murine allergic rhinitis. J Immunol. 2007;179(10):7157-65. PubMed PMID: 17982108.
27. Ozaki K, Spolski R, Feng CG, Qi CF, Cheng J, Sher A, Morse HC, 3rd, Liu C, Schwartzberg PL, Leonard WJ. A critical role for IL-21 in regulating immunoglobulin production. Science. 2002;298(5598):1630-4. doi: 10.1126/science.1077002. PubMed PMID: 12446913.
28. Suto A, Nakajima H, Hirose K, Suzuki K, Kagami S, Seto Y, Hoshimoto A, Saito Y, Foster DC, Iwamoto I. Interleukin 21 prevents antigen-induced IgE production by inhibiting germ line C(epsilon) transcription of IL-4-stimulated B cells. Blood. 2002;100(13):4565-73. doi: 10.1182/blood-2002-04-1115. PubMed PMID: 12393685.
29. Talay O, Yan D, Brightbill HD, Straney EE, Zhou M, Ladi E, Lee WP, Egen JG, Austin CD, Xu M, Wu LC. IgE(+) memory B cells and plasma cells generated through a germinal-center pathway. Nat Immunol. 2012;13(4):396-404. doi: 10.1038/ni.2256. PubMed PMID: 22366892.
30. Wu LC, Zarrin AA. The production and regulation of IgE by the immune system. Nat Rev Immunol. 2014;14(4):247-59. doi: 10.1038/nri3632. PubMed PMID: 24625841.
31. Abbas AK, Janeway CA. Immunology: Improving on nature in the twenty-first century. Cell. 2000;100(1):129-38. doi: Doi 10.1016/S0092-8674(00)81689-X. PubMed PMID: WOS:000084722600011.
32. Glinka A, Wu W, Delius H, Monaghan AP, Blumenstock C, Niehrs C. Dickkopf-1 is a member of a new family of secreted proteins and functions in head induction. Nature. 1998;391(6665):357-62. Epub 1998/02/05. doi: 10.1038/34848. PubMed PMID: 9450748.
33. Cruciat CM, Dolde C, de Groot RE, Ohkawara B, Reinhard C, Korswagen HC, Niehrs C. RNA helicase DDX3 is a regulatory subunit of casein kinase 1 in Wnt-beta-catenin signaling. Science. 2013;339(6126):1436-41. Epub 2013/02/16. doi: 10.1126/science.1231499. PubMed PMID: 23413191.
34. Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. The human microbiome project. Nature. 2007;449(7164):804-10. Epub 2007/10/19. doi: 10.1038/nature06244. PubMed PMID: 17943116; PubMed Central PMCID: PMC3709439.
35. Erb-Downward JR, Thompson DL, Han MK, Freeman CM, McCloskey L, Schmidt LA, Young VB, Toews GB, Curtis JL, Sundaram B, Martinez FJ, Huffnagle GB. Analysis of the lung microbiome in the "healthy" smoker and in COPD. PLoS One. 2011;6(2):e16384. doi: 10.1371/journal.pone.0016384. PubMed PMID: 21364979; PubMed Central PMCID: PMC3043049.

36. Hilty M, Burke C, Pedro H, Cardenas P, Bush A, Bossley C, Davies J, Ervine A, Poulter L, Pachter L, Moffatt MF, Cookson WO. Disordered microbial communities in asthmatic airways. PLoS One. 2010;5(1):e8578. doi: 10.1371/journal.pone.0008578. PubMed PMID: 20052417; PubMed Central PMCID: PMC2798952.
37. Huang YJ, Nariya S, Harris JM, Lynch SV, Choy DF, Arron JR, Boushey H. The airway microbiome in patients with severe asthma: Associations with disease features and severity. J Allergy Clin Immunol. 2015. doi: 10.1016/j.jaci.2015.05.044. PubMed PMID: 26220531.
38. Morris A, Beck JM, Schloss PD, Campbell TB, Crothers K, Curtis JL, Flores SC, Fontenot AP, Ghedin E, Huang L, Jablonski K, Kleerup E, Lynch SV, Sodergren E, Twigg H, Young VB, Bassis CM, Venkataraman A, Schmidt TM, Weinstock GM, Lung HIVMP. Comparison of the respiratory microbiome in healthy nonsmokers and smokers. Am J Respir Crit Care Med. 2013;187(10):1067-75. doi: 10.1164/rccm.201210-1913OC. PubMed PMID: 23491408; PubMed Central PMCID: PMC3734620.
39. Zemanick ET, Sagel SD, Harris JK. The airway microbiome in cystic fibrosis and implications for treatment. Curr Opin Pediatr. 2011;23(3):319-24. doi: 10.1097/MOP.0b013e32834604f2. PubMed PMID: 21494150.
40. Dickson RP, Huffnagle GB. The Lung Microbiome: New Principles for Respiratory Bacteriology in Health and Disease. PLoS pathogens. 2015;11(7):e1004923. Epub 2015/07/15. doi: 10.1371/journal.ppat.1004923. PubMed PMID: 26158874; PubMed Central PMCID: PMC4497592.
41. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, Owens SM, Betley J, Fraser L, Bauer M, Gormley N, Gilbert JA, Smith G, Knight R. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J. 2012;6(8):1621-4. Epub 2012/03/10. doi: 10.1038/ismej.2012.8. PubMed PMID: 22402401; PubMed Central PMCID: PMC3400413.
42. Goodrich JK, Di Rienzi SC, Poole AC, Koren O, Walters WA, Caporaso JG, Knight R, Ley RE. Conducting a microbiome study. Cell. 2014;158(2):250-62. doi: 10.1016/j.cell.2014.06.037. PubMed PMID: 25036628.
43. Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. Diversity, stability and resilience of the human gut microbiota. Nature. 2012;489(7415):220-30. Epub 2012/09/14. doi: 10.1038/nature11550. PubMed PMID: 22972295; PubMed Central PMCID: PMC3577372.
44. Blumberg R, Powrie F. Microbiota, disease, and back to health: a metastable journey. Sci Transl Med. 2012;4(137):137rv7. Epub 2012/06/08. doi: 10.1126/scitranslmed.3004184. PubMed PMID: 22674557.
45. Goleva E, Jackson LP, Harris JK, Martin RJ, Leung DYM. Microbiome of the Lower Airways Alters Corticosteroid Responsiveness in Asthma. J Allergy Clin Immun. 2013;131(2):Ab138-Ab. PubMed PMID: WOS:000316550800491.
46. Proceedings of the ATS workshop on refractory asthma: current understanding, recommendations, and unanswered questions. American Thoracic Society. Am J Respir Crit Care Med. 2000;162(6):2341-51. Epub 2000/12/09. doi: 10.1164/ajrccm.162.6.ats9-00. PubMed PMID: 11112161.
47. Buss G. [Biologics in asthma: what's new?]. Revue medicale suisse. 2015;11(456-457):20, 2-4. Epub 2015/03/25. PubMed PMID: 25799646.
48. Darveaux J, Busse WW. Biologics in asthma--the next step toward personalized treatment. The journal of allergy and clinical immunology In practice. 2015;3(2):152-60; quiz 61. Epub 2015/03/11. doi: 10.1016/j.jaip.2014.09.014. PubMed PMID: 25754716.
49. Palm NW, de Zoete MR, Cullen TW, Barry NA, Stefanowski J, Hao L, Degnan PH, Hu J, Peter I, Zhang W, Ruggiero E, Cho JH, Goodman AL, Flavell RA. Immunoglobulin A coating identifies colitogenic bacteria in inflammatory bowel disease. Cell. 2014;158(5):1000-10. Epub 2014/08/30. doi: 10.1016/j.cell.2014.08.006. PubMed PMID: 25171403; PubMed Central PMCID: PMC4174347.
50. Goleva E, Jackson LP, Harris JK, Robertson CE, Sutherland ER, Hall CF, Good JT, Gelfand EW, Martin RJ, Leung DYM. The Effects of Airway Microbiome on Corticosteroid Responsiveness in Asthma. Am J Resp Crit Care. 2013;188(10):1193-201. doi: 10.1164/rccm.201304-0775OC. PubMed PMID: WOS:000326963600011.
51. Spitzer MH, Gherardini PF, Fragiadakis GK, Bhattacharya N, Yuan RT, Hotson AN, Finck R, Carmi Y, Zunder ER, Fantl WJ, Bendall SC, Engleman EG, Nolan GP. IMMUNOLOGY. An interactive reference framework for modeling a dynamic immune system. Science. 2015;349(6244):1259425. Epub 2015/07/15. doi: 10.1126/science.1259425. PubMed PMID: 26160952; PubMed Central PMCID: PMC4537647.
*52. Chupp GL, Lee CG, Jarjour N, Shim YM, Holm CT, He S, Dziura JD, Reed J, Coyle AJ, Kiener P, Cullen M, Grandsaigne M, Dombret M, Aubier M, Pretolani M, Elias JA. A chitinase-like protein in the lung and

circulation of patients with severe asthma. New Engl J Med. 2007;357(20):2016-27. doi: Doi 10.1056/Nejmoa073600. PubMed PMID: WOS:000250894500004.
*53. Yao Y, Liu R, Shin MS, Trentalange M, Allore H, Nassar A, Kang I, Pober JS, Montgomery RR. CyTOF supports efficient detection of immune cell subsets from small samples. Journal of immunological methods. 2014;415:1-5. Epub 2014/12/03. doi: 10.1016/j.jim.2014.10.010. PubMed PMID: 25450003; PubMed Central PMCID: PMC4269324.
*54. Strauss-Albee DM, Fukuyama J, Liang EC, Yao Y, Jarrell JA, Drake AL, Kinuthia J, Montgomery RR, John-Stewart G, Holmes S, Blish CA. Human NK cell repertoire diversity reflects immune experience and correlates with viral susceptibility. Science translational medicine. 2015;7(297):297ra115. Epub 2015/07/24. doi: 10.1126/scitranslmed.aac5722. PubMed PMID: 26203083; PubMed Central PMCID: PMC4547537.
55. Krishnaswamy S, Spitzer MH, Mingueneau M, Bendall SC, Litvin O, Stone E, Pe'er D, Nolan GP. Systems biology. Conditional density-based analysis of T cell signaling in single-cell data. Science. 2014;346(6213):1250689. Epub 2014/10/25. doi: 10.1126/science.1250689. PubMed PMID: 25342659; PubMed Central PMCID: PMC4334155.
*56. Cohn L, Elias JA, Chupp GL. Asthma: mechanisms of disease persistence and progression. Annu Rev Immunol. 2004;22:789-815. Epub 2004/03/23. doi: 10.1146/annurev.immunol.22.012703.104716. PubMed PMID: 15032597.
*57. Gomez JL, Crisafi GM, Holm CT, Meyers DA, Hawkins GA, Bleecker ER, Jarjour N, Severe Asthma Research Program I, Cohn L, Chupp GL. Genetic variation in chitinase 3-like 1 (CHI3L1) contributes to asthma severity and airway expression of YKL-40. The Journal of allergy and clinical immunology. 2015;136(1):51-8 e10. Epub 2015/01/17. doi: 10.1016/j.jaci.2014.11.027. PubMed PMID: 25592985; PubMed Central PMCID: PMC4494869.
*58. Lee CG, Hartl D, Lee GR, Koller B, Matsuura H, Da Silva CA, Sohn MH, Cohn L, Homer RJ, Kozhich AA, Humbles A, Kearley J, Coyle A, Chupp G, Reed J, Flavell RA, Elias JA. Role of breast regression protein 39 (BRP-39)/chitinase 3-like-1 in Th2 and IL-13-induced tissue responses and apoptosis. The Journal of experimental medicine. 2009;206(5):1149-66. Epub 2009/05/06. doi: 10.1084/jem.20081271. PubMed PMID: 19414556; PubMed Central PMCID: PMC2715037.
*59. Wright PL, Yu J, Di YP, Homer RJ, Chupp G, Elias JA, Cohn L, Sessa WC. Epithelial reticulon 4B (Nogo-B) is an endogenous regulator of Th2-driven lung inflammation. The Journal of experimental medicine. 2010;207(12):2595-607. Epub 2010/10/27. doi: 10.1084/jem.20100786. PubMed PMID: 20975041; PubMed Central PMCID: PMC2989775.
*60. Bai F, Town T, Qian F, Wang P, Kamanaka M, Connolly TM, Gate D, Montgomery RR, Flavell RA, Fikrig E. IL-10 signaling blockade controls murine West Nile virus infection. PLoS pathogens. 2009;5(10):e1000610. Epub 2009/10/10. doi: 10.1371/journal.ppat.1000610. PubMed PMID: 19816558; PubMed Central PMCID: PMC2749443.
*61. Zhang X, Shan P, Sasidhar M, Chupp GL, Flavell RA, Choi AM, Lee PJ. Reactive oxygen species and extracellular signal-regulated kinase 1/2 mitogen-activated protein kinase mediate hyperoxia-induced cell death in lung epithelium. American journal of respiratory cell and molecular biology. 2003;28(3):305-15. Epub 2003/02/21. doi: 10.1165/rcmb.2002-0156OC. PubMed PMID: 12594056.
*62. Lam TT, Nguyen TP, Montgomery RR, Kantor FS, Fikrig E, Flavell RA. Outer surface proteins E and F of Borrelia burgdorferi, the agent of Lyme disease. Infection and immunity. 1994;62(1):290-8. Epub 1994/01/01. PubMed PMID: 8262642; PubMed Central PMCID: PMC186099.
*63. Pedra JH, Sutterwala FS, Sukumaran B, Ogura Y, Qian F, Montgomery RR, Flavell RA, Fikrig E. ASC/PYCARD and caspase-1 regulate the IL-18/IFN-gamma axis during Anaplasma phagocytophilum infection. Journal of immunology. 2007;179(7):4783-91. Epub 2007/09/20. PubMed PMID: 17878377.
*64. Town T, Bai F, Wang T, Kaplan AT, Qian F, Montgomery RR, Anderson JF, Flavell RA, Fikrig E. Toll-like receptor 7 mitigates lethal West Nile encephalitis via interleukin 23-dependent immune cell infiltration and homing. Immunity. 2009;30(2):242-53. Epub 2009/02/10. doi: 10.1016/j.immuni.2008.11.012. PubMed PMID: 19200759; PubMed Central PMCID: PMC2707901.
*65. Wang P, Bai F, Zenewicz LA, Dai J, Gate D, Cheng G, Yang L, Qian F, Yuan X, Montgomery RR, Flavell RA, Town T, Fikrig E. IL-22 signaling contributes to West Nile encephalitis pathogenesis. PloS one. 2012;7(8):e44153. Epub 2012/09/07. doi: 10.1371/journal.pone.0044153. PubMed PMID: 22952908; PubMed Central PMCID: PMC3429482.
*66. Lee N, You S, Shin MS, Lee WW, Kang KS, Kim SH, Kim WU, Homer RJ, Kang MJ, Montgomery RR, Dela Cruz CS, Shaw AC, Lee PJ, Chupp GL, Hwang D, Kang I. IL-6 receptor alpha defines effector memory CD8+ T cells producing Th2 cytokines and expanding in asthma. Am J Respir Crit Care Med.

2014;190(12):1383-94. Epub 2014/11/13. doi: 10.1164/rccm.201403-0601OC. PubMed PMID: 25390970; PubMed Central PMCID: PMC4299645.
*67. Qian F, Chung L, Zheng W, Bruno V, Alexander RP, Wang Z, Wang X, Kurscheid S, Zhao H, Fikrig E, Gerstein M, Snyder M, Montgomery RR. Identification of genes critical for resistance to infection by West Nile virus using RNA-Seq analysis. Viruses. 2013;5(7):1664-81. Epub 2013/07/25. doi: 10.3390/v5071664. PubMed PMID: 23881275; PubMed Central PMCID: PMC3738954.
*68. Whittaker L, Niu NQ, Temann UA, Stoddard A, Flavell RA, Ray A, Homer RJ, Cohn L. Interleukin-13 mediates a fundamental pathway for airway epithelial mucus induced by CD4 T cells and interleukin-9. American journal of respiratory cell and molecular biology. 2002;27(5):593-602. doi: 10.1165/rcmb.4838. PubMed PMID: WOS:000179189000010.

Yale SCHOOL OF MEDICINE

CAROLYN W. SLAYMAN, PHD
Deputy Dean for Academic and Scientific Affairs

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September 1, 2015

Gang Dong, MD, Ph.D.
National Institute of Allergy and Infectious Diseases (NIAID)
T: 240-627-3508
gdong@mail.nih.gov

Re: RFA-AI-15-032; Asthma and Allergic Diseases Cooperative Research Centers (U19)

Dear Dr. Dong:

On behalf of Yale University School of Medicine, I write to express strongest possible support for the attached proposal. The principal investigator is Dr. Geoffrey Chupp, Professor of Medicine and Director of the Yale Center for Asthma and Airway Disease (YCAAD) in the Section of Pulmonary, Critical Care, and Sleep Medicine, Department of Internal Medicine. As described in the proposal, Dr. Chupp and his outstanding team of scientists seek to identify novel innate and adaptive immune endotypes of severe asthma.

This project takes perfect advantage of the translational nature of YCAAD, its accomplishments to date, and its mission to be a destination program where patients with uncontrollable asthma can receive state-of-the-art care and participate in cutting-edge research. The assembled team of clinicians and investigators in immunobiology, computational biology, translational medicine, and pulmonary genomics has the experience and the ability to carry out the proposed studies.

As described, 200 subjects with asthma will be enrolled and provide sputum and blood samples for *in vitro* studies including RNAseq and CyTOF at the single cell level. Data will be analyzed using novel network and cluster approaches, and immune cell functions will be correlated with well-characterized clinical definitions of disease severity. Ultimately, the results can be expected to yield significant insights into the pathogenesis of severe disease.

This project will leverage substantial core resources at the School of Medicine, including the Yale Center for Genome Analysis, Yale Center for Single Cell Analysis, and Yale Center for Precision Pulmonary Medicine. Together with the Yale Center for Clinical Investigation, which serves as the home for our CTSA award and offers a broad range of services to support clinical and translational research, these centers provide a very strong infrastructure for the studies proposed by Dr. Chupp and his colleagues.

Dean Alpern and I commit our full support to the success of this AADCRC, including the administration of the opportunity fund (IOF) if the grant is awarded.

Please feel free to contact me if you or the members of the study section need any further information.

Sincerely yours,

Carolyn Slayman

Carolyn W. Slayman, PhD
Deputy Dean

OMB Number: 4040-0001
Expiration Date: 06/30/2016

APPLICATION FOR FEDERAL ASSISTANCE

SF 424 (R&R)

5. APPLICANT INFORMATION **Organizational DUNS*:** 0432075620000

Legal Name*: YALE UNIVERSITY
Department:
Division:
Street1*: OFFICE OF SPONSORED PROJECTS
Street2: 25 Science Park
City*: NEW HAVEN
County:
State*: CT: Connecticut
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 065208237

Person to be contacted on matters involving this application

Prefix:	First Name*:	Middle Name:	Last Name*:	Suffix:
	Marybeth		Brandi	

Position/Title: Proposal Manager
Street1*: 25 Science Park
Street2: 150 Munson Street
City*: New Haven
County:
State*: CT: Connecticut
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 06520-8237
Phone Number*: 203-737-3495 Fax Number: Email: marybeth.brandi@yale.edu

7. TYPE OF APPLICANT*

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Administrative Core

12. PROPOSED PROJECT

Start Date*	Ending Date*
07/01/2016	06/30/2021

OMB Number: 4040-0010
Expiration Date: 06/30/2016

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	YALE UNIVERSITY
Duns Number:	0432075620000
Street1*:	300 Cedar St
Street2:	TAC S440
City*:	NEW HAVEN
County:	
State*:	CT: Connecticut
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	065208237
Project/Performance Site Congressional District*:	CT-003

Project/Performance Site Location 1

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	YALE UNIVERSITY
DUNS Number:	0432075620000
Street1*:	300 Cedar St
Street2:	TAC441B
City*:	NEW HAVEN
County:	
State*:	CT: Connecticut
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	065208237
Project/Performance Site Congressional District*:	CT-003

File Name

Additional Location(s)

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ❍ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ❍ Yes ❍ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6

If NO, is the IRB review Pending? ❍ **Yes** ❍ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ❍ **Yes** ● **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ❍ Yes ❍ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. Is proprietary/privileged information included in the application?* ❍ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ❍ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ❍ Yes ❍ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ❍ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ❍ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Core_A_Project_Summary_Asthma_U19.pdf
8. Project Narrative*	
9. Bibliography & References Cited	Core_A_and_D_References_Cited.pdf
10.Facilities & Other Resources	
11.Equipment	

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

Project Summary

An Administrative Core will coordinate and facilitate all activities within the Yale U19 Asthma and Allergic Disease Cooperative Research Center (AADCRC) Group. This Core will be responsible for the overall organization, management, decision-making, and utilization of institutional resources. The Administrative Core will support the full complement of administrative tasks related to the Yale Asthma U19 Program entitled: Next Generation Endotyping of Asthma Heterogeneity. The Principle Investigator, Dr. Geoffrey Chupp will oversee the Administrative Core, and Ms. Susan Ardito will be Administrator of the Core. Ms. Ardito has 15 years of experience managing NIH and privately funded grants for the Division of Pulmonary and Critical Care Medicine (PCCSM), and Dr. Chupp has 20 years of experience on the faculty at the Yale School of Medicine which includes leadership positions as the Director of the: 1) PCCSM Fellowship, 2) Yale Center for Asthma and Airway Disease (YCAAD), 3) Yale New Haven Hospital (YNHH) Bronchoscopy, and 4) Pulmonary Function Laboratory. The Administrative Core will: 1) Provide oversight and consultation to each of the Research Projects and Scientific Cores to ensure that scientific objectives are met and that there is optimal interaction and utilization of resources, 2) Monitor and assist each group so that their goals are achieved and emergent problems are expeditiously addressed, 3) Support administrative tasks related to subject enrollment and scheduling, subject payment, and the preparation and timely submission of regulatory documents, 4) Provide fiscal management and ensure cost-effective utilization of U19 resources, 5) Promote the communication of the research team and dissemination of AADCRC research and technology, and organize the presentation and publication of data. This aim also will ensure data sharing, protection of intellectual property, and long-term data storage.

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: GEOFFREY Middle Name L Last Name*: CHUPP Suffix:

Position/Title*: Associate Professor
Organization Name*: YALE UNIVERSITY
Department:
Division:
Street1*: 300 Cedar Street, TAC S441B
Street2: PO Box 208057
City*: New Haven
County:
State*: CT: Connecticut
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 065208057

Phone Number*: 203-785-3627 Fax Number: 203-785-3826 E-Mail*: geoffrey.chupp@yale.edu

Credential, e.g., agency login: GCHUPP

Project Role*: Other (Specify) Other Project Role Category: Project Lead

Degree Type: Degree Year:

	File Name
Attach Biographical Sketch*:	Chupp_Bio_Asthma_U19.pdf
Attach Current & Pending Support:	

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2016 **End Date*:** 06-30-2017 **Budget Period: 1**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	GEOFFREY	L	CHUPP		Project Lead		0.6			9,165.00	2,841.00	12,006.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **12,006.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
1	Secretarial/Clerical	1.8			11,171.00	6,468.00	17,639.00
1	**Total Number Other Personnel**					**Total Other Personnel**	**17,639.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**29,645.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2016 **End Date*:** 06-30-2017 **Budget Period: 1**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	7,000.00
2. Foreign Travel Costs	
Total Travel Cost	**7,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2016 **End Date*:** 06-30-2017 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	3,500.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**3,500.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**40,145.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Modified total direct cost	66.5	40,145.00	26,696.00
		Total Indirect Costs	**26,696.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**66,841.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name:
Core_A_Budget_Justification_Asthma_U19.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2017 **End Date*:** 06-30-2018 **Budget Period: 2**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	GEOFFREY	L	CHUPP		Project Lead		0.6			9,165.00	2,841.00	12,006.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **12,006.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
1	Secretarial/Clerical	1.8			11,506.00	6,662.00	18,168.00
1	**Total Number Other Personnel**					**Total Other Personnel**	**18,168.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**30,174.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2017 **End Date*:** 06-30-2018 **Budget Period: 2**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	7,000.00
2. Foreign Travel Costs	
Total Travel Cost	**7,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2017 **End Date*:** 06-30-2018 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	3,500.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**3,500.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**40,674.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Modified total direct cost	66.5	40,674.00	27,048.00
		Total Indirect Costs	**27,048.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**67,722.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: Core_A_Budget_Justification_Asthma_U19.pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2018 **End Date*:** 06-30-2019 **Budget Period: 3**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	GEOFFREY	L	CHUPP		Project Lead		0.6			9,165.00	2,841.00	12,006.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **12,006.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
1	Secretarial/Clerical	1.8			11,851.00	6,862.00	18,713.00
1	**Total Number Other Personnel**					**Total Other Personnel**	**18,713.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**30,719.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2018 **End Date*:** 06-30-2019 **Budget Period: 3**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	7,000.00
2. Foreign Travel Costs	
Total Travel Cost	**7,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2018 **End Date*:** 06-30-2019 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	3,500.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**3,500.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**41,219.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Modified total direct cost	66.5	41,219.00	27,410.00
		Total Indirect Costs	**27,410.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**68,629.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name:
Core_A_Budget_Justification_Asthma_U19.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2019 **End Date*:** 06-30-2020 **Budget Period: 4**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	GEOFFREY	L	CHUPP		Project Lead		0.6			9,165.00	2,841.00	12,006.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **12,006.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
1	Secretarial/Clerical	1.8			12,206.00	7,068.00	19,274.00
1	**Total Number Other Personnel**					**Total Other Personnel**	**19,274.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**31,280.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2019 **End Date*:** 06-30-2020 **Budget Period: 4**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	7,000.00
2. Foreign Travel Costs	
Total Travel Cost	**7,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2019 **End Date*:** 06-30-2020 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	3,500.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**3,500.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**41,780.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Modified total direct cost	66.5	41,780.00	27,784.00
		Total Indirect Costs	**27,784.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**69,564.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: Core_A_Budget_Justification_Asthma_U19.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2020 **End Date*:** 06-30-2021 **Budget Period: 5**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	GEOFFREY	L	CHUPP		Project Lead		0.6			9,165.00	2,841.00	12,006.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **12,006.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
1	Secretarial/Clerical	1.8			12,573.00	7,280.00	19,853.00
1	**Total Number Other Personnel**					**Total Other Personnel**	**19,853.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**31,859.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2020 **End Date*:** 06-30-2021 **Budget Period: 5**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	7,000.00
2. Foreign Travel Costs	
Total Travel Cost	**7,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2020 **End Date*:** 06-30-2021 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	3,500.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**3,500.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**42,359.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Modified total direct cost	66.5	42,359.00	28,168.00
		Total Indirect Costs	**28,168.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**70,527.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: Core_A_Budget_Justification_Asthma_U19.pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

CORE A Administration Core

BUDGET JUSTIFICATION

Key Personnel

Geoffrey Chupp, M.D., (Principal Investigator and Core A Lead). Dr. Chupp is Professor of Medicine, Pulmonary and Critical Care Medicine, Yale University School of Medicine. He is the founder and Director of the Yale Center for Asthma and Airways Disease (YCAAD) and Cardiopulmonary Function Laboratory at Yale New Haven Hospital. He is an experienced clinical investigator who has developed novel approaches to genomic profiling of the blood and sputum in airway diseases including asthma and emphysema. He has developed an interdisciplinary, collaborative team (including Dr. Rajeevan and Dr. Yan) of researchers to develop novel approaches to evaluate genomic data acquired from individuals with asthma. His responsibility as PI is to oversee all aspects of these studies and to ensure the overall success of this project, from coordinating analyses with the research team to publishing the results. Dr. Chupp will chair U19 monthly progress meetings and meetings of the Executive Committee to oversee general progress of the project as a whole and to ensure optimal efficiency and collaboration among the various components. He will meet weekly with the Administrative Core to review recent progress, identify obstacles, and revise strategies to optimize project success. Dr. Chupp will prepare and submit progress reports to NIAID and liaise with program officials from the NIAID regarding all aspects of the U19 project operations. He will dedicate 0.6 calendar months annually to the Administration Core of this grant.

Non-Key Personnel

Susan Ardito, (Senior Administrative Assistant). Ms. Ardito is a Senior Administrative Assistant in the Section of Pulmonary, Critical Care and Sleep Medicine and presently supports Dr. Chupp's research and clinical activities. She will be responsible for coordinating committee meetings, teleconferences, and the preparation of scientific review reports, and manuscript preparation and submission specific to the U19. She is experienced in coordinating the activities necessary for a collaborative, multi-investigator award. Ms. Ardito will meet weekly with the Core Director, Dr. Chupp, to ensure efficient operation of the Core and Yale Asthma U19 program. Her effort in this regard will be 1.8 calendar months annually.

Other Expenses

Supplies
$3,500 is budgeted annually for purchase of supplies and services including poster preparation for presentation at national meetings, communications, and monthly meeting costs.

Domestic Travel
$7,000 per year is budgeted to defray travel costs for the PIs and the other investigators to attend meetings at NIH and other national meetings.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		60,030.00
Section B, Other Personnel		93,647.00
Total Number Other Personnel	5	
Total Salary, Wages and Fringe Benefits (A+B)		153,677.00
Section C, Equipment		0.00
Section D, Travel		35,000.00
1. Domestic	35,000.00	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		17,500.00
1. Materials and Supplies	17,500.00	
2. Publication Costs	0.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	0.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	0.00	
9. Other 2	0.00	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		206,177.00
Section H, Indirect Costs		137,106.00
Section I, Total Direct and Indirect Costs (G + H)		343,283.00
Section J, Fee		0.00

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

1. Project Director / Principal Investigator (PD/PI)

Prefix:
First Name*: GEOFFREY
Middle Name: L
Last Name*: CHUPP
Suffix:

2. Human Subjects

Clinical Trial? ● No ○ Yes
Agency-Defined Phase III Clinical Trial?* ○ No ○ Yes

3. Permission Statement*

If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?

○ Yes ● No

4. Program Income*

Is program income anticipated during the periods for which the grant support is requested? ○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

Budget Period*	Anticipated Amount ($)*	Source(s)*

PHS 398 Cover Page Supplement

5. Human Embryonic Stem Cells

Does the proposed project involve human embryonic stem cells?* ● No ○ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s): ❑ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

6. Inventions and Patents (For renewal applications only)

Inventions and Patents*: ○ Yes ○ No

If the answer is "Yes" then please answer the following:

Previously Reported*: ○ Yes ○ No

7. Change of Investigator / Change of Institution Questions

❑ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:

First Name*:

Middle Name:

Last Name*:

Suffix:

❑ Change of Grantee Institution

Name of former institution*:

PHS 398 Research Plan

Please attach applicable sections of the research plan, below.

OMB Number: 0925-0001

1. Introduction to Application (for RESUBMISSION or REVISION only)	
2. Specific Aims	Core_A_Specific_Aims.pdf
3. Research Strategy*	Core_A_Research_Strategy.pdf
4. Progress Report Publication List	
Human Subjects Sections	
5. Protection of Human Subjects	
6. Inclusion of Women and Minorities	
7. Inclusion of Children	
Other Research Plan Sections	
8. Vertebrate Animals	
9. Select Agent Research	
10. Multiple PD/PI Leadership Plan	
11. Consortium/Contractual Arrangements	
12. Letters of Support	
13. Resource Sharing Plan(s)	
Appendix (if applicable)	
14. Appendix	

Specific Aims

An Administrative Core will coordinate and facilitate all activities within the Yale U19 Asthma and Allergic Disease Cooperative Research Center (AADCRC) Group. This Core will be responsible for the overall organization, management, decision-making, and utilization of institutional resources. The Administrative Core will pursue the following Aims:

1. **Provide oversight and consultation to each of the Research Projects and Scientific Cores to ensure that scientific objectives are met and that there is optimal interaction and utilization of resources. The Administrative Core will monitor and assist each group so that their goals are achieved and emergent problems are expeditiously addressed. Support administrative tasks related to subject enrollment and scheduling, payment and the preparation and timely submission of regulatory documents.**

2. **Provide fiscal management and ensure cost-effective utilization of U19 resources.**

3. **Promote the communication of the research team and dissemination of AADCRC research and technology, and organize the presentation and publication of data. This aim also will ensure data sharing, protection of intellectual property, and long-term data storage.**

Research Strategy

Core A. Administration

A. Introduction and Specific Aims

An Administrative Core will coordinate and facilitate all activities within the Yale U19 Asthma and Allergic Disease Cooperative Research Center (AADCRC) Group. This Core will be responsible for the overall organization, management, decision-making, and utilization of institutional resources. The Administrative Core will pursue the following Aims:

1. **Provide oversight and consultation to each of the Research Projects and Scientific Cores to ensure that scientific objectives are met and that there is optimal interaction and utilization of resources. The Administrative Core will monitor and assist each group so that their goals are achieved and emergent problems are expeditiously addressed. Support administrative tasks related to subject enrollment and scheduling, payment and the preparation and timely submission of regulatory documents.**

2. **Provide fiscal management and ensure cost-effective utilization of U19 resources.**

3. **Promote the communication of the research team and dissemination of AADCRC research and technology, and organize the presentation and publication of data. This aim also will ensure data sharing, protection of intellectual property, and long-term data storage.**

B. Research Plan

B1. Leadership and Organizational Structure

Geoffrey Chupp MD, the PI of the AADCRC Center, will direct the Administrative Core with aid in the day-to-day operations by a Senior Administrative Assistant, Ms. Susan Ardito. Major decisions within the U19 will be made by the Administrative Core in conjunction with an Executive Committee comprised of Research Project Leads: Joseph Craft, MD, and Mark Gerstein, PhD, and Scientific Core Leads: Lauren Cohn, MD, and Ruth Montgomery, PhD. The Executive Committee together can also identify a member to provide additional support to the U19 group should any member be unable to serve. The role of the Administrative Core in coordinating the operations of the U19 and the lines of interaction and authority are shown in the figure below.

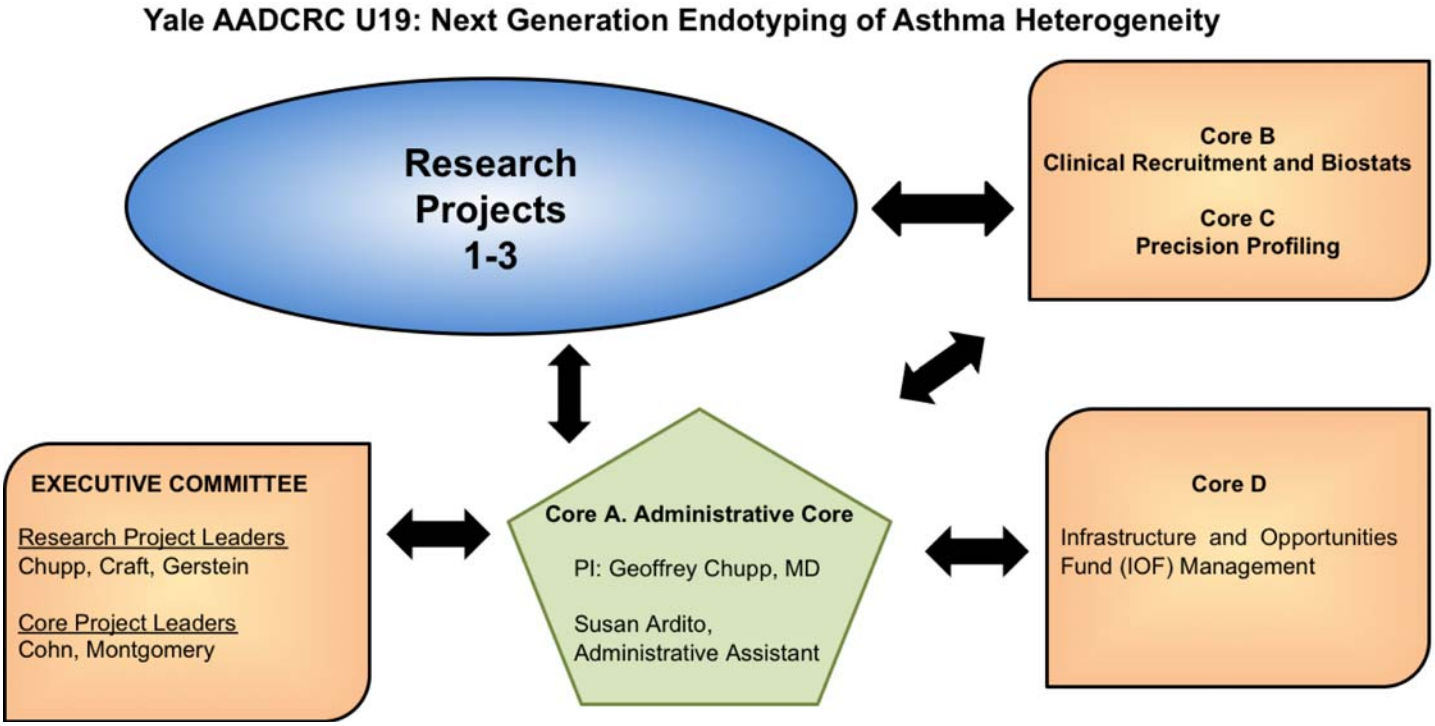


Figure 1: Administrative structure of the Yale Asthma U19 Administrative Core

B2. Role of Director and Leadership Components

Director. Dr. Chupp will administer the Center, which is distinct from his role as Lead of Project 1 (Mechanisms of YKL-40 and Transcriptomic Endotypes in Asthma), or his research and clinical activities as part of a faculty member in the Division of Pulmonary, Critical Care and Sleep Medicine. Dr. Chupp will oversee all Center operations, including the progress of the Research and Core Projects and will have close knowledge of all aspects of U19 operations, including scientific progress, personnel, finance, and day-to-day operations. He will meet weekly with the Senior Administrative Assistant, Ms. Susan Ardito, in order to review all administrative and financial aspects of the Center. Dr. Chupp will chair monthly scientific progress meetings and meetings of the Executive Committee to review preliminary results, discuss subject recruitment, and collaborate on future experiments. He will ensure that all aspects of the U19 research group work together at optimal efficiency, and with a high level of collaboration among the various components. He will prepare and submit progress reports to NIAID and will liaise with program officials from NIAID regarding all aspects of the Center operations. Finally, Dr. Chupp will support the efforts of Ruth Montgomery, PhD, in directing the Infrastructure and Opportunities Fund of this U19.

Dr. Chupp's qualifications for administering the U19 Center, beyond those listed in the biosketch, include the following experience: he has been pursuing translational research in airway disease for the past 20 years, specifically severe asthma and heterogeneity of asthma. His research career has focused on the identification of novel pathobiologic determinants of asthma through genomic characterization of airway and blood profiles in individuals with this disabling lung disease. He has authored numerous papers and invited reviews related to this topic. At the Yale School of Medicine, his laboratory is equipped with state-of-the-art technologies to support genomic research including resources at the Center for Precision Pulmonary Medicine (P2Med), that he co-directs with Dr. Naftali Kaminski, section chief of the Pulmonary, Critical Care and Sleep Medicine at Yale. He is the former Pulmonary Fellowship Program Director and Director of Bronchoscopy and has a track record of successful interdisciplinary national and international collaborations. Thus, Dr. Chupp is well experienced in the administrative challenges in bringing together the diverse groups of scientists that are part of the Yale Asthma U19 team.

Administrative Assistant. Ms. Susan Ardito is a Senior Administrative Assistant within the Department of Medicine and presently supports Dr. Chupp's research and clinical activities. Ms. Ardito has nearly 20 years of experience at Yale, including coordination between multiple PIs, financial expense reports and editing of academic documents. She is experienced in coordinating the activities necessary for a collaborative, multi-investigator award. Ms. Ardito will organize meetings, teleconferences, arrange for travel for the investigators, and assist in the preparation of scientific reports. Ms. Ardito will meet regularly with the U19 leadership and ensure complete financial and administrative support of this collaborative project.

Executive Committee. An Executive Committee comprised of Dr. Chupp and the Research Project Leads: Joseph Craft, MD, and Mark Gerstein, PhD, and Scientific Core Leaders: Lauren Cohn, MD, and Ruth Montgomery, PhD, will ensure regular communication within the group. These individuals have the closest knowledge of research within their individual projects and can best identify potential problems and propose timely solutions. The Executive Committee will meet monthly with Dr. Chupp to formally review all ongoing activities of the U19 Center and provide advice to maintain optimal interactions between groups and productivity and efficient use of Center resources.

B3. Implementation of Specific Aims

1. Provide oversight and consultation to each of the Research Projects and Scientific Cores to ensure that scientific objectives are met and that there is optimal interaction and utilization of resources. The Administrative Core will monitor and assist each group so that their goals are achieved and emergent problems are expeditiously addressed. Support administrative tasks related to subject enrollment and scheduling, payment and the preparation and timely submission of regulatory documents.

1.1 Oversight of the Research Projects. The Administrative Core will meet monthly with the U19 Executive Committee to review scientific progress and ensure that objectives are met and that there is optimal utilization of resources within the Center. These regularly scheduled meetings will review scientific data, monitor patient recruitment for the NextGen and Adenotonsillectomy studies, and HIC/HIPAA compliance. As scientific findings develop, the committee may refine research direction or suggest collaboration with other laboratories or

AADCRC Centers. The utilization of Core research and services will be reviewed and, if indicated, recommendations for the re-allocation of Center resources will be made and implemented. Fiscal and other resources also will be prioritized as necessary as the research projects develop. Committee decisions will be made by majority consensus with the Administrative Director/Principal Investigator acting as final decision maker in the event of an impasse. Every attempt will be made to provide a full airing and resolution of issues within these monthly meetings, and potential conflicts resolved in a professional and sensitive manner. Ms. Ardito will record minutes and forward these to the Executive Committee for approval. Given the collaboration history of Yale Asthma U19 team, we believe significant disagreements are unlikely, but we are prepared to initiate these interventions immediately to achieve rapid resolution and to preserve the scientific goals of the Yale Asthma U19 program. Almost all the investigators laboratories are on the same floor in the Anlyan Center at the Yale School of Medicine, so these discussions are easily scheduled and resolution will be swift.

1.2 Oversight of the Scientific and Pilot Projects Cores. The monthly meeting of the PI and the Executive Committee will discuss progress within the Cores; for instance, status of enrollment targets in the Clinical Recruitment and Biostatistics Core B, or data and distribution to and from the Precision Profiling Core C. Emergent issues and future work plans will be reviewed on an ongoing basis. If indicated, the PI will provide assistance and intercede and arbitrate if needed in any institutional, personnel, or other structural problems that may arise. Ms. Ardito will record minutes and forward these to the attendees for their approval.

2. Provide Fiscal Management and Ensure Cost-effective Utilization of Center Resources.

2.1 Overall Business and Accounting Management. Ms. Ardito will manage day-to-day financial operations under the direction of the PI. She will work closely with the Pulmonary, Critical Care and Sleep Section's business office and with the School of Medicine's Grants and Contracts Administration to manage U19 Center finances. All financial issues will be discussed as part of the agenda for the monthly meetings of the Administrative Core and the Executive Committee. The Research Project and Scientific and Pilot Core Leads have real-time access to financial information (expenditures, cost recovery) in order to make efficient use of Center resources and prevent cost overruns. Ms. Ardito will assist Dr. Chupp in the preparation of financial reports for the NIH.

2.2 Oversight and Accounting Management of the Individual Cost Centers. The U19 Center will have at its disposal a full-time business manager plus an accountant who will be responsible for financial reporting. These individuals part of the Division of Pulmonary, Critical Care and Sleep Medicine business office and are supported by departmental and institutional funds. Yale has implemented a transparent and accessible web-based reporting system for investigators and program administrators to view all financial grant and contract transactions. Investigators receive electronically updated monthly reports that detail all financial transactions, including purchases of goods and services from external and internal vendors and service centers along with a running total that details dollar amounts. This information includes individual categories (personnel, supplies) that remain to be expended for each budget year. This reporting system has been adopted by all administrative units within the University, and it provides reporting for individual components or program grants. In the context of this U19, the Administrative Core will receive a detailed financial breakdown of the total grant and its constituent components, including the Research Projects and Scientific Cores.

3. Promote the Communication and Dissemination of U19 Center Research and Technology, and Organize the Presentation and Publication of Data. Ensure Data Sharing, Protection of Intellectual Property, and Long-term Data Storage.

3.1 HIPC Steering Committee meeting. The PI will serve on the AADCRC steering committee which functions to govern the AADCRC collaborative research program and maximize the utilization of resources. The PI will participate in discussions including regular teleconferences and meetings in Bethesda, MD as needed.

3.2 Executive Committee Meetings. The monthly meetings of the Administrative Core and Executive Committee will provide a venue for the Research Project Leads to update each other on research progress, identify the most promising experimental findings for continued development, and to propose collaborative opportunities.

3.2 Joint Laboratory Meetings. In order to facilitate closer, interdisciplinary interaction among the scientists working within the U19 Center, a monthly meeting that will involve the entire participating laboratories will occur. Each meeting will be hosted on a rotating basis by one laboratory and feature data presentation

related directly to the specific aims of the program. These meetings will be one hour in duration, allow sufficient time for presentation and discussion, and will be attended by as many team members as are available, including but not limited to co-investigators, post-doctoral fellows, laboratory technicians, computational scientists, medical students and graduate students.

3.3 Research-in-Progress Lectures. Data presentation will occur more formally within the Department of Medicine's Research-in-Progress (RIP) and Human Translational Immunology lecture series that occur monthly. These seminars are widely attended and will serve to more broadly inform the University community about research in human immunology and asthma. When suitable, Abstracts for presentation will be submitted to national and international meetings, such as the American Academy of Allergy, Asthma and Immunology (AAAAI), ATS (American Thoracic Society), European Respiratory Society (ERS), AAI (American Association of Immunology), the ASCI (American Society for Clinical Investigation), the SLB (Society for Leukocyte Biology, and Aspen Lung, Keystone and Gordon conferences.

3.4 Research Reports and Publications. The PI will be responsible for the organization and submission of yearly progress reports; these will be prepared in coordination with the Project PI's. Accepted versions of final, peer-reviewed manuscripts emanating from U19 research will be deposited on-line to PubMed Central in accord with NIH Public Access Policy (grants.nih.gov/grants/guide/notice-files/NOT-OD-08-033).

3.5 Resource Sharing. The PI will be responsible for ensuring resource and data sharing for the U19. We will comply with the AADCRC Data Sharing Plan, which is designed to enable the widest dissemination of data, while also protecting the privacy of study participants and the utility of the data, by de-identifying and masking potentially sensitive data elements, consistent with HIPAA considerations. This approach is fully compliant with the NIH public data sharing policy (grants.nih.gov/grants/policy/data_sharing). Completed data sets will remain private for up to 3 years or until the online publication of the first manuscript. When the earlier of these two conditions is met, the data set(s) supporting the publication will be made available through relevant public databases (e.g., Gene Expression Omnibus (GEO) and the Sequence Read Archive (SRA)). Additional details of our data sharing mechanisms are described in the Resource Sharing plans of each project and core included within each part of this proposal. Research resources generated with funds from this grant will be freely distributed to qualified academic investigators for non-commercial research. All U19 Center investigators will adhere to the NIH Grants Policy on Sharing of Unique Research Resources including the "Sharing of Biomedical Research Resources: Principles and Guidelines for Recipients of NIH Grants and Contracts" issued in December 1999 (ott.od.nih.gov/policy/rt_guide_final). Specifically, material transfers would be made with no more restrictive terms than in the Simple Letter Agreement or the UBMTA and without reach through requirements. Should any intellectual property arise which requires a patent, we would ensure that the technology remains widely available to the research community in accordance with the NIH Principles and Guidelines document.

3.6 Protection and Licensing of Intellectual Property. New intellectual property created as a result of U19 Center research will be safeguarded by University policies as implemented by Yale's Office of Cooperative Research (yale.edu/ocr/disclose). This office is located in a central administrative building and it is dedicated to the patenting and licensing of technology from Yale laboratories. The Office of Cooperative Research has demonstrated experience translating research findings into products, please see the Yale Drug Pipeline, as an example of technologies that are in pre-clinical and clinical development (yale.edu/ocr/news/studies/docs/YalePipeline_Apr2008.pdf). Dr. John Puziss, Senior Associate Director at the Office of Cooperative Research, has worked closely with the Program PI in the past, and he will monitor new research findings and technology for its novelty, inventiveness, and potential applicability. He will attend meetings of the Executive Committee on a quarterly basis, during which intellectual property issues will be on the agenda and discussed. With Dr. Puziss's advice, disclosures will be submitted to the Office of Cooperative Research, patents will be filed, and the inventions marketed for commercialization by his office team.

3.7 Resource and Data Sharing and Long-term Data Storage. Detailed procedures for data sharing and long-term data storage are described in our Data Management and Bioinformatics Core. Briefly, we will employ a database management infrastructure already established in our current YCAAD project that records subject enrollment and compiles laboratory and research results on subject samples, and that facilitates data sharing between investigators. The clinical database, based on the Online Collaborative Research Environment (OnCore) (Forte, WI), is maintained by the Yale Center for Clinical Investigation (YCCI) and a dedicated database manager, in adherence to HIPAA regulations to ensure the security of personal identifying information. Long-term data storage will be accomplished by submitting all data to NIAID's Immunology Database and Analysis Portal (ImmPort) system (immport.niaid.nih.gov), which serves as the data repository for the NIAID's Division of

Allergy, Immunology, and Transplantation-funded investigators. Core Lead Montgomery has extensive experience preparing data for submission to ImmPort as part of a current U19 HIPC project, as well as her N01 Biodefense Contract on Special Populations. In relevant cases, ImmPort submissions also require raw data be deposited in relevant public repositories. For example, gene expression microarray data is deposited in GEO. Data will be shared through these public repositories, and the ImmPort database. We will make available final research data from our study in accordance with the NIH data sharing policy, and also in accordance with the AADCRC steering committee policies.

3.8 University-wide Research Seminars. A key component of scientific cohesiveness and the dissemination of research within the U19 Center is our active participation in a University-wide seminar series dedicated to asthma, human immunology and the study of NIAID Category A, B, or C Priority or emerging/re-merging Pathogens. The Yale School of Medicine presently hosts two successful and widely attended weekly seminar series: one in Human Translational Immunology and one in Microbial Pathogenesis. We will arrange to support one monthly slot from these series for the invitation of U19 investigators or scientists from outside institutions to present their research. Speakers will be proposed by U19 investigators and invited by the Executive Committee. The speakers and their sponsorship will be advertised in the University calendar, and Department, Sectional calendars as well.

References Cited

None

OMB Number: 4040-0001
Expiration Date: 06/30/2016

APPLICATION FOR FEDERAL ASSISTANCE

SF 424 (R&R)

5. APPLICANT INFORMATION **Organizational DUNS*:** 0432075620000

Legal Name*: YALE UNIVERSITY
Department:
Division:
Street1*: OFFICE OF SPONSORED PROJECTS
Street2: 25 Science Park
City*: NEW HAVEN
County:
State*: CT: Connecticut
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 065208237

Person to be contacted on matters involving this application

Prefix:	First Name*:	Middle Name:	Last Name*:	Suffix:
	Marybeth		Brandi	

Position/Title: Proposal Manager
Street1*: 25 Science Park
Street2: 150 Munson Street
City*: New Haven
County:
State*: CT: Connecticut
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 06520-8237
Phone Number*: 203-737-3495 Fax Number: Email: marybeth.brandi@yale.edu

7. TYPE OF APPLICANT*

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Clinical Recruitment and Biostatistics Core

12. PROPOSED PROJECT

Start Date*	Ending Date*
07/01/2016	06/30/2021

OMB Number: 4040-0010
Expiration Date: 06/30/2016

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	YALE UNIVERSITY
Duns Number:	0432075620000
Street1*:	300 Cedar St
Street2:	TAC S440
City*:	NEW HAVEN
County:	
State*:	CT: Connecticut
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	065208237
Project/Performance Site Congressional District*:	CT-003

Project/Performance Site Location 1

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	YALE UNIVERSITY
DUNS Number:	0432075620000
Street1*:	300 Cedar St
Street2:	TAC 460
City*:	NEW HAVEN
County:	
State*:	CT: Connecticut
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	065208237
Project/Performance Site Congressional District*:	CT-003

File Name

Additional Location(s)

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ● **Yes** ❍ **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ❍ Yes ● No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6

If NO, is the IRB review Pending? ❍ **Yes** ❍ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ❍ **Yes** ● **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ❍ Yes ❍ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. Is proprietary/privileged information included in the application?* ❍ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ❍ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ❍ Yes ❍ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ❍ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ❍ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Core_B_Project_Summary.pdf
8. Project Narrative*	
9. Bibliography & References Cited	Core_B_References_Cited.pdf
10.Facilities & Other Resources	CoreB-Facilities.pdf
11.Equipment	CoreB-Equipment.pdf

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

Project Summary

The Clinical Recruitment and Biostatistics Core (Core B) will acquire essential clinical data and human samples, and provide database and analytic support for each of the Projects in the human-based Program. The Yale Center for Asthma and Airway Diseases (YCAAD) supports the infrastructure of a well-established, finely tuned clinical research center designed by Core Lead Cohn and Principle Investigator Chupp to obtain human data and biospecimens for airway diseases research. The clinical research tools developed over a decade in YCAAD include rapid recruitment from a large, urban population of asthmatic subjects with a range of disease activity and severity and a study visit that delivers high quality clinical and physiological data, and biospecimens including sputum and blood. Through this mechanism we have conducted human-based research that led to the discovery of genes and immunological pathways associated with asthma and developed novel methods to characterize disease heterogeneity using transcriptomic data and defined novel endotypes of asthma. In *Next Generation Endotyping of Asthma Heterogeneity* we will take these studies further to define in-depth the airway single cell signatures, airway cell populations and immune pathways that exist in heterogeneous cohort of individuals with asthma. The Clinical Recruitment and Biostatistics Core (CRBC) is the starting point of the three driving Projects in this Program. Each Project will harness the strengths of this Core to obtain essential human data and biospecimens to conduct the proposed studies. The Clinical Recruitment Component of the Core will recruit and enroll asthmatic and control subjects to participate in the proposed clinical studies, obtain both clinical and physiological data, and biological samples, including sputum and blood and tonsil tissue. The Biostatistical Component of the Core will support a custom built, web-based, HIPAA compliant database for data entry, biospecimen management and a portal for retrieval of de-identified data for analysis, as well as extensive biostatistical support for analyses of the complex datasets generated in this proposal. Each of the Projects will take advantage of human clinical data and biological specimens obtained from the Core. Human samples obtained through this Core will be transferred to the Precision Profiling Core C for analyses, and to investigators for customized functional studies. Data generated will then be entered and stored in the database or on a large server accessible to YCAAD for biostatistical analyses in the Core. Through Core B, these projects will have an efficient mechanism to conduct these cutting-edge studies to define heterogeneity in asthma.

Facilities and Other Resources - Core B

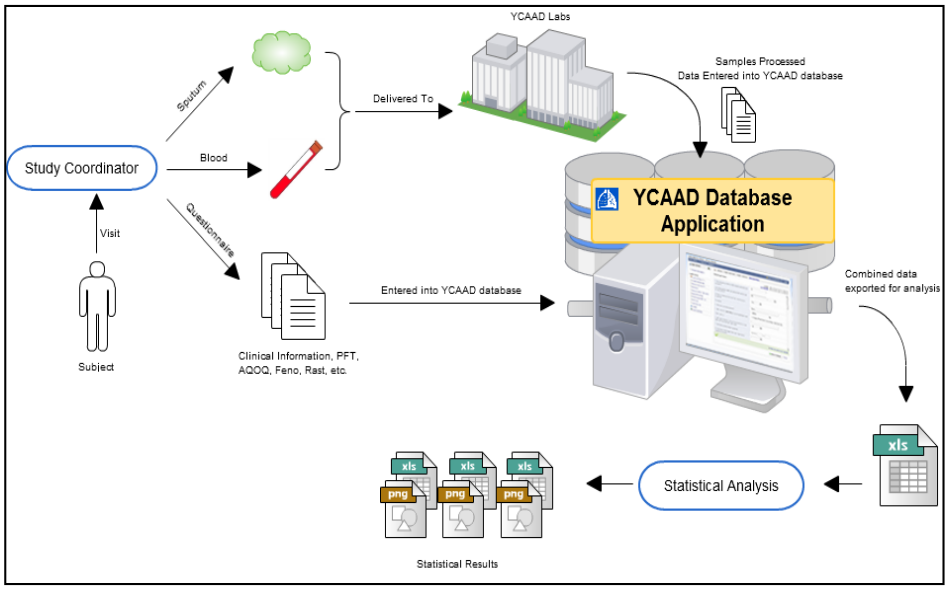
Yale Research Environment Enables Accessibility to Core Materials

The Clinical Recruitment and Biostatistics Core oversees recruitment of study subjects through our clinical research center, the Yale Center for Asthma and Airway Diseases (YCAAD). YCAAD will collect clinical data and biological samples and perform physiological testing, process and distribute samples, upload data and perform biostatistical analyses. The center for these activities is on the fourth floor of The Anlyan Center (TAC) on the Yale School of Medicine campus – a building designed to facilitate transdisciplinary collaboration for the advancement of science. The academic office of Dr. Cohn and the Core B laboratory and the P2Medicine Biostatistics Center (Dr. Yan) are located on the fourth floor of The Anlyan Center (TAC), as are the offices and laboratories of Drs. Montgomery (PI Core C) and Dr. Chupp. The Department of Immunobiology is located on the 5th floor of the Anlyan Center where Drs. Craft and Flavell have offices and laboratories. YCAAD operates in a building adjacent to the Anlyan Center. Other investigators are located nearby on the Yale Campus. This close physical structure enables rapid processing and distribution of samples, casual conversations and ease of collaboration, which has led to the interactive studies outlined in this proposal.

Research Facilities

Clinical Research Facilities

Yale Center for Asthma and Airways Disease (YCAAD). Yale-New Haven Hospital is the largest referral center in the state, and YCAAD is the only dedicated adult asthma center in the region. Directed by Drs. Chupp and Cohn, and staffed by 2 additional providers, Dr. Gomez and a physican assistant, this active and growing center receives 2000 visits annually and is the hub of the asthma clinical/translational research program at Yale. Recruitment of subjects, one of the primary functions of this Core, will take place in YCAAD. For 15 years YCAAD has been operating with a mission to study human asthma in a longitudinal, high throughput fashion. Using the YCAAD phenotyping protocol (see appendices), we have enrolled over 800 subjects (≥12 years of age). The protocol comprises a 2 hour study visit that includes an extensive coordinator-administered asthma questionnaire (Appendix), lung function testing, hypertonic saline sputum induction (Appendix) and blood drawing for genomic level analyses of RNA isolated from the blood and sputum. We currently enroll new asthma subjects at a rate of ~100 per year and reevaluate ~70 subjects for follow up visits. This infrastructure is central to the studies outlined in this proposal and will accommodate enrollment of 200 new subjects in the first 24 months, with follow-up visits at 9 month intervals, as described.



Research Laboratories. The YCAAD bench research laboratory is located in the Section of Pulmonary, Critical Care and Sleep Medicine on the 4th floor of The Anlyan Center building. The laboratory occupies ~1000 square feet of space in a large, open laboratory structure with shared equipment and resources and issituated close to the YCAAD Clinical Center. Samples obtained from subjects in YCAAD, including blood and sputum, can be quickly transported to the bench laboratory to be processed and distributed to Core C and other laboratories, as needed. The samples are barcoded and scanned into a system for identification and tracking. The bench laboratories are stocked and equipped for the cell and molecular investigations described in this application. The laboratory is a Level II Biosafety facility and contains tissue culture, cell biology, molecular biology, darkroom, and computing facilities.

P²Med Biostatistical Center. The biostatistical component of this Core includes both database and biostatistics/informatics capabilities. These are described in detail in Research Strategy. P²Med is part of the Section of Pulmonary, Critical Care and Sleep Medicine and is a specially designed resource on the 4th floor of the TAC building with the infrastructure for human research including database management of biorepository samples and data, and biostatistical analyses served by extensive computing facilities. Dr. Yan, P²Med Director, has an office in the Center. Along with Dr. Chupp, she has developed the YCAAD informatics systems that will be used for this U19 grant.

YCAAD Informatics System: An essential resource of the YCAAD research program is the web-based informatics platform that was constructed to handle the high flow of subjects and data required for these studies (see figure). Custom built, in collaboration with the Yale Center for Medical Informatics (YCMI) (which serves as a focus for training, research, and institutional computer systems development and supports a range of computing and informatics projects), YCMI provided the infrastructure for the development of the YCAAD web-based database platform which is central to the translational asthma research program. This secure, HIPAA-compliant, web-based portal allows program personnel, including research coordinators, laboratory technicians, genomics specialists, and biostatisicians, the ability to upload and download de-identified clinical, laboratory, and genomic data from different locations. Clinical data is directly entered into the system at the time of the phenotyping visit from a HIPAA-compliant, protected and encrypted iPad. Experimental results are uploaded by laboratory scientists, and a customized query application easily downloads data files with the requisite clinical phenotype and experimental data in any desired file format. This system greatly increases the pace of scientific discovery and resource sharing.

To advance the capabilities of the YCAAD database to interact with electronic medical record in the Yale New Haven Hospital (YNHH) network and enhance subject data extraction in quantity and quality, we are in the process of migrating the database to the OnCore system. OnCore is a suite of clinical and translational research modules consisting of software for research, patient registry and biospecimen management that is integrated with Epic, the electronic medical record of the YNHH network. Clinical data will be entered into OnCore by study coordinators using a web interface that identifies users by Yale NetID and password. The 'Study_Number' and 'Visit_Date' makes up the unique identifier that will be used throughout the system for a subject visit. Laboratory personnel will access the biospecimen module and use the unique identifier to store, track, monitor, and enter information relevant to individual samples. Since OnCore is not equipped to store high throughput data such as gene expression, protein levels or genotype data, YCAAD will maintain a copy of the OnCore data. Code has been written so the every night data will be extracted from OnCore into the YCAAD server so that all of the research data can be managed from one database. This new system will be in place at the start of these studies.

Pulmonary Function Laboratory. The adult Pulmonary Function Testing (PFT) Laboratory consists of over 1200 square feet, located in Yale-New Haven Hospital and adjacent to YCAAD. The PFT Lab is directed by Dr. Chupp and staffed by five certified PFT technicians who are expert in performing all routine and many specialized pulmonary function tests. The equipment is all networked through a file server to a Dek 386 450-D2LP-based database. The laboratory performs over 8000 tests a year and has extensive experience in the performance of pulmonary function tests for clinical and translational research studies. All studies are done in accordance with ATS guidelines.

Yale Center for Clinical Investigation. The Yale Center for Clinical Investigation (YCCI) is a multidisciplinary resource funded through a Clinical Translational Science Award (CTSA) from the National Institutes of Health. Launched in January 2006, the YCCI supports and facilitates clinical and translational research and training across the entire medical campus. This collaborative was designed to foster and support the development of clinical and translational research at Yale. Through YCCI funding, the YCAAD research program was developed and continues to be closely associated with the YCCI. YCCI supports OnCore, which will integrate YCAAD subject visits with Epic, the electronic medical record of the YNHH network and enhance our ability to analyze large and complex datasets. We are working with YCCI in the transition to OnCore, which is in process at this time (see above). The YCCI is integrated with all aspects of human-based research at Yale, and the interactive environment at the Medical School encourages involvement in YCCI.

Computers

The PI and team members on this grant each have desktop computers (Apple or IBM-compatible) with connections color printers and scanners. E-mail and internet access are provided through the Yale University Information Technology Network with connections to the University's mainframe system, impressive biostatistical support, access to computer-focused literature and genetic searches.

Offices

The Drs. Cohn and Yan have academic research offices on the 4th floor of The Anlyan Center and access to a full-time administrative assistant.

Animals
Not applicable.

Equipment - Core B

YCAAD Laboratories

Clinical Research. YCAAD study visits are conducted in a research laboratory in a building adjacent to the Anlyan Center and the hospital and clinics. This laboratory is equipped with 3 portable spirometers, 2 Nioxx Mino machines to measure exhaled nitric oxide, a computer and printer. For additional testing the adult Pulmonary Function Testing (PFT) Laboratory is adjacent to the YCAAD clinical research laboratory and is equipped with two Collins DS560 systems for performing spirometry, 3 Nioxx machines, static lung volume and diffusing capacity measurements. There is a Collins body plethysmograph, Sensormedics computerized dry rolling spirometer, computerized Stedwell survey plus wet spirometer, and a custom-built dry pneumotech for methacholine challenge testing. This equipment is networked through a file server to a Dek 386 450-D2LP-based database.

Bench Laboratories. Dr. Cohn and Dr. Chupp have adjacent laboratories within the Pulmonary Research Laboratories that provide space and equipment for the bench laboratory activities in YCAAD. Here, sputum and blood samples will be processed and distributed to the Precision Profiling Core (Core C) or to individual investigators for biological studies. These laboratories occupy ~1000 square feet in The Anlyan Center at Yale School of Medicine. The laboratories are equipped for cellular immunology, microbiology and molecular biology and include the following equipment: a Dako Auto stainer (Dako) with the capability to do automated immunohistochemistry, DNA cyclers, gel apparatuses and power supplies, tissue culture hoods with laminar flow, chemical hoods, water jacketed CO2 incubators, Coulter counter, microcentrifuges, 2 inverted and 2 light microscopes, tissue homogenizer, cytospin, ELISA reader, spectrophotometer, real-time PCR machine and liquid nitrogen cell bank, refrigerators, -20 and -80 degree freezers, 2 refrigerated centrifuges, liquid scintillation counter, gamma counter, pH meters, balances, refrigerators, fast protein liquid chromatography apparatus (FPLC, Pharmacia), HPLC (Waters), vertical SDS gels (Hoeffer), 2-D gels, UV gel viewer, Polaroid camera system, submerged gel system, dot-blot apparatus, hot wire bag sealer, Geiger counter, baking oven, freeze-dryer, 37^{o} C rotating incubator, speed vac concentrator, ultracentrifuge, super speed centrifuge, laser densitometer, fluorescent microscope (Nikon Microphot-FXA). The Anlyan research facility is equipped with a luminometer, scintillation counter, cell harvesters, ultra- and high-speed centrifuges and ELISPOT reader, fluorescent microscope, Cytometry Facility, confocal microscopy. Additional equipment is detailed in Core C. Glass washing and autoclave facilities are adjacent to the laboratories. A -80^{o} freezer dedicated to human samples; this is locked and has a remote sensing alarm to notify an on-call member of the research team if there is a temperature abnormality. Four additional -80^{o} C freezers are located in the laboratory. Laboratories are equipped with desktop computers for each member and a printer. E-mail and internet access are provided via the Yale University Information Technology Network. This provides access also to the University's mainframe system, impressive biostatistical support, access to computer focused literature evaluation and genetic searches.

P2 Med (Precision Pulmonary Medicine) Biostatistics Center

The P2MED Center on the 4th floor of The Anlyan Center is the biostatistical hub of the Core where Dr. Yan has her office and computers. P2 Med is equipped with 3 iMACs, 3 Dell dual processor workstations served by a color laser printer and 3 external hard drives. All computers are networked and loaded with analysis software. The Center also has a high performance computing cluster queue maintained by the Yale Information Technology Services (ITS). The queue includes 8 computing nodes (20 cores and 128Gb RAM per node) and a 50 Tb high performance general parallel file system (GPFS) for storage. For sequencing analysis we have access to the resources at the Yale Center for Statistical Genomics and Proteomics and all of the software required including CLC-Bio, FastQC, SAMtools, TopHat2, Cufflinks2 and other software as required. Software for word-processing, spreadsheets, statistics, graphics, database and image analysis is installed, including: JMP, Adobe Photoshop and Adobe Illustrator. The academic software packages for gene expression analysis include: tools for normalization and statistical reading of microarray results, including D-chip, RMA, and Cyclic Loess, Genespring (Including the NGS module); a variety of clustering and visualization tools including: Cluster, Treeview, Expander, BRB Array tools, and GEDI. Statistical analysis and classification packages include: Scoregene, R, SAM & PAM, the unique probabilistic relational modeling viewer, Genomica, as well as a license for Spotfire. The YCAAD Database is on a server maintained by the Yale School of Medicine and accessible through the Yale intranet. This database was custom built for YCAAD and is a secure, HIPAA compliant, password protected database that can be used by multiple investigators simultaneously from multiple sites. This database efficiently integrates clinical data and genomic expression data that will be uploaded by the Center for Genome Analysis. Combined, this informatic hardware infrastructure will facilitate the success of these studies.

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: LAUREN Middle Name E Last Name*: COHN Suffix:

Position/Title*: Associate Professor of Medicine
Organization Name*: YALE UNIVERSITY
Department:
Division:
Street1*: YALE UNIVERSITY SCH OF MEDICIN
Street2: PULMONARY & CRITICAL CARE
City*: NEW HAVEN
County:
State*: CT: Connecticut
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 065208057

Phone Number*: (203) 737-1459 Fax Number: (203) 785-3826 E-Mail*: LAUREN.COHN@YALE.EDU

Credential, e.g., agency login: LECOHN

Project Role*: Other (Specify) Other Project Role Category: Project Lead

Degree Type: MD,BA Degree Year:

	File Name
Attach Biographical Sketch*:	Cohn_Bio_Asthma_U19.pdf
Attach Current & Pending Support:	

PROFILE - Senior/Key Person

Prefix:	First Name*: XITING	Middle Name	Last Name*: YAN	Suffix:

Position/Title*:	Assistant Professor (Pulmonary)
Organization Name*:	YALE UNIVERSITY
Department:	
Division:	
Street1*:	300 Cedar St
Street2:	TAC-S469B
City*:	New Haven
County:	
State*:	CT: Connecticut
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	065200000

Phone Number*:	Fax Number:	E-Mail*:
203-785-5567		xiting.yan@yale.edu

Credential, e.g., agency login: XITINGYAN

Project Role*:	Other Project Role Category:
Other (Specify)	Co-Lead

Degree Type:	Degree Year:
PHD	

	File Name
Attach Biographical Sketch*:	Yan_Bio_Asthma_U19.pdf
Attach Current & Pending Support:	

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2016 **End Date*:** 06-30-2017 **Budget Period: 1**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	LAUREN	E	COHN		Project Lead		2.4			20,797.00	6,447.00	27,244.00
2.	Xiting		Yan		Co-Lead		2.4			16,995.00	5,268.00	22,263.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **49,507.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
3	Database Manager, Research Coordinator, Technician	7.8			54,331.00	16,843.00	71,174.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**71,174.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**120,681.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2016 **End Date*:** 06-30-2017 **Budget Period: 1**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2016 **End Date*:** 06-30-2017 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	37,049.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Study Subject Payment	9,750.00
Total Other Direct Costs	**46,799.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**167,480.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Modified total direct cost	66.5	167,480.00	111,374.00
		Total Indirect Costs	**111,374.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**278,854.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: Core_B_Budget_Justification_Asthma_U19.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2017 **End Date*:** 06-30-2018 **Budget Period: 2**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	LAUREN	E	COHN		Project Lead		2.4			21,420.00	6,640.00	28,060.00
2.	Xiting		Yan		Co-Lead		2.4			21,006.00	6,512.00	27,518.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **55,578.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
3	Database Manager, Research Coordinator, Technician	7.8			55,961.00	17,348.00	73,309.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**73,309.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**128,887.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2017 **End Date*:** 06-30-2018 **Budget Period: 2**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2017 **End Date*:** 06-30-2018 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	26,655.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Study Subject Payment	9,750.00
Total Other Direct Costs	**36,405.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**165,292.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Modified total direct cost	66.5	165,292.00	109,919.00
		Total Indirect Costs	**109,919.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**275,211.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: Core_B_Budget_Justification_Asthma_U19.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2018 **End Date*:** 06-30-2019 **Budget Period: 3**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	LAUREN	E	COHN		Project Lead		2.4			22,063.00	6,840.00	28,903.00
2.	Xiting		Yan		Co-Lead		2.4			21,636.00	6,707.00	28,343.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **57,246.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
3	Database Manager, Research Coordinator, Technician	7.8			57,640.00	17,868.00	75,508.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**75,508.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**132,754.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2018 **End Date*:** 06-30-2019 **Budget Period: 3**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2018 **End Date*:** 06-30-2019 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	21,785.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Study Subject Payment	9,750.00
Total Other Direct Costs	**31,535.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**164,289.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Modified total direct cost	66.5	164,289.00	109,252.00
		Total Indirect Costs	**109,252.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**273,541.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: Core_B_Budget_Justification_Asthma_U19.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2019 **End Date*:** 06-30-2020 **Budget Period: 4**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	LAUREN	E	COHN		Project Lead		2.4			22,725.00	7,045.00	29,770.00
2.	Xiting		Yan		Co-Lead		2.4			22,285.00	6,908.00	29,193.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **58,963.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
3	Database Manager, Research Coordinator, Technician	6.6			50,549.00	15,670.00	66,219.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**66,219.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**125,182.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2019 **End Date*:** 06-30-2020 **Budget Period: 4**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2019 **End Date*:** 06-30-2020 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	21,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Study Subject Payment	9,750.00
Total Other Direct Costs	**30,750.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**155,932.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Modified total direct cost	66.5	155,932.00	103,695.00
		Total Indirect Costs	**103,695.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**259,627.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: Core_B_Budget_Justification_Asthma_U19.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2020 **End Date*:** 06-30-2021 **Budget Period: 5**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	LAUREN	E	COHN		Project Lead		2.4			23,407.00	7,256.00	30,663.00
2.	Xiting		Yan		Co-Lead		3.6			34,430.00	10,673.00	45,103.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **75,766.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
3	Database Manager, Research Coordinator, Technician	6.0			47,406.00	14,697.00	62,103.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**62,103.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**137,869.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2020 **End Date*:** 06-30-2021 **Budget Period: 5**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2020 **End Date*:** 06-30-2021 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	18,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Study Subject Payment	2,000.00
Total Other Direct Costs	**20,000.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**157,869.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Modified total direct cost	66.5	157,869.00	104,983.00
		Total Indirect Costs	**104,983.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**262,852.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: Core_B_Budget_Justification_Asthma_U19.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

CORE B

BUDGET JUSTIFICATION

Key Personnel

Lauren Cohn, M.D., (Lead). Dr. Cohn is Associate Professor of Medicine, Section of Pulmonary, Critical Care and Sleep Medicine, Yale University School of Medicine. She has been Co-Director of the Yale Center for Asthma and Airways Disease (YCAAD) for the past 10 years. She has been integral to establishment of current protocols and workflow for subject recruitment and study visits, and is involved in all aspects of clinical and translational research in YCAAD. As Core B Lead, she will oversee the recruitment of subject and study visits, biological sample acquisition and processing. She will ensure that the database is secure and running smoothly and that the biostatistics core is accessible to all investigators. She will ensure the overall success of this project, from coordinating analyses with the research team to publishing the results. Dr. Cohn will dedicate 2.4 calendar months annually to this project. Note that Dr. Cohn spends 50% of her time as an attending physician at the Connecticut VA Health System.

Xiting Yan, Ph.D., (Co-Lead) Dr. Yan is Assistant Professor and Director of the Center for Pulmonary Personalized Medicine (P2MED) in the Section of Pulmonary, Critical Care and Sleep Medicine of the Yale School of Medicine. She was trained in statistics, computational biology and bioinformatics. She has specific expertise in transcriptomic analyses utilizing programs such as SAS, R, Genespring, Partek, MetaCore, and has designed a novel approach to hierarchical clustering analysis. Dr. Yan will be responsible for all aspects of biostatistical analysis and will work closely with investigators in Project 3 to integrate new analytic methods for these large data sets. Dr. Yan will devote 2.4 calendar months in Years 1-4, and 3.6 calendar months in Year 5, as more data will be available for analysis.

Non-Key Personnel

Naiqian Niu, M.D., (Technician). Dr. Niu is a Senior Research Associate, Pulmonary, Critical Care, and Sleep Medicine, Yale University School of Medicine. Dr. Niu is an accomplished cell and molecular biologist and member of the Pulmonary Section for 15 years. She has extensive experience in all the technical aspects required for these studies and has been processing samples for YCAAD for several years. Dr. Niu will be responsible for the processing of all biologic samples including isolation of cells and RNA to be sent for processing in the Precision Profiling Core. She will be responsible for tracking samples and entering laboratory sample data into the database. Dr. Niu will devote 3.0 calendar months annually for Years 1-3, and in as study visits decrease in Years 4 and 5, her annual effort will be reduced to 2.4 calendar months.

Haseena Rajeevan, Ph.D., (Database Manager) is Associate Research Scientist, Department of Anesthesia, Yale University School of Medicine. Dr. Rajeevan has a Ph.D. in atmospheric sciences and a master's degree in software engineering. She has a scientific focus in asthma and genetic variation related to atmospheric fluctuations. In addition, she has continuously improved the YCAAD database over the past 3 years and is working on software development to migrate data from the YCAAD database to OnCore, which will integrate clinical data with Epic, the Yale-New Haven Health System electronic medical record. In this project Dr. Rajeevan will be responsible for maintaining the database, including troubleshooting and repairing database problems, updating questionnaires, quality assurance checks, monitoring back-up systems, and she will manage and train Investigators, coordinators and laboratory personnel in the use of OnCore. Dr. Rajeevan will devote 1.2 calendar months annually.

Nicole Grant, M.S., (Research Coordinator). Ms. Grant is a Research Coordinator in the Section of Pulmonary, Critical Care and Sleep Medicine, Yale University School of Medicine. She assumes a prominent role in recruitment of subjects, protocol development and oversight of workflow for sample acquisition for the studies proposed in this grant and communicates regularly with Drs. Chupp and Cohn, laboratory research staff and other collaborators. Her duties encompass consenting subjects, conducting YCAAD phenotyping protocol visits including phlebotomy and sputum induction. Ms. Grant's effort in this proposal will be 3.6 calendar months in Years 1-3, then decline to 3.0 calendar months in Year 4, and 2.4 calendar months in Year 5 as study visits are completed.

Other Expenses

Materials and Supplies
The budget includes purchase of clinical supplies for sputum induction and blood drawing, laboratory testing, and other basic laboratory supplies.

Year 1: $37,049
Year 2: $26,655
Year 3: $21,785
Year 4: $21,000
Year 5: $18,000

Study Subject Payments
$9,750 is budgeted annually Years 1-4, and $2000 in Year 5 for payments to study subjects.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		297,060.00
Section B, Other Personnel		348,313.00
Total Number Other Personnel	15	
Total Salary, Wages and Fringe Benefits (A+B)		645,373.00
Section C, Equipment		0.00
Section D, Travel		0.00
1. Domestic	0.00	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		165,489.00
1. Materials and Supplies	124,489.00	
2. Publication Costs	0.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	0.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	41,000.00	
9. Other 2	0.00	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		810,862.00
Section H, Indirect Costs		539,223.00
Section I, Total Direct and Indirect Costs (G + H)		1,350,085.00
Section J, Fee		0.00

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

1. Project Director / Principal Investigator (PD/PI)

Prefix:
First Name*: GEOFFREY
Middle Name: L
Last Name*: CHUPP
Suffix:

2. Human Subjects

Clinical Trial? ● No ○ Yes
Agency-Defined Phase III Clinical Trial?* ○ No ○ Yes

3. Permission Statement*

If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?

○ Yes ● No

4. Program Income*

Is program income anticipated during the periods for which the grant support is requested? ○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

Budget Period*	Anticipated Amount ($)*	Source(s)*

PHS 398 Cover Page Supplement

5. Human Embryonic Stem Cells

Does the proposed project involve human embryonic stem cells?* ● No ❍ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s): ❑ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

6. Inventions and Patents (For renewal applications only)

Inventions and Patents*: ❍ Yes ● No

If the answer is "Yes" then please answer the following:

Previously Reported*: ❍ Yes ❍ No

7. Change of Investigator / Change of Institution Questions

❑ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:

First Name*:

Middle Name:

Last Name*:

Suffix:

❑ Change of Grantee Institution

Name of former institution*:

PHS 398 Research Plan

Please attach applicable sections of the research plan, below.

OMB Number: 0925-0001

1. Introduction to Application (for RESUBMISSION or REVISION only)	
2. Specific Aims	Core_B_Specific_Aims.pdf
3. Research Strategy*	Core_B_Research_Strategy.pdf
4. Progress Report Publication List	
Human Subjects Sections	
5. Protection of Human Subjects	Protection-HumanSubjects_Asthma_U19.pdf
6. Inclusion of Women and Minorities	Inclusion-Women_Minorities_Asthma_U19.pdf
7. Inclusion of Children	Inclusion-Children_Asthma_U19.pdf
Other Research Plan Sections	
8. Vertebrate Animals	
9. Select Agent Research	
10. Multiple PD/PI Leadership Plan	
11. Consortium/Contractual Arrangements	
12. Letters of Support	
13. Resource Sharing Plan(s)	ResourceSharingPlan_Asthma_U19.pdf
Appendix (if applicable)	
14. Appendix	

Specific Aims

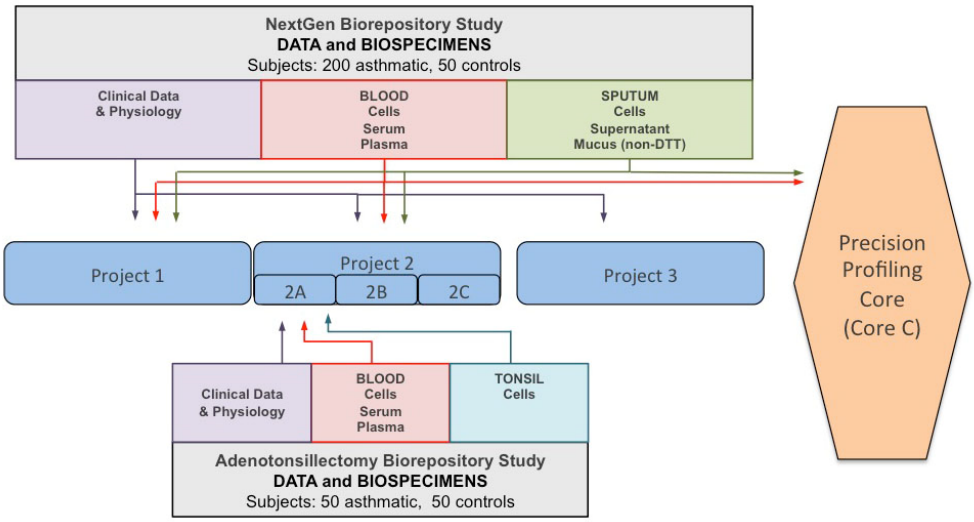
The Clinical Recruitment and Biostatistics Core (Core B) will serve this Program and contribute to each of the Projects though acquisition of clinical data and human samples, and provide database and analytic support. The Yale Center for Asthma and Airway Diseases (YCAAD) supports the infrastructure of a well-established, finely tuned clinical research center designed by Core Lead Cohn and Principle Investigator Chupp to obtain human data and biospecimens for airway diseases research. The clinical research tools developed over a decade in YCAAD include rapid recruitment from a large, urban population of asthmatic subjects with a range of disease activity and severity and a study visit that delivers high quality clinical and physiological data, and biospecimens including sputum and blood. Through this mechanism we have conducted human-based research that led to the discovery of genes and immunological pathways associated with asthma and developed novel methods to characterize disease heterogeneity using transcriptomic data to define endotypes of asthma. In *Next Generation Endotyping of Asthma Heterogeneity* we will take these studies further to define in-depth the airway single cell signatures, airway cell populations and immune pathways that exist in heterogeneous cohort of individuals with asthma. The Clinical Recruitment and Biostatistics Core (CRBC) is the starting point of the three driving Projects in this Program. Each Project will harness the strengths of this Core to obtain essential human data and biospecimens to conduct the proposed studies. The Clinical Recruitment Component of the Core will recruit and enroll asthmatic and control subjects to participate in the proposed clinical studies, obtain both clinical and physiological data, and biological samples, including sputum and blood and tonsillar tissue. The Biostatistical Component of the Core will support a custom built, web-based, HIPAA compliant database for data entry, biospecimen management and a portal for data retrieval and analysis, as well as extensive biostatistical support for analyses of the complex datasets generated in this proposal. Each of the Projects will take advantage of human clinical data and biological specimens obtained from the Core.

Aim 1: Clinical Recruitment Component: Recruit and acquire clinical data and human samples for proposed studies.

We will use an existing, highly efficient program in the Yale Center for Asthma and Airway Diseases to recruit subjects for clinical studies proposed by the three Projects in this U19 grant. NextGen will enroll 200 subjects with asthma and 50 control subjects to obtain extensive clinical data, perform lung physiology and acquire sputum and blood samples to investigate asthma heterogeneity using next generation sequencing and single cell technologies to. The Core will also support a study to obtain tonsillar tissue for immunological investigations into asthma heterogeneity. Samples obtained in the Core will be distributed to the Precision Profiling Core for next generation analyses and to investigators for customized testing.

Aim 2: Biostatistics Component: Management of Database and Biostatistical Support

The Projects will be supported by a custom-built, web-based, HIPAA compliant database for real-time clinical data entry, biospecimen tracking and recording of biological data. Biostatistical support for the projects includes association analyses and novel methods of analysis of pathways developed by biostatistician Dr. Xiting Yan. After research and development of novel computational analytic tools in Project 3, the Biostatistics Component will use these methods to analyze bulk and single cell RNA-Seq data generated from subjects in these clinical studies.



Core B: Clinical Recruitment and Biostatistics Core
Research Strategy

Significance
This U19 "*Next Generation Endotyping of Asthma Heterogeneity*" will harness the multidisciplinary expertise of Yale investigators in human asthma, immunobiology, genetics, computational biology and biostatistics to define further the pathobiology driving heterogeneity in asthma using novel immune-, microbiome- and gene expression-driven pathways. The Clinical Research and Biostatistics Core (Core B) will be responsible for providing samples, clinical data and biostatistical support for the clinical trials proposed herein. The Core has two distinct components, a Clinical Research Component that will be responsible for subject recruitment, clinical data and biological sample collection and a Biostatistics Component that will oversee data management and biostatistical analyses of clinical, biological and genomic data. Figure 1 shows how Core B will interact with each Project to provide essential services and biological samples to the investigators to accomplish our goals.

Benefits of a Shared Core
Each investigator has proposed human studies designed to understand the heterogeneity of asthma using next generation and single cell technologies. This Core serves as the starting point to obtain human data and biologic samples for the proposed studies. This Core has well-trained personnel and a fifteen-year track record with the capability to recruit subjects, obtain data and process human samples of high quality adequate for the proposed analyses and an integrated database and asthma-focused biostatistics program. This Core will allow investigators in pulmonary, immunobiology and computational sciences access to human data and biological samples to focus their scientific expertise in the study of human asthma.

Qualifications of the Core Lead and Personnel
Core Lead Cohn is an experienced translational investigator with a clinical and research focus in asthma. She is Co-Director of the Yale Center for Asthma and Airway Diseases (YCAAD). She has been active for the past ten years in subject recruitment for the YCAAD phenotyping protocol. Dr. Cohn's research in YCAAD is focused on lung immunology, mucus and the airway epithelium. Dr. Cohn manages personnel in the YCAAD laboratories and oversees processing of samples. She has been a key investigator leading to publications generated from YCAAD data. She has an active clinical practice in asthma in YCAAD and the Connecticut VA Health System. **Dr. Yan** is director of the Center for Pulmonary Personalized Medicine (P^2MED). She has worked with YCAAD investigators for eight years. She is trained in statistics and computational biology and bioinformatics and is expert in standard data analysis, design of novel computational models and she understands basic biology. Through her work in YCAAD she developed a novel unsupervised clustering approach to identify clinically relevant and validated subtypes of asthma from genomics data by integrating known biologic pathway information. Her expertise in endotypes of asthma is essential as she manages the biostatical analyses in Core B. **Dr. Rajeevan** has been administrating and enhancing the YCAAD database for the past 3 years and is actively engaged in migrating the database to OnCore to connect with the hospital electronic record system. For the proposed studies, her expertise will be crucial to expand the database to accommodate the new and larger datasets and expanded workflow. Ultimately, future studies will leverage this powerful database in combination with disease endotypes to advance the study of precision medicine (1).

Preliminary Studies
YCAAD Recruitment and Phenotyping Capabilities. The Yale Center for Asthma and Airways Diseases is part of the Advanced Lung Disease Program in the Section of Pulmonary, Critical Care and Sleep Medicine at Yale University School of Medicine and at Yale-New Haven Hospital. YCAAD delivers cutting-edge clinical care to the full spectrum of asthmatic patients and conducts clinical and translational research studies. Under the direction of Drs. Chupp and Cohn, YCAAD is the only dedicated adult asthma center between New York and Boston, with approximately 3000 patient visits per year. Fifteen years ago, with funding through the Yale Center for Clinical Investigation (YCCI), we established the YCAAD phenotyping protocol to collect airway and blood samples from subjects with airway disease for human-based pathogenesis research. Patients are recruited predominantly through YCAAD from our own group practice. Through a clinical protocol entitled, *Mechanisms and Mediators of Asthma and Chronic Obstructive Pulmonary Disease (COPD)* (Yale HIC 0102012268), asthmatic and non-asthmatic subjects participate in a 2-hour study visit, involving collection of clinical and physiologic data and biologic specimens (DNA, RNA, serum, plasma, sputum cell pellets and cell cytospins). Blood and induced sputum samples are processed to characterize cell populations and isolate RNA and DNA for genomic profiling. With funding through the NIH, FAMRI and other sources, our phenotyping protocol has now enrolled over 800 subjects ranging in age from 12 to 90 years. As of July 2015, 412 asthmatic and 52 control subjects completed the YCAAD phenotyping protocol that includes sputum induction.

The YCAAD population characteristics are shown (Table1). This cohort is similar to other urban asthma populations: predominantly female, racially mixed, overweight, and includes subjects with a spectrum of disease severities. High quality RNA is generated from sputum from these subjects, as shown (RNA integrity number (RIN) > 7.0.). This high-throughput translational research pipeline has led to publications highlighting novel pathways that define severity in asthma, including YKL-40 and a novel transcriptional signature in cells from blood and sputum (2-7).

YCAAD, in collaboration with Dr. David Karas, a Yale-associated otolaryngologist, created a second biorepository of blood, adenoid and tonsil tissue to study biological pathways in asthmatic children undergoing adenotonsillectomy (*Correlation of Gene Expression Profile with Clinical Response to Adenotonsillectomy,* Yale HIC 1009007345). The characteristics of this population, the "adenotonsillectomy cohort," show a young, racially mixed population and includes a large control group without asthma (Table 2). These data show a significant improvement in asthma control in children after tonsillectomy, a significant drop in serum YKL-40 and cytokines, including IL-5 (8).

These ongoing YCAAD clinical protocols are the mechanisms by which new subjects will be enrolled in the proposed clinical studies in Projects 1, 2, and 3. Our publications and recruitment data show that we have the capacity to enroll, phenotype and acquire adequate biological materials for the proposed studies for all Projects. The proposed studies will be possible through recent efforts of the YCAAD research team to develop and refine protocols for these studies and through availability of technologies that require very low sample volumes and/or cell numbers.

Table 1. YCAAD Cohort

	Control	Asthma
Prevalence (N)	56	412
	42.1 ±	49.1 ± 14.2
Age at Visit (years)	14.7	
Female sex, N (%)	37 (66)	293(71)
Race		
White - N (%)	51 (91)	282 (68)
Black - N (%)	1 (2)	80 (19)
Other - N (%)	4 (7)	50 (12)
Hispanic Origin - N (%)	6 (11)	51 (12)
BMI (Kg/m^2)	26.5 ± 4.6	29.9 ± 7.6
History of Atopy - N (%)	26 (46)	362 (88)
History of Hospitalization - N (%)	1 (2)	180 (44)
History of Intubations - N (%)	0 (0)	53 (13)
OCS tapers in past year- N (%)	1 (2)	230 (56)
ACT Score	NA	16.3 ± 5.7
ICS use yes or no – N (%)	0 (0)	301 (73)
	0 (0)	502.5 ±
ICS dose per day (µg)		432.3
Chronic OCS use (%)	0 (0)	32 (8)
FEV1- % of predicted value		
Pre β_2 agonist use	96 ± 13	76 ± 22
Post β_2 agonist use	100 ± 15	83 ± 21
	78.25 ±	113.02 ±
Sputum Characteristics		
Mucus Cell Concentration	129.14 [P]	175.41 [P]
Squamous (%)	25.5 ± 25.1	16.2 ± 20.1
Viability (%)	56.4 ±	57.0 ± 19.9

YCAAD Biostatistical Expertise. Dr. Xiting Yan is a longstanding member of the YCAAD research team with expertise in statistics and computational biology and bioinformatics. Dr. Yan is a Yale Center for Clinical Investigation (YCCI) Scholar with a focus on defining endotypes of lung disease to improve and "personalize" the approach to management of chronic lung diseases. Dr. Yan received her first Ph.D. in applied statistics from Peking University in China in 2006. During this training, she was introduced to all the challenges of biostatistics and recognized that she wanted additional training to be at the cutting-edge of translational research in disease pathogenesis. This led to her second Ph.D. in computational biology and bioinformatics. In collaboration with YCAAD investigators Dr. Yan developed a novel clustering method in asthma, based on pathways instead of individual genes (9). The TEA clusters are detailed further in Project 1. This clustering method is knowledge-based and focuses on the genes that have been assigned to known biological pathways defined in Kyoto Encyclopedia of Genes and Genomes (KEGG). This reduces the number of genes analyzed from ~22,000 to ~5,500, which significantly reduces the "background noise" gene expression signals. Dr. Yan brings her expertise in biostatistics and computational biology of asthma to the Biostatistics Component of Core B.

Table 2. Adenotonsillectomy Cohort

	Controls	Asthma
Prevalence (N)	54	100
Age Mean [Range]	5.7 [2-18]	6.5 [2-17]
Male Gender (%)	50	63
Race (%)		
White/Caucasian	72	77
African American	26	17
Asian	2	0
Other	0	6
Latino Ethnicity (%)	21	44
BMI Percentile		
Mean [Range]	68 [0.2-99]	65 [0-100]
Location (%)		
Rural	15	12
Suburban	59	51
Urban	26	36
Declined	0	1
Second Hand Smoke	29	31
Asthma Comorbidities (%)		
Sinusitis	20	39

Aim 1. Clinical Recruitment Component: Recruit and acquire clinical data and human samples for proposed studies.

Overview:

- **NextGen Biorepository Study**: 200 new asthmatic and 50 control subjects will be recruited for a longitudinal study with 3 visits. On the initial visit each subject will participate in the YCAAD

phenotyping protocol (detailed below) and on 2 follow-up visits at 9 and 18 months and will have re-assessments of physiology, collection of biologic samples and updates in medical history.

- **Adenotonsillectomy Biorepository Study**: 50 asthmatic and 50 control subjects will be recruited to obtain tonsil tissue.

A. Study title: NextGen Biorepository Study

1.Study objectives: To collect clinical data and human sputum and blood from a heterogeneous cohort of individuals with asthma to define in-depth the airway single cell signatures, airway cell populations and immune pathways that define endotypes of disease.

2. Study population and design: We will enroll **200 subjects (≥12 years old) with asthma and 50 non-asthmatic control subjects** to participate in NextGen (Yale HIC 0102012268). Each subject will return for 2 follow-up visits to establish longitudinal stability of our findings in cluster, single cell, RNA-Seq, and cellular subpopulation analyses and for customized functional studies developed by each of the projects based on the baseline dataset analysis. Sample size calculations for the projects are outlined below. Adults and children (≥ age 12 years) with asthma and non-asthmatic controls will be recruited from the population in New Haven and the surrounding region through the YCAAD and the NetHaven Practice-Based Research Network (PBRN) of primary care practices, a network of approximately 30 adult primary care, adolescent, and family medicine clinicians who care for an estimated 600-800 individuals with asthma with a commitment to community-based clinical/translational research. Print, radio, and internet advertising are also leveraged on a routine basis to enhance enrollment into this YCAAD clinical trials. In the Greater New Haven area, over 9% of 427,000 adults suffer from asthma, and this population has been adequate to provide a continuous stream of subjects for YCAAD studies for the past 10 years. YCAAD has enrolled over 800 subjects into its asthma cohort. These subjects are immediately available for enrollment into this study.

Enrollment Criteria:

- Inclusion Criteria
  1) Clinician diagnosis of asthma based on NAEPP guidelines
  2) age ≥ 12 yrs
  3) historical evidence of variable airflow obstruction determined by: (a) >12% improvement in FEV_1 after an inhaled β_2 agonist, inhaled corticosteroids or an oral prednisone burst; (b) methacholine reactivity with a provocative concentration causing a 20% decrease in FEV_1 (PC20) of <8 mg/ml; or (c) 20% diurnal variation of peak expiratory flow rates on 2 days over a 2-3 week period.
- Exclusion Criteria:
  1) >10 pack-year smoking history
  2) active smoking within the past year
  3) other chronic lung disease, asthma variant (e.g. chronic obstructive pulmonary disease, allergic bronchopulmonary aspergillosis, etc.)
  4) patients will be excluded if they have other severe chronic conditions (CHF, renal failure, liver disease, chronic viral infections) or cannot safely undergo the studies required for participation
  5) inability to generate an adequate sputum sample at the initial visit.

3. Data and sample acquisition. After informed consent, subjects will be administered a questionnaire by the study coordinator (see Appendix), Ms. Grant, that includes a detailed asthma and medical history, asthma activity questionnaire (Asthma Control Test), Asthma Quality of Life Questionnaire, medication history, smoking and smoke exposure history, occupational and environmental history, allergy history, sinus disease history, gastroesophageal reflux history, obesity history, and medication compliance history. Inhaled corticosteroid dose will also be documented using a dose equivalent conversion to fluticasone proprionate to allow for ICS use-comparison among subjects (see Appendix). All data will be entered in real-time, using an encrypted iPad, into the YCAAD web-based database/OnCore (see Database, below). Following the interview, blood is drawn and processed for isolation of DNA, RNA (Tempus RNA isolation tubes, Applied Biosystems), serum and plasma. Additional samples are drawn and sent to the clinical laboratory for CBC and differential, serum IgE level, ImmunoCAP testing for 22 common aeroallergens and routine chemistries. Following phlebotomy, patients undergo FeNO testing and spirometry in adherence with American Thoracic Society guidelines before and after short-acting bronchodilator administration. Then, airway samples will be acquired with nebulized hypertonic saline to induce sputum samples containing mucus plugs, as previously described. Typically, 75% of subjects generate high quality sputum samples. Subjects unable to provide an adequate sputum sample on the initial visit will not be enrolled, but will have 2 opportunities to return for sputum induction within 4 weeks of the initial visit. Sputum samples are processed by removal of mucus plugs using a dissecting microscope and washed to remove squamous cell contamination. Typical samples are 300-400 mg and 200 mg is used for RNA, thus one sample typically supplies adequate material for all of the

studies listed. The mucus plug will be cut into two parts (Figure 2). The first 200mg will be processed to separate the cellular and aqueous compartments using DTT. The aqueous phase will be aliquotted and stored at -80. A small number of cells will be retained for a cell differential and viability count (DiffQuik, trypan blue exclusion). The remaining cells will be delivered to the Precision Profiling Core (CoreC) for processing for RNA isolation and preparation for CyTOF or single cell capture on the Fluidigm C1 apparatus (see Precision Profiling Core C). The second portion of the mucus plug will be frozen and distributed to Project 2C for analysis of sputum microbiome, since use of DTT destroys the binding of IgA to bacteria. Poor sample quality is determined by contamination with greater than 20% squamous cells (approximately 20% of sputum samples acquired) and poor quality RNA (approximately 20% of sputum samples processed). Samples contaminated with squamous cells will not be processed further for bulk RNA or microbiome analysis since these techniques cannot eliminate the "noise" contributed by oral contamination. With our new technologies, these lower airway mucus samples can be retained for single cell analysis since both the CyTOf and Fluidigm allow gating out of contaminating cells, Fluidigm by size, CyTOF by surface markers. After processing, typically 20% of YCAAD sputum samples result in inadequate quality RNA with a RIN < 4.0. Yet, for RNA-Seq of single cells, we expect the loss of samples to be lower as described in Core C. Overall, based on our past experience and new technologies that will allow use of samples previously unusable, we expect to have loss of at most 40% for standard bulk RNA processing and much lower for the single cell methods proposed. Therefore, we reasonably predict a maximum loss of 25% of sputum samples. We can expect, therefore to have 150 asthmatic and 37 control samples from an enrollment of 200 asthmatic and 50 control subjects. We expect that there may be more than these predictions, as a large proportion of samples will be subjected to single cell RNA-Seq and CyTOF.

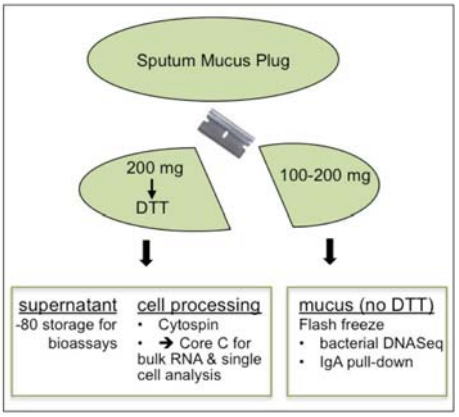


Figure 2. Asthma U19 mucus sample allocation

4. Study duration and timeline. Asthmatic and control subjects will have three study visits: An initial visit, and 2 follow-up visits at 9 and 18 months. Follow-up visits will be scheduled at 9 and 18 months, with a window of ± 1 month, and will include re-assessments of physiology, collection of biologic samples and updates in medical history. At times, when necessary for additional biological studies, subjects may be invited to return to have re-sampling of blood or sputum for functional studies as needed by individual investigators. Based on our experience, approximately 75% of subjects will generate sputum samples with a mucus plug of adequate quality for processing. Subjects will not be enrolled in NextGen unless an adequate sputum sample is generated, but will have a four-week rescreen window. Subjects unable to produce adequate sputum samples at subsequent Visits 2 or 3 will also have a window of four weeks to return for sputum sampling. Subjects will be enrolled in NextGen in the first 24 months of funding. The final study visits will be completed by month 42 of the funding period.

5. Sample size: Project 1. *Single Cell Mechanisms of YKL-40 and Transcriptomic Endotypes of Asthma***.** 200 asthmatic individuals will be recruited during the course of this proposal for the studies outlined in Project 1 to characterize the immunobiology of the YKL-40 endotypes and TEA clusters. For the analysis of clinical features including, mucus cell concentration, cell viability, post-bronchodilator FEV1/FVC ratio, and bronchodilator response, and cytokine levels, non-parametric tests will be used such as Mann-Whitney U and Kruskal Wallis tests will be used, as the data is usually not normally distributed. We calculated power for gene expression studies because these analyses require the largest sample size to achieve a genome-wide false discovery rate probability <0.05 (Table 1). Assuming that genes less than 2-fold change between two groups are regarded as "no change," Sizepower (R package) was used to calculate the power with various values of the parameters – false discovery rate (FDR) and the anticipated standard deviation (SD) of the difference in log-expression between groups. The SD of sputum gene expression presented above ranges from 0.4-0.6. FDR is controlled by the mean number of false positives, fixing the anticipated number of genes in an experiment that are not

Sample Size	SD	Table 3: Power for gene expression For genome-wide FDR				
		0.05	0.1	0.15	0.2	0.3
50	0.5	100	100	100	100	100
	0.75	99.99	100	100	100	100
	1.00	98.42	99.13	99.41	99.56	99.72
	1.25	87.46	91.56	93.54	94.76	96.21
	1.50	68.49	76.10	80.25	83.02	86.62
75	0.5	100	100	100	100	100
	0.75	100	100	100	100	100
	1.00	99.95	99.98	99.99	99.99	100
	1.25	97.97	98.86	99.22	99.41	99.63
	1.50	89.08	92.77	94.52	95.58	96.84
100	0.5	100	100	100	100	100
	0.75	100	100	100	100	100
	1.00	100	100	100	100	100
	1.25	99.75	99.88	99.92	99.95	99.97
	1.50	98.17	98.17	98.72	99.02	99.36

differently expressed at 10,000. Table 1 demonstrates that we have adequate power for an FDR of 0.05 if we generate bulk RNA-Seq from 150 sputum samples in the YCAAD biorepository to analyze. Bivariate correlations among continuous variables will be determined using Spearman rank correlation, and multiple comparisons will be controlled using the Hochberg method (10).

Project 1 has the largest sample size, therefore 200 asthmatic subjects are adequate to serve the Projects with clinical data and biospecimens. Projects 2 B and 2C each require non-asthmatic control samples and **50 control subjects** are estimated to serve the statistical needs of both of these projects. Samples size calculations for each Project in NextGen are detailed in the text of the Projects.

B. Study Title: Adenotonsillectomy Biorepository Study

1. Study objectives: To obtain clinical data, tonsil tissue and blood to determine the role of follicular B helper T (Tfh) cells in driving pathogenic IgE responses in endotypes of asthma.

2. Study population and design: 50 subjects with asthma and 50 non-asthmatic controls will be recruited to participate in the *Correlation of Gene Expression Profile with Clinical Response to Adenotonsillectomy* (Yale HIC 1009007345). A sample size calculation for this clinical study is below. Pediatric patients ages 2–18, with and without asthma, who are undergoing adenotonsillectomy for standard indications at Yale New Haven Children's Hospital and North Haven Surgery Center will be recruited. Informed parental consent and child assent (patients age ≥ 7) will be obtained according to protocol. On the day of surgery, a study member will administer a questionnaire to the subject's parent. Definition of asthma will be based on a previous physician diagnosis prior to surgery. The YCAAD pediatric questionnaire is a modified version of the YCAAD adult questionnaire (see Appendix). Children age 6 and above will attempt spirometry if they are able to comply with the instructions. Intraoperatively, the anesthesiology team will obtain venous blood. Tonsils will be removed and excess tissue, after samples are sent to pathology, will be allocated. Tonsil tissue and blood will be picked up by Core B staff, and fresh blood and tonsil tissue will be distributed to Project 2A (Craft) for processing and analysis. Total IgE and Immunocap for aeroallergens will be measured on serum samples. Excess tissue will be processed for RNA, and from blood, serum, plasma and RNA will be stored as described previously (8).

3. Study duration and timeline: Subjects will be recruited over a 48-month period.

4. Sample Size: Project 2A. *T follicular helper cells and IgE in endotypes of asthma.* 50 asthmatic and 50 control subjects will be enrolled in this study. For the objective to compare the Tfh cell proportion between asthmatics and controls, if there are 50 samples per group and we assume that both groups have the same standard deviation (denoted by SD) across samples, there is an 80% power to detect a minimum difference of 0.57xSD between the mean of the two groups at the 0.05 statistical significance level. Preliminary data from a previous study estimated the standard deviation of the CD4+CXCR5+ T cell frequency to be 0.8, 4.1 and 5.0 in the healthy controls and mild asthma patients, respectively (11). Therefore, using the conservative estimation of SD=5.0, we have 80% power to detect a minimum difference of 2.85 between the two groups. The preliminary data also suggested a difference of 7.8 between healthy controls and mild asthma, and 14.5 between healthy controls and severe asthma, which are both much higher than the minimum detectable difference. Therefore, we have enough statistical power to identify the difference of CD4+CXCR5+ T cell frequency between asthmatics and controls. For the objective to correlate the total IgE level with the number of CD4+CXCR5+ T cells, preliminary data from the previous study suggested a Pearson correlation coefficient of 0.631. Based on this preliminary estimation of the correlation coefficient, if there are 50 asthmatic samples, we will have 99.99% statistical power to identify the suggested correlation at the 0.05 significance level.

Sample Distribution (Figure 1): Samples will be distributed by Dr. Niu to investigators and Core C. Samples for bulk RNA, CyTOF, Fluidigm (Single cell RNA-Seq) will be delivered to Core C. Other distributions include: To Project 2A (for analysis of Tfh cells), Whole blood from NextGen and from adenotonsillectomy study, whole blood and tonsil tissue; to Project 2B, aliquots of plasma for measurement of Dkk-1; to Project 2B, sputum samples (non-DTT-treated). To Projects 1 and 2B, distribution of sputum cells to activate prior to CyTOF analysis.

Potential problems and alternate approaches: We expect to be able to meet enrollment in the NextGen trial given that YCAAD has met enrollment of this magnitude in the past. One limitation to enrollment is the ability to provide an adequate sputum sample containing a mucus plug. We will allow subjects to return for 2 repeat attempts, and in in the hands of our experienced staff of coordinators, this typically results in obtaining a good sample. Poor quality sputum samples have less bearing in these studies, as the majority of samples will be sent for single cell analysis and this permits sputum with squamous cells to be used. If we encounter problems with inadequate samples due to splitting samples for the microbiome studies in Project 2C, we will attempt to reduce the mass of sputum directed to them. As indicated in Project 2C, they will be testing if smaller amounts

of mucus can be used, since they had more than adequate bacteria in the samples used for preliminary studies.

Aim 2. Biostatistics Component: Management of Database and Biostatistical Support
The Biostatistics Component of this U19 is funded through this Core and will manage both the database and provide biostatistical support for the projects. It serves as a crucial resource for both the Clinical Research Component of Core B and the Precision Profiling Core (Core C) integrating analyses of clinical data and complex data sets of the genomic and single cell data using algorithms developed in Project 3 and delivered to Core C.

2A. Database management and availability for use by all YCAAD team members. YCAAD developed a customized, web-based informatics platform that was constructed to handle the high flow of subjects and data required for high frequency of enrollment, data acquisition and biospecimen tracking. This is a secure, HIPAA-compliant, web-based portal that allows program personnel, including research coordinators, laboratory technicians, genomics specialists, and biostatisticians, the ability to upload and download de-identified clinical, laboratory, and genomic data from different locations. Clinical data is directly entered into the system at the time of the phenotyping visit from a HIPAA-compliant, protected and encrypted iPad. Experimental results are uploaded by laboratory scientists, and a customized query application easily downloads data files with the requisite clinical phenotype and experimental data in any desired file format. This system has greatly increased the pace of scientific discovery and resource sharing.

To advance the capabilities of the YCAAD database to interact with electronic medical record in the Yale New Haven Hospital (YNHH) network and enhance subject data extraction in quantity and quality, we are in the process of migrating the database to the OnCore system. YCAAD will be active in OnCore at the time of funding of this program. OnCore is a suite of clinical and translational research modules consisting of software for research, patient registry and biospecimen management that is integrated with Epic, the electronic medical record of the YNHH network. Clinical data for the NextGen study and the adenotonsillectomy studies will be entered will be entered directly into OnCore by study coordinators using a web interface that identifies users by Yale NetID and password. The 'Study_Number' and 'Visit_Date' makes up the unique identifier that will be used throughout the system for a subject visit. Dr. Niu in the laboratory will access the biospecimen module and use the unique identifier to store, track, monitor, and enter information relevant to individual samples. OnCore is not equipped to store high throughput data such as gene expression, protein levels or genotype data. This data is handled by the Yale Center for Genomic Analysis, directed by Co-Lead Shrikant Mane (See Precision Profiling Core C). Therefore, code has been written so that every night data will be extracted from OnCore into the YCAAD server so that all of the research data can be managed from one database. During this process, data quality is checked and if errors are identified, error emails are sent to YCAAD research coordinators. This feature will significantly enhance data quality and minimize data cleaning requirements and downstream issues with the data. Investigators in this U19 program, including project and core leads and biostatisticians will have access to de-identified data in the YCAAD database for analysis functions.

2B. Biostatistical Support. Biostatistical support includes a broad expertise in applied and theoretical computational biology. Dr. Yan will work with investigators to find associations between molecular signals and asthma clinical features, as described above. She will perform advanced analyses of complex data sets generated and help with modeling for the Asthma MAP project. The specific analyses planned for these studies also includes a) Assignment of TEA clusters to the samples in NextGen for Project 1. b) Analyses to define associations of Tfh cells (Project 2A) and Dkk-1 (Project 2B) with clinical and genomic data. c) Microbiome/IgA coated differentiation analysis (Project 2C). d) Analysis of bulk and single cell RNA-Seq data from NextGen for all studies.

Potential problems and alternate approaches: The YCAAD database has now been in use for more than a decade and is constantly being updated and upgraded to meet the needs of YCAAD. The migration to OnCore is proceeding rapidly now and we expect it to be active at the time of funding of this U19. If there are delays, we will continue to use the current database. Such an eventuality would only limit laboratory data capture, such as CBC and IgE from Epic, thus does not impair our ability to proceed with these studies. If at any time the statistical issues stretch beyond the expertise of Dr. Yan, there is extensive biostatistical, informatics and computational support at Yale, beyond our Core. Then, we will request assistance through the Yale Center for Clinical Investigation (see facilities) where Dr. Yan is a Scholar and has many close contacts, the Yale Center for Statistical Genomics and Proteomics and its director Dr. Hongyu Zhao, a collaborator on many past projects and former mentor of Dr. Yan, and the Yale Center for Genome Analysis and Dr. Shrikant Mane, a co-lead of Core C.

Protection of Human Subjects

Risks to Human Subjects

a. Human Subjects Involvement and Characteristics. Over the course of this project, we plan to recruit 200 asthmatic and 50 non-asthmatic subjects through the Yale Center for Asthma and Airway Diseases (YCAAD). Each subject will return approximately every 9 months and complete 3 visits over the 5-year grant period. At each of the 3 visits the subject will be administered a questionnaire and perform physiological testing, and blood and sputum samples will be obtained. Asthmatic and non-asthmatic subjects recruited for these studies will be 12 years old or greater. Through a distinct HIC protocol, for these studies we will also recruit asthmatic and non-asthmatic children (age 7-17 years old) undergoing adenotonsillectomy, to donate tonsillar tissue, blood samples, and nasal brushings to the YCAAD biorepository.

Drs. Cohn and Chupp have recruited subjects for the past 15 years through the *Mechanisms and Mediators of Asthma and Chronic Obstructive Pulmonary Disease (COPD) human investigation* protocol (Yale HIC 0102012268, PI Chupp, Co-I Cohn). Over 800 subjects have been recruited to date. Tonsillar tissue will be obtained as part of the *Correlation of Gene Expression Profile with Clinical Response to Adenotonsillectomy* protocol (Yale HIC 1009007345, PI Karas, Co-I Chupp). This is a repository is to investigate the relationship between adenotonsillar hypertrophy and asthma and airway diseases, and identify genes, molecules and pathways that are associated with disease development, progression, and severity.

YCAAD Study Protocol: Exclusions will be based on health considerations. We will exclude any patient with a contraindication to any of the required testing including sputum induction, spirometry, or phlebotomy. These exclusions include severe cardiovascular, renal, or liver disease, bleeding diathesis, or advanced neuromuscular disease. A centerpiece of the YCAAD translational research program and this proposal is the airways disease phenotyping protocol developed to study human asthma. This protocol has been developed to acquire the full complement of clinical information, physiologic measurements and biologic samples to perform genotype-phenotype studies. Following informed consent, subjects are administered a questionnaire by the study coordinator. This includes a detailed asthma and medical history, asthma activity questionnaire (Asthma Control Test), Asthma Quality of Life Questionnaire, and details about medications, histories of smoking, occupational and environmental exposures, history of allergy, sinus disease, gastroesophageal reflux, obesity, and medication compliance. All data is uploaded into a HIPAA compliant web-based database developed by YCAAD. Blood is drawn and processed for the isolation of DNA, RNA, serum and plasma. Samples are aliquoted and banked for future studies. Blood is sent to the clinical laboratory for CBC and differential, serum IgE level, ImmunoCap testing for 22 common aeroallergens and routine chemistries. Following phlebotomy, patients undergo FeNO testing and spirometry (in adherence with ATS guidelines) before and after short acting bronchodilator administration. Sputum induction is done using hypertonic saline and immediately processed. Mucus plugs are removed using a dissecting microscope to remove squamous cell contamination, the cellular and aqueous compartments are separated, cell counts and differentials are quantified, and aliquots of supernatants, cell protein, and cellular RNA are stored for later analysis. The protocol is practical, efficient, and enrolls large numbers of subjects in a timely fashion. We will phenotype up to 5 subjects weekly. Subjects will be scheduled to return for repeat sampling as described, which is important for determination of the stability of their disease and their biological samples.

Asthma Inclusion criteria. Recruitment through YCAAD is designed to enroll a wide-range of asthmatic subjects in terms of age, disease severity and ongoing treatment. Inclusion criteria for asthmatic subjects are: (1) ≥ 12 years of age; (2) < 10 pack years of tobacco, and have not smoked for ≥ 1 year. Asthmatic subjects must have a diagnosis of asthma defined by the following criteria (based on the National Asthma and Education and Prevention Panel Guidelines): (1) a history of chronic symptoms (>2 years) compatible with asthma (determined by a study investigator/pulmonary physician specializing in asthma); and (2) historical evidence of variable airflow obstruction determine by: (a) > 12% improvement in FEV_1 after an inhaled $beta_2$ agonist, inhaled corticosteroids, or an oral prednisone burst; (b) methacholine reactivity with a provocative concentration causing a 20% decrease in FEV_1(PC20) of <8 mg/ml; or (c) 20% diurnal variation of peak expiratory flow rates on 2 days over a 2-3 week period. Patients will be excluded if they cannot safely undergo the studies required for participation or have other chronic lung disease or asthma variant disease (ABPA, Churg-Strauss Syndrome, vocal cord dysfunction, etc.). Normal subjects must have no history of any chronic lung disease. Non-asthmatic control subjects will be excluded if they have a diagnosis of any ongoing acute or chronic lung disease, have smoked ≥10 pack-years or have abnormal spirometry.

Adenotonsillectomy Study Protocol. Parents and patients identified by providers as potential subjects will be

invited to participate in the research study after they have been consented independently for their planned procedure of adenotonsillectomy. The parent and child will complete a YCAAD pediatric questionnaire and an asthma activity score, and spirometry will be done for those children aged six years or older who are capable of completing the test. Intraoperatively, a venous blood sample (5-10ml) and nasal swab sample will be obtained. After a portion of the tissue is sent to surgical pathology, as is standard for adenotonsillectomy, a portion of the removed tonsils and/or adenoid tissue will be taken for study purposes. An additional specimen will be held in the pathology department, flash frozen in OCT in the event more tissue is needed for analysis. Samples will be processed and banked with the assigned de-identified study subject number. A second study visit will take place six months following surgery and includes an interim history using the follow-up questionnaire focused on stability of upper airway and asthma symptoms. Repeat PFT testing will be performed in children aged six years or older, if possible. An additional venous blood sample will be obtained for the repository. Subjects may decline any of the testing at any of these visits.

b. Sources of Materials. In this proposal, the following materials will be collected from human subjects: clinical data (questionnaire, history and physical examination data, lung function data and relevant laboratory test results), blood, sputum, nasal brushings and tonsils/adenoids. Clinical data will be identified with the study subject's name. This information will be maintained in a locked file and these records are kept separate from other clinical records to assure confidentiality. Only Drs. Cohn, Chupp, Niu, and study coordinators will have access to these confidential records. All research specimens, including blood, and sputum specimens are identified by a patient code for correlation purposes. All samples and datasets will be de-identified and only known to study investigators involved in consent and sample acquisition. Investigators and staff will receive only de-identified data and samples for their studies and analyses. All data sets uploaded and samples delivered to Core C and the Yale Center for Genomic Analysis will be de-identified.

The studies in this proposal will adhere to the U.S. Department of Health and Human Services Office for the Protection from Research Risk (OPRR). All information relating to participating subjects will be de-identified. Specimens may only be used in accordance with the conditions stipulated in the Yale Human Investigation Committee (HIC) (see approval letters in Appendix).

c. Potential Risks. Risks associated with the YCAAD study (HIC 0102012268) including pulmonary function tests, blood draw and sputum induction are minimal (adults) and greater than minimal (children 12-17 years old) and are explained to subjects in detail at the time of consent (see consent forms in Appendix). Bronchospasm is a risk of sputum induction, but the safety of sputum induction in asthmatic subjects, even those with severe asthma has been well documented in several studies (ref). Spirometry is repeatedly tested during the procedure to monitor for bronchospasm, and if wheezing or a significant drop in FEV1 occurs, subjects are given bronchodilators. Wheezing, if it occurs, is usually transient and treatable with bronchodilators. A Data and Safety Monitoring (DSM) Plan and DSM Committee are in place to assess adverse events (see Appendix). To date, we have had no serious adverse events in our laboratory, even in severe asthma subjects undergoing sputum induction. Clinical Recruitment and Biostatistics Core Lead (Dr. Cohn) or a research coordinator will monitor all procedures performed. After any procedure performed in YCAAD, subjects are instructed to contact the YCAAD on-call physician if any problem occurs. YCAAD is covered 24 x 7 by pulmonary physicians who are familiar with the management of acute bronchospasm in asthmatic patients. Risks associated with the adenotonsillectomy study (Yale HIC 1009007345) are minimal for the blood draw and there is no added risk of apportioning adenoid and tonsil tissue to the biorepository. Emergency equipment and personnel are immediately available in the event of an adverse reaction at all times during these study visits.

Adequacy of Protection Against Risks

a. Recruitment and Informed Consent. Subjects will be recruited from YCAAD and from the pediatric ENT offices of Dr. Karas. Once a subject is deemed eligible for this study, informed consent will be obtained. The study procedures, risks and benefits will be clearly explained to each subject. All questions will be answered, and time will be allowed for the subject to make an informed decision before signing the consent form. If the subject agrees to enroll, a consent form (see Appendix) will be reviewed and explained, and the subject or consenting adult will be asked to sign it.

b. Protections Against Risk. Risks will be minimized by appropriate subject exclusions, close medical supervision throughout the protocol, and adaptation of testing to identify patients who might be at higher risk. Subjects experiencing adverse events will have access to full inpatient medical facilities of the Yale-New

Haven Hospital if more intensive treatment is necessary to reverse an asthma attack or complication during the study. All patients will be under full-time coordinator supervision throughout every phase of the YCAAD procedures. In the adenotonsillectomy protocol, at least one physician and/or nurse will be present throughout the study visits and procedures. Adverse events will be reported to the Yale IRB (Human Investigation Committee) and the DSMC.

Potential Benefits of the Proposed Research to Human Subjects and Importance of the Knowledge to be Gained

These studies are not designed to provide direct benefit to the participants. However, the knowledge gained from this work will enhance information about the pathogenesis of asthma and may lead to improved diagnosis, treatment and prevention of asthma in the future. All subjects receive asthma education and copies of blood work and lung function tests as part of their visit, and have full access to a YCAAD physician, if desired. If any major test abnormalities are encountered, YCAAD physicians counsel the subjects and provide referrals to appropriate specialists as needed.

Inclusion of Women and Minorities

Subjects will be recruited through the Clinical Recruitment and Biostatistics Core (Core B) and through the office of Dr. Karas for adenotonsillectomy without any bias of race or gender. The YCAAD asthma and control populations and the children undergoing adenotonsillectomy are racially diverse and reflect the population in New Haven and surrounding communities. All minority groups already take part in these research programs and we will continue to actively recruit women and minorities as these studies go forward. Based on demographics from past studies, the asthmatic population in YCAAD is approximately 71% female and 31% Black and Hispanic. The demographics of the adenotonsillectomy population is approximately 37% female, 14% Black and 36% Hispanic.

OMB Number: 0925-0002

Planned Enrollment Report

Study Title: NextGen Biorepository Study

Domestic/Foreign: Domestic

Comments:

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/Alaska Native	0	0	0	0	**0**
Asian	3	2	0	0	**5**
Native Hawaiian or Other Pacific Islander	0	0	0	0	**0**
Black or African American	20	18	8	8	**54**
White	85	84	12	10	**191**
More than One Race	0	0	0	0	**0**
Total	**108**	**104**	**20**	**18**	**250**

Study 1 of 2

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

OMB Number: 0925-0002

Planned Enrollment Report

Study Title: Adenotonsillectomy Biorepository Study

Domestic/Foreign: Domestic

Comments:

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/Alaska Native	0	0	0	0	**0**
Asian	2	2	0	0	**4**
Native Hawaiian or Other Pacific Islander	0	0	0	0	**0**
Black or African American	12	10	6	4	**32**
White	28	22	8	6	**64**
More than One Race	0	0	0	0	**0**
Total	**42**	**34**	**14**	**10**	**100**

Study 2 of 2

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

Inclusion of Children

The proposal will recruit children between the ages of 2 and 21 years (consent and assent form are included in Appendix 1 and 2). The investigative team for Yale #HIC0102012268 will request that the participants (12-21 years old) undergo phlebotomy, sputum induction, lung function testing and complete an asthma questionnaire with the subject and his/her parent/legal guardian. Venipuncture and pulmonary function testing and questioning are part of the routine care of children with asthma. Sputum induction is a low-risk procedure and is outlined in the Appendix. All interviews and procedures will be performed in the Yale Center for Asthma and Airways Disease. Overall, the study poses more than a minimal risk to minor subjects, but its benefits outweigh these risks. The investigative team for Yale # HIC1009007345 will request that the participant (2-18 years old) who was previously scheduled to undergo adenotonsillectomy, consent to have blood drawn intraoperatively, perform spirometry before surgery, if capable, and agree to donate excess tonsil tissue to the biorepository for analyses. These procedures are assessed to be of minimal risk to child subjects. The investigative team has experience caring for children should any adverse events arise during sample acquisition.

Inclusion of children in this study is important since we are interested in the relationship of transcriptomic endophenotypes across the spectrum of asthma. Our studies thus far suggest that there are stable Transcriptional Endophenotypes of Asthma (TEA) in children and adults with severe asthma and, in particular, near fatal asthma. Endotypes seen in adult asthmatics may be evident in young subjects, and this may help us predict the course of disease as they age. Further, we may be able to discern which patients will benefit from more aggressive care and elucidate molecular pathways involved in the pathogenesis of severe asthma. We will follow subjects longitudinally and will define how transcriptomic signatures relate with progression of this disease.

References

1. Collins FS, Varmus H. A new initiative on precision medicine. N Engl J Med. 2015;372(9):793-5. doi: 10.1056/NEJMp1500523. PubMed PMID: 25635347.
2. Chupp GL, Lee CG, Jarjour N, Shim YM, Holm CT, He S, Dziura JD, Reed J, Coyle AJ, Kiener P, Cullen M, Grandsaigne M, Dombret MC, Aubier M, Pretolani M, Elias JA. A chitinase-like protein in the lung and circulation of patients with severe asthma. N Engl J Med. 2007;357(20):2016-27. doi: 10.1056/NEJMoa073600. PubMed PMID: 18003958.
3. Ober C, Tan Z, Sun Y, Possick JD, Pan L, Nicolae R, Radford S, Parry RR, Heinzmann A, Deichmann KA, Lester LA, Gern JE, Lemanske RF, Jr., Nicolae DL, Elias JA, Chupp GL. Effect of variation in CHI3L1 on serum YKL-40 level, risk of asthma, and lung function. N Engl J Med. 2008;358(16):1682-91. doi: 10.1056/NEJMoa0708801. PubMed PMID: 18403759; PMCID: PMC2629486.
4. Ramaprakash H, Shibata T, Duffy KE, Ismailoglu UB, Bredernitz RM, Moreira AP, Coelho AL, Das AM, Fursov N, Chupp GL, Hogaboam CM. Targeting ST2L potentiates CpG-mediated therapeutic effects in a chronic fungal asthma model. Am J Pathol. 2011;179(1):104-15. doi: 10.1016/j.ajpath.2011.03.032. PubMed PMID: 21640974; PMCID: PMC3123853.
5. Vicencio AG, Chupp GL, Tsirilakis K, He X, Kessel A, Nandalike K, Veler H, Kipperman S, Young MC, Goldman DL. CHIT1 mutations: genetic risk factor for severe asthma with fungal sensitization? Pediatrics. 2010;126(4):e982-5. doi: 10.1542/peds.2010-0321. PubMed PMID: 20819891.
6. Gomez JL, Crisafi GM, Holm CT, Meyers DA, Hawkins GA, Bleecker ER, Jarjour N, Severe Asthma Research Program I, Cohn L, Chupp GL. Genetic variation in chitinase 3-like 1 (CHI3L1) contributes to asthma severity and airway expression of YKL-40. J Allergy Clin Immunol. 2015;136(1):51-8 e10. doi: 10.1016/j.jaci.2014.11.027. PubMed PMID: 25592985; PMCID: PMC4494869.
7. Wright PL, Yu J, Di YP, Homer RJ, Chupp G, Elias JA, Cohn L, Sessa WC. Epithelial reticulon 4B (Nogo-B) is an endogenous regulator of Th2-driven lung inflammation. J Exp Med. 2010;207(12):2595-607. doi: 10.1084/jem.20100786. PubMed PMID: 20975041; PMCID: PMC2989775.
8. Levin JC, Gagnon L, He X, Baum ED, Karas DE, Chupp GL. Improvement in asthma control and inflammation in children undergoing adenotonsillectomy. Pediatr Res. 2014;75(3):403-8. doi: 10.1038/pr.2013.237. PubMed PMID: 24452590; PMCID: PMC3943680.
9. Yan X, Chu JH, Gomez J, Koenigs M, Holm C, He X, Perez MF, Zhao H, Mane S, Martinez FD, Ober C, Nicolae DL, Barnes KC, London SJ, Gilliland F, Weiss ST, Raby BA, Cohn L, Chupp GL. Noninvasive analysis of the sputum transcriptome discriminates clinical phenotypes of asthma. American journal of respiratory and critical care medicine. 2015;191(10):1116-25. Epub 2015/03/13. doi: 10.1164/rccm.201408-1440OC. PubMed PMID: 25763605; PMCID: 4451618.
10. Benjamini Y aH, Y. . Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society Series B 1995;57:289–300.
11. Gong F, Su Q, Jiang D, Chen J, Pan Y, Huang X. High frequency of circulating follicular helper T cells in patients with bronchial asthma. Clinical laboratory. 2014;60(6):963-8. PubMed PMID: 25016701.

Resource Sharing Plan

Data generated in these studies will be freely available to members of the research community with a goal to enable dissemination of data, while also protecting the privacy of the participants and the utility of the data, by de-identifying and masking potentially sensitive data elements in compliance with the NIH public data sharing policy. All other resources developed in the course of the proposed studies will be available by request to qualified academic investigators for non-commercial research.

For all studies, we will follow the National Institute of Health's Genomic Data Sharing Policy. The raw datasets corresponding to expression, genomic, and genetic data generated by these studies will be submitted to Gene Expression Omnibus (GEO) or the Sequence Read Archive (SRA) for use by other investigators. As datasets are analyzed, then validated, we will proceed with deposition in ImmPort according to a timeline negotiated with the Program Officer. Sample data in the YCAAD biorepository are, and will continue to be, available on the internet through the YCCI research accelerator, a publicly accessible platform for scientific collaboration (ycci.researchaccelerator.org). In addition, tools, pipelines, derived datasets and analyses will be made available through the website (asthmaMAP.gersteinlab.org) which will serve as an organizational tool for the participants in this cooperative proposal as well as a repository and resource for the greater research community. Details of the contents and construction of the asthma MAP website are in Project 3, Research Proposal Aim 3.

Yale University School of Medicine and all investigators will adhere to the NIH Grants Policy on Sharing of Unique Research Resources including the “Sharing of Biomedical Research Resources: Principles and Guidelines for Recipients of NIH Grants and Contracts” issued in December 1999. Specifically, material transfers would be made with no more restrictive terms than in the Simple Letter Agreement or the UBMTA and without reach through requirements. Should any intellectual property arise which requires a patent, we would ensure that the technology remains widely available to the research community in accordance with the NIH Principles and Guidelines document. In addition, we will provide relevant protocols and published data upon request. Accepted versions of final, peer-reviewed manuscripts emanating from this research will be deposited on-line to PubMed Central in accord with NIH Public Access.

OMB Number: 4040-0001
Expiration Date: 06/30/2016

APPLICATION FOR FEDERAL ASSISTANCE

SF 424 (R&R)

5. APPLICANT INFORMATION **Organizational DUNS*:** 0432075620000

Legal Name*: YALE UNIVERSITY
Department:
Division:
Street1*: OFFICE OF SPONSORED PROJECTS
Street2: 25 Science Park
City*: NEW HAVEN
County:
State*: CT: Connecticut
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 065208237

Person to be contacted on matters involving this application

Prefix:	First Name*:	Middle Name:	Last Name*:	Suffix:
	Marybeth		Brandi	

Position/Title: Proposal Manager
Street1*: 25 Science Park
Street2: 150 Munson Street
City*: New Haven
County:
State*: CT: Connecticut
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 06520-8237
Phone Number*: 203-737-3495 Fax Number: Email: marybeth.brandi@yale.edu

7. TYPE OF APPLICANT*

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Precision Profiling Core

12. PROPOSED PROJECT

Start Date*	Ending Date*
07/01/2016	06/30/2021

OMB Number: 4040-0010
Expiration Date: 06/30/2016

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	YALE UNIVERSITY
Duns Number:	0432075620000
Street1*:	300 Cedar St
Street2:	TAC S440
City*:	NEW HAVEN
County:	
State*:	CT: Connecticut
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	065208237
Project/Performance Site Congressional District*:	CT-003

Project/Performance Site Location 1

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	YALE UNIVERSITY
DUNS Number:	0432075620000
Street1*:	300 Cedar St
Street2:	TAC S416, S420 and S460
City*:	NEW HAVEN
County:	
State*:	CT: Connecticut
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	065208237
Project/Performance Site Congressional District*:	CT-003

File Name

Additional Location(s)

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ● **Yes** ❍ **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ❍ Yes ● No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6

If NO, is the IRB review Pending? ❍ **Yes** ❍ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ❍ **Yes** ● **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ❍ Yes ❍ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. Is proprietary/privileged information included in the application?* ❍ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ❍ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ❍ Yes ❍ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ❍ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ❍ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Core_C_Project_Summary.pdf
8. Project Narrative*	
9. Bibliography & References Cited	Core_C_References_Cited.pdf
10.Facilities & Other Resources	Core_C_Facilities.pdf
11.Equipment	Core_C_Equipment.pdf

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

Project Summary

Our program investigates biologic mechanisms that lead to heterogeneity of asthma and in particular to severe asthma. Recent studies by PI Chupp have identified biomarkers of severe disease and the current program is designed to reveal detailed mechanistic underpinning of severe asthma. The purpose of this Core is to provide validated, standardized, quantitative, in depth multiparameter profiling and transcriptomic analyses of airway and blood samples. This data will be integrated with clinical data in Project 3 for computational modeling defining cellular functions relevant to the heterogeneity of asthma.

Facilities and Other Resources - Core C

Laboratory

The Montgomery laboratory is located in The Anlyan Center for Medical Research at the Yale University School of Medicine, which also houses the laboratories of Drs. Chupp, Cohn, Craft, Bothwell, and Flavell, making collaborations between our groups very easy. Dedicated laboratory space for this project includes over 1,600 square feet of bench space with shared areas for microscopy, tissue culture, centrifugation and cold/warm rooms. The shared platforms for analysis in this core (CyTOF, MesoScale, Fluidigm C1) are all located on the same floor of The Anlyan Center, adjacent to the labs of Core Lead Montgomery and several of the Project Leads.

Data Management

Our project employs an administrative database system for tracking clinical and lab data that is detailed in Core B.

CyTOF Facility

Yale has recently opened a shared facility for CyTOF 2: Time-of-Flight mass spectrometer (medicine.yale.edu/intmed/rheumat/cytof/index.aspx) for high-speed acquisition of highly multi-parametric single cell data. It is in the The Anlyan Center adjacent to the office and laboratory of Core Lead Montgomery and in the same building as the laboratories for Project Leads generating samples for this project. Our CyTOF is housed in a custom designed suite with secure key card entry, internet access, controlled humidity, vibration, temperature, and enhanced ventilation requirements for CyTOF. The ~300 square feet of laboratory space includes a wet bench area for sample preparation and custom conjugation of antibodies.

Our facility holds a repository of metal-conjugated antibodies as well as reagents for antibody labeling which are available to users at cost. CyTOF currently detects up to 42 parameters simultaneously in a single tube (with a theoretical limit of 100 parameters). Specific panels are described in the Core text.

CyTOF Data Management and Analysis

Our CyTOF facility employs a custom database for reagent inventory and cost recovery income. This system has optimized for our facility and is fully operational. In addition, Yale has established an enterprise site for data analysis with Cytobank. CyTOF data files are uploaded to Cytobank for analysis and password protected sharing with collaborating investigators. Use of Cytobank for analysis is available for CyTOF facility users. Data files are available for analysis tools such as SPADE, ViSNE, and CITRUS or for more detailed analytic plans as detailed in Project 3 of the proposal. This site is HIPAA compliant.

MesoScale Discovery

The Meso Scale (MSD) QuickPlex SQ 120 instrument for multiplex detection of cytokines (MSD) is housed adjacent to the Core Lead's lab. MSD's products are based on patterned arrays of selected targets (MULTI-ARRAY® technology) and electrochemiluminescence detection. MSD's instruments require a very small sample size, and use highly-efficient, custom designed optics and ultra-sensitive photodetectors to collect and quantitatively measure light emitted from the microplates. MSD reads wells in ~ 2 min and supports immunoassay in a dynamic range > 5 logs. MSD technology has enhanced sensitivity (< 1.0 pg/ml) and reduced background signals compared to standard colorimetric or fluorescent ELISA kits. The platform has proven to have minimal background and sufficient sensitivity and dynamic range for the proposed studies. MSD kits to be employed are detailed in the Projects and the Core.

Fluidigm C1 Single Cell Auto Prep System

Single cell suspensions will be prepared for RNA sequencing studies using the C1 Single-Cell Auto Prep System available adjacent to PI Chupp's lab. The C1 Integrated Fluidic Circuit (IFC) allows the user to capture up to 96 single cells, wash, stain, image, lyse, preamplify and harvest in a 96-well format. Following capture in the IFC, C1 platform performs specific reverse transcription of RNA from single cells, and targeted preamplification of cDNA. The C1 platform streamlined protocol will be used for studies of expression patterns of RNA species from up to 96 individual cells as detailed in Core C.

Yale Center for Genome Analysis (YCGA)

RNA sequencing will be conducted with a state-of-the-art Nucleic Acid Sequencing Center launched in 2010 on Yale's West Campus—a full service facility dedicated to providing microarray and high-throughput DNA sequence analysis services using various technologies (info.med.yale.edu/wmkeck). The Genome Center occupies approximately 7000 square feet of laboratory space and is equipped with 10 Illumina HiSeqs, one each of Pacific Bioscience, Ion Torrent and MiSeq sequencing systems. Including Illumina HiSeq 2500 sequencers that can produce approximately 300 million paired-end reads per flow cell lane per day. The high throughput and low cost of their sequencing platform makes next generation sequencing feasible for all Yale faculty.

Clinical

No clinical enrollment will take place under this Core. Samples for analysis will derive from protocols outlined in Core B and in Projects 1 and 2.

Animal

No animal studies will take place under this Core.

Computer

We have established network services for Apple or Intel processor computers, and 3 Dell computers, 2 digital cameras, 2 scanners and 3 printers and 2 copiers are available for use by the students, fellows and staff. All computer stations are equipped for word processing, data analysis and the preparation of lectures or presentations. In the CyTOF facility we have established network services for Apple or Intel processor computers including the instrument control and data handling computer system with CyTOF MS-1 software. Computers are available in the facility for data analysis use by the staff and users.

Office

Dr. Montgomery's office is across the hall from the CyTOF facility and in the same hallway as the Mesoscale and Fluidigm C1 instruments.

Equipment - Core C

CyTOF Mass Cytometry

CyTOF2 with inductively coupled plasma ion source with free running RF generator and a balanced load coil interface. The CyTOF2 includes a 5-stage vacuum system with 2-stage plasma-vacuum interface and TOF turbo-pump; ion-neutral decoupling ion deflector, low-mass cut-off quadrupole and point-to-parallel focusing lens system; Orthogonal TOF analyzer operated at >75000 spectra/second TOF cycle frequency; Discrete dynode ion detector.

The CyTOF2 detection system is based on 1 GS/s 8-bit signal digitizing boards; Direct sampling spray chamber for individual cell assays, Auto fill Syringe pump, and microconcentric nebulizer. In addition, our instrument uses CyTOF Autosampler Model AS5 that handles up to three 96-well plates, a 2-position 4-port automated valve for simultaneous flush/sample, and adjustable injection volume up to 0.95 ml.

We have established network services for Apple or Intel processor computers including Instrument control and data handling computer system with CyTOF MS-1 software. Computers are available in the facility for data analysis use by the staff and users.

MesoScale Discovery

A Meso Scale (MSD) QuickPlex SQ 120 is in place in PI Chupp's lab for sensitive detection of multiple analytes in a 96 well format. Features include electrochemoluminescence detection by CCD camera, plate motion control, and a plate barcode reader. MSD reads wells in ~ 2 min and supports immunoassay in a dynamic range > 5 logs. MSD technology has enhanced sensitivity (< 1.0 pg/ml) and reduced background signals compared to standard colorimetric or fluorescent ELISA kits.

Fluidigm C1 Single Cell Auto Prep System

Single cell suspensions will be prepared for RNA sequencing studies using the C1 Single-Cell Auto Prep System available adjacent to PI Chupp's lab. The C1 Integrated Fluidic Circuit (IFC) allows the user to capture up to 96 single cells, wash, stain, image, lyse, preamplify and harvest in a 96-well format. Following capture in the IFC, C1 platform performs specific reverse transcription of RNA from single cells, and targeted preamplification of cDNA. The C1 platform streamlined protocol will be used for studies of expression patterns of RNA species from up to 96 individual cells as detailed in Core C.

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix:	First Name*: RUTH	Middle Name R	Last Name*: MONTGOMERY	Suffix:

Position/Title*:	Associate Professor
Organization Name*:	YALE UNIVERSITY
Department:	
Division:	
Street1*:	YALE UNIVERSITY SCH OF MEDICINE
Street2:	DEPT OF INTERNAL MEDICINE
City*:	NEW HAVEN
County:	
State*:	CT: Connecticut
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	065200000

Phone Number*: (203) 785-7039 Fax Number: (203) 785-7053 E-Mail*: RUTH.MONTGOMERY@YALE.EDU

Credential, e.g., agency login: RMONTGOMERY

Project Role*: Other (Specify) Other Project Role Category: Project Lead

Degree Type: PHD,BA Degree Year:

	File Name
Attach Biographical Sketch*:	Montgomery_Bio_Asthma_U19.pdf
Attach Current & Pending Support:	

PROFILE - Senior/Key Person

Prefix: First Name*: SHRIKANT Middle Name M Last Name*: MANE Suffix:

Position/Title*:
Organization Name*: YALE UNIVERSITY
Department:
Division:
Street1*: Yale University
Street2: Dept Of Mole Biophy & Biochem
City*: New Haven
County:
State*: CT: Connecticut
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 065110000

Phone Number*: (203) 737-2229 Fax Number: (203) 785-7919 E-Mail*: SHRIKANT.MANE@YALE.EDU

Credential, e.g., agency login: SMMANE

Project Role*: Other (Specify) Other Project Role Category: Co-Lead

Degree Type: PHD Degree Year:

	File Name
Attach Biographical Sketch*:	Mane_Bio_Asthma_U19.pdf
Attach Current & Pending Support:	

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2016 **End Date*:** 06-30-2017 **Budget Period: 1**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	RUTH	R	MONTGOMERY		Project Lead		1.2			18,330.00	5,682.00	24,012.00
2.	Shrikant		Mane		Co-Lead		0.24			3,666.00	1,136.00	4,802.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **28,814.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	4.8			18,535.00	5,746.00	24,281.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Associate	4.8			33,685.00	10,443.00	44,128.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**68,409.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**97,223.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2016 **End Date*:** 06-30-2017 **Budget Period: 1**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	950.00
2. Foreign Travel Costs	
Total Travel Cost	**950.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2016 **End Date*:** 06-30-2017 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	69,202.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**69,202.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**167,375.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Modified total direct cost	66.5	167,375.00	111,305.00
		Total Indirect Costs	**111,305.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**278,680.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: Core_C_Budget_Justification_Asthma_U19.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2017 **End Date*:** 06-30-2018 **Budget Period: 2**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	RUTH	R	MONTGOMERY		Project Lead		1.2			18,330.00	5,682.00	24,012.00
2.	Shrikant		Mane		Co-Lead		0.24			3,666.00	1,136.00	4,802.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **28,814.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	4.8			19,091.00	5,918.00	25,009.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Associate	4.8			34,696.00	10,757.00	45,453.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**70,462.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**99,276.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2017 **End Date*:** 06-30-2018 **Budget Period: 2**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	950.00
2. Foreign Travel Costs	
Total Travel Cost	**950.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2017 **End Date*:** 06-30-2018 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	58,808.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**58,808.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**159,034.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Modified total direct cost	66.5	159,034.00	105,757.00
		Total Indirect Costs	**105,757.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**264,791.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: Core_C_Budget_Justification_Asthma_U19.pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2018 **End Date*:** 06-30-2019 **Budget Period: 3**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	RUTH	R	MONTGOMERY		Project Lead		1.2			18,330.00	5,682.00	24,012.00
2.	Shrikant		Mane		Co-Lead		0.24			3,666.00	1,136.00	4,802.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **28,814.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	4.8			19,664.00	6,096.00	25,760.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Associate	4.8			35,737.00	11,078.00	46,815.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**72,575.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**101,389.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2018 **End Date*:** 06-30-2019 **Budget Period: 3**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	950.00
2. Foreign Travel Costs	
Total Travel Cost	**950.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2018 **End Date*:** 06-30-2019 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	57,153.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**57,153.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**159,492.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Modified total direct cost	66.5	159,492.00	106,063.00
		Total Indirect Costs	**106,063.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**265,555.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: Core_C_Budget_Justification_Asthma_U19.pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2019 **End Date*:** 06-30-2020 **Budget Period: 4**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	RUTH	R	MONTGOMERY		Project Lead		1.2			18,330.00	5,682.00	24,012.00
2.	Shrikant		Mane		Co-Lead		0.24			3,666.00	1,136.00	4,802.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **28,814.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	4.8			20,254.00	6,279.00	26,533.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Associate	4.8			36,809.00	11,411.00	48,220.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**74,753.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**103,567.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2019 **End Date*:** 06-30-2020 **Budget Period: 4**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	950.00
2. Foreign Travel Costs	
Total Travel Cost	**950.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2019 **End Date*:** 06-30-2020 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	62,771.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**62,771.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**167,288.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Modified total direct cost	66.5	167,288.00	111,246.00
		Total Indirect Costs	**111,246.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**278,534.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name:
Core_C_Budget_Justification_Asthma_U19.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2020 **End Date*:** 06-30-2021 **Budget Period: 5**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	RUTH	R	MONTGOMERY		Project Lead		1.2			18,330.00	5,682.00	24,012.00
2.	Shrikant		Mane		Co-Lead		0.24			3,666.00	1,136.00	4,802.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **28,814.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	4.8			20,862.00	6,467.00	27,329.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Associate	4.8			37,913.00	11,753.00	49,666.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**76,995.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**105,809.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2020 **End Date*:** 06-30-2021 **Budget Period: 5**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	950.00
2. Foreign Travel Costs	
Total Travel Cost	**950.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2020 **End Date*:** 06-30-2021 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	58,013.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**58,013.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**164,772.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Modified total direct cost	66.5	164,772.00	109,574.00
		Total Indirect Costs	**109,574.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**274,346.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: Core_C_Budget_Justification_Asthma_U19.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

CORE C

BUDGET JUSTIFICATION

Ruth R. Montgomery, Ph.D., (Core Lead) Dr. Montgomery is an Associate Professor in Internal Medicine and a cell biologist with extensive experience with innate immune cells in human translational studies. Dr. Montgomery has overseen successful enrollment of >1500 healthy individuals and >150 immunosuppressed subjects for studies of differential innate immune responsiveness in human cohorts. She directs the Yale CyTOF facility, and has been responsible for all aspects of operation including overseeing space renovation, installation of the instrument, staff and user training, cost recovery, and the development of our antibody repository and database. She is senior author on recent publications defining immune status in human cohorts and defining immune elements of susceptibility to infection in studies which employ the assays platforms proposed in this core (CyTOF, MSD, RNASeq). Dr. Montgomery will oversee all aspects of coordination with the investigators in Core B and the research projects for preparation and validation of human samples proposed here to investigate asthma heterogeneity. In particular, she will oversee the CyTOF Core facility, development and customization of the CyTOF antibody panels for Projects 1-2, and coordinate with Dr. Mane for guidance of transcriptional platforms, and with Project 3 investigators for analysis of data generated. She will oversee the work of Dr. Yao for CyTOF experiments and Ms. Wang for MSD and RNASeq and will ensure quality control of reagents and compliance with all Biosafety, HIC/HIPAA regulations. She will attend regular progress meetings and coordinate with other investigators to achieve the project goals. Her effort on this grant will be 1.2 calendar months annually.

Shrikant Mane, PhD. (Co-Lead). Dr. Mane is an experienced molecular biologist and Senior Research Scientist at Yale. He has been the Director of the Yale Center for Genome Analysis (YCGA) since 2001 and The Keck Biotechnology Resource Laboratory at Yale since 2009. He has published more than 100 articles, holds 2 patents, and has amassed over 25 years of research experience in both academic and private industry. He oversees a successful and productive genomic facility and ongoing collaborative studies with the investigators of this proposal, in particular Drs. Chupp, Gerstein, and Montgomery. Dr. Mane will provide expert guidance for the RNASeq studies in this Core, including both bulk and single cell measurements. In addition, Dr. Mane is at the forefront of new technological developments and cutting edge genomic technologies at Yale that may be relevant to the conduct of the proposed studies on sputum cells. Dr. Mane will attend regular progress meetings and coordinate with Core Lead Montgomery for optimal sample processing. Dr. Mane's effort on this grant will be 0.24 calendar months annually.

Non-Key Personnel

Yi Yao, Ph.D., (Postdoctoral Associate). Dr. Yao is a highly qualified immunologist with expertise in immune signaling of monocytes, macrophages and dendritic cells. She has worked with Dr. Montgomery for 2 years and has rapidly established herself as one of the leaders in our CyTOF group. She is the lead author in our methods study defining lower limits of detection of immune cells by CyTOF. She will coordinate studies with investigators in Projects 1-2 to profile expression and functional efficiency of airway cells from human subjects as detailed in the Research Projects. Dr. Yao will coordinate with Project 3 investigators for analysis of CyTOF data generated. Dr. Yao will attend regular progress meetings and will be supervised by Dr. Montgomery. Dr. Yao's effort on this grant will be 4.8 calendar months annually.

Xiaomei Wang, (Research Associate). Ms. Wang has >20 years of lab experience and quality control assurance and has worked with Dr. Montgomery for 15 years to evaluate the functions of human immune cells. She will coordinate with investigators in Core B and Projects 1-2 to isolate RNA from study samples for RNASeq transcriptional profiling and she will quantify cytokines by MSD. She is experienced with both assays. Ms. Wang will be supervised by Dr. Montgomery. Her effort on this grant will be 4.8 calendar months annually.

Other Expenses

Travel

We request $950 per year for travel to allow the Core Lead and investigators to attend scientific meetings with Program investigators (for train travel, hotel, and per diem).

Supplies

We request funds ($69,202) to assess samples from the Research Projects using MSD, CyTOF, and RNA-Seq (bulk and single cell). These funds are based on a per subject cost for the experiment types and thus are shared across Projects 1 and 2, and include the purchase of general laboratory supplies including pipettes and tips, chemicals, paper towels, etc. We estimate lower expenses for MSD cytokine assays (human 10-plex is $1,550 per kit) with the majority of the funds being for RNA-Seq for human samples (with spike-in target genes as detailed in the text) and CyTOF studies. Airway cell RNA-Seq studies will be conducted in batches across multiple years of the project to insure coverage of study groups and sufficient funds for processing. These assays require molecular reagents to extract total RNA with high integrity from samples, transcription, library preparation, and sequencing at Yale's Keck facility, which can be done from as little as 1-2 nanograms of RNA. While sequencing costs approach $1-2000 per run, we estimate expenses for sequencing of both bulk and single cell RNA-Seq to decline in the coming years based on barcoding of samples and lower sequencing costs through Yale's Keck facility.

We request funds for CyTOF antibody reagents for immunoprofiling panels of study samples as detailed in the Research Projects. The expenses include purchase of commercial antibodies (~$300 per 100 tests) as well as in-house conjugations ($75/antibody), anti-fluorescence antibodies, and purchases through our collaboration with the Longwood CyTOF. Total expenses for CyTOF antibody panels are approximately $12,000 for a 40-marker panel for 100 samples, although this expense is likely to decrease with barcoding of samples and in-house reagent availability. We expect to process approximately 200 samples per year from the Projects, with some differences in the number of samples from each project in a given year. This estimate includes funds for instrument time and for consumable supplies required for the operation of the CyTOF such as argon gas, NormJect Luer syringes, CyTOF tuning and washing solution, and parts as needed including Nebulizer, torch body, ball joint injector, spray chamber, sample capillary assembly, load coil, skimmer-reducer assembly, sampler cone, nebulizer arm-O-ring; labware such as gloves, Kim wipes, and pipette tips.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		144,070.00
Section B, Other Personnel		363,194.00
Total Number Other Personnel	10	
Total Salary, Wages and Fringe Benefits (A+B)		507,264.00
Section C, Equipment		0.00
Section D, Travel		4,750.00
1. Domestic	4,750.00	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		305,947.00
1. Materials and Supplies	305,947.00	
2. Publication Costs	0.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	0.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	0.00	
9. Other 2	0.00	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		817,961.00
Section H, Indirect Costs		543,945.00
Section I, Total Direct and Indirect Costs (G + H)		1,361,906.00
Section J, Fee		0.00

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

1. Project Director / Principal Investigator (PD/PI)

Prefix:
First Name*: GEOFFREY
Middle Name: L
Last Name*: CHUPP
Suffix:

2. Human Subjects

Clinical Trial? ● No ○ Yes
Agency-Defined Phase III Clinical Trial?* ○ No ○ Yes

3. Permission Statement*

If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?

○ Yes ● No

4. Program Income*

Is program income anticipated during the periods for which the grant support is requested? ○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

Budget Period*	Anticipated Amount ($)*	Source(s)*

PHS 398 Cover Page Supplement

5. Human Embryonic Stem Cells

Does the proposed project involve human embryonic stem cells?* ● No ❍ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s): ❑ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

6. Inventions and Patents (For renewal applications only)

Inventions and Patents*: ❍ Yes ● No

If the answer is "Yes" then please answer the following:

Previously Reported*: ❍ Yes ❍ No

7. Change of Investigator / Change of Institution Questions

❑ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:

First Name*:

Middle Name:

Last Name*:

Suffix:

❑ Change of Grantee Institution

Name of former institution*:

PHS 398 Research Plan

Please attach applicable sections of the research plan, below.

OMB Number: 0925-0001

1. Introduction to Application (for RESUBMISSION or REVISION only)	
2. Specific Aims	Core_C_Specific_Aims.pdf
3. Research Strategy*	Core_C_Research_Strategy.pdf
4. Progress Report Publication List	
Human Subjects Sections	
5. Protection of Human Subjects	Protection-HumanSubjects_Asthma_U19.pdf
6. Inclusion of Women and Minorities	Inclusion-Women_Minorities_Asthma_U19.pdf
7. Inclusion of Children	Inclusion-Children_Asthma_U19.pdf
Other Research Plan Sections	
8. Vertebrate Animals	
9. Select Agent Research	
10. Multiple PD/PI Leadership Plan	
11. Consortium/Contractual Arrangements	
12. Letters of Support	
13. Resource Sharing Plan(s)	ResourceSharingPlan_Asthma_U19.pdf
Appendix (if applicable)	
14. Appendix	

Tracking Number: GRANT12004239 Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

Specific Aims

Asthma afflicts approximately 7% of the U.S. population and millions of patients worldwide with persistent/chronic airway inflammation, episodic wheezing, and shortness of breath (1, 2). Although many individuals easily control symptoms with standard medication regimen, a subgroup (~5%) of "refractory" asthmatics have severe, persistent symptoms despite the use of maximal medical therapy (3, 4). A closer understanding of the biologic mechanisms responsible for this heterogeneity is essential if we are to develop more personalized and effective treatment strategies for severe asthma. The overall goal of this proposal is to understand **the mechanisms of heterogeneity of asthma by in depth investigation of airway cell populations** and their function, biomarkers of disease, and immune mediated exacerbations of asthma. Our investigation is based on a well-characterized cohort of asthmatic subjects with mild or refractory asthma from the Yale Center for Asthma and Airway Diseases (YCAAD) directed by PI Chupp and co-directed by Dr. Cohn. YCAAD has enrolled > 700 asthmatic subjects over the last 9 years for the integrated characterization of disease phenotypes associated with gene expression analysis (6-10). Characterization of certain functional and genomic markers, including circulating biomarker YKL-40, and **t**ranscriptomic **e**ndophenotypes of **a**sthma (TEA clusters), polymorphism in the promoter of the CHI3L1 gene is associated with YKL-40 levels have recently been found to correlate with clinical asthma and distinguish classes of severe patients (6-8, 11).

Our goal is to identify functional cell subsets relevant to a mechanistic understanding of disease severity that may lead to tailored therapeutics based on cellular activity phenotypes. Efficient and reproducible detection of airway cell states is paramount to our understanding of the pathogenesis of severe asthma. To maximize the immune profiles of samples generated in our projects and the shared analysis and interpretation of influences on asthma severity, we will establish a **Precision Profiling Core** to conduct and analyze multiparameter immune markers using shared platforms. The purpose of this Core is to provide standardized, quantitative, in depth profiling of tissues that allows the investigators of Projects 1 and 2 to integrate data for computational modeling in Project 3. The Core will provide complementary data of airway cells from clinical samples for multiparameter analysis including CyTOF single cell profiling, multiplex cytokine measurements (MSD), and transcriptional profiling by RNA-Seq on PBMCs, bulk sputum, and single-cell RNA-seq for sputum. Standardization, both at the assay and analysis levels, will allow comparisons and integration of data across projects. Data from the 3 platforms will be provided to **Project 3 Asthma MAP: Computational Tools and Clustering for the Study of Asthma Heterogeneity,** to provide a multidimensional profile of airway and immune cell function in severe asthma according to the following Specific Aims:

Aim 1. Quantitative Multiparameter Immune phenotyping and functional measurements. We will employ CyTOF (Cytometry by Time-Of-Flight), which provides unprecedented detail for cellular analysis of immune subsets, to quantify markers of airway and immune cell subsets and functions. Multiparameter antibody panels for immune cell phenotyping will be customized for specific markers and pathways relevant to disease severity. Data files to Project 3 for extraction of cell subpopulation information, visualization, and analysis.

Aim 2. Quantitative multiplex cytokine measurements. We will determine the concentration of secreted mediators to assess differential cytokine production

Aim 3. Transcriptional profiling using bulk and single cell RNA-Seq. We will quantify gene expression patterns in batched analysis of human airway cells by RNA-Seq to probe the intrinsic differences between cells of interest and to define molecular signatures of airway cells. Performing bulk RNA-seq with single-cell RNA-seq will synergize our research and provide the most complete profiling resource to date of asthmatic patients.

	Project 1 Chupp	Project 2 Craft, Bothwell, Flavell	Project 3 Gerstein, Krishnaswamy
Assessment/Tissue	sputum, serum	sputum, plasma tonsils, PBMCs,	
CyTOF immune panels	X	X	X
Multiplex cytokine ELISA	X	X	X
RNA-Seq (bulk)	X	X	X
RNA-Seq (single cells)	X	X	X

Table 1. Cohort samples for shared interrogation platforms and analysis

Significance. Here we will conduct deep phenotypic and transcriptomic analyses of cells from clinical samples to define cellular functions relevant to the heterogeneity of asthma. We anticipate these analyses will reveal variance in the numbers, phenotypes, and functional status of cells from mild and severe asthmatics, and as a function of disease status. Data will be provided to Project investigators for integration of transcriptional, functional, and single cell data to understand mechanisms of asthma heterogeneity.

Benefits of a shared core. Our program investigates biologic mechanisms that lead to heterogeneity of asthma and in particular to severe asthma. Recent studies by PI Chupp have identified biomarkers of severe disease and the current program is designed to reveal detailed mechanistic underpinning of severe asthma. The purpose of this Core is to provide standardized, quantitative, in depth profiling of tissues that allows the investigators of Projects 1 and 2 to integrate data for computational modeling in Project 3. Variation is a major issue in the investigation of cellular responses in human populations, thus we have developed common platforms and reagents to measure defined responses in diverse investigations of airway responses.

Qualifications of the Core Leader and personnel. Core leader Montgomery is an experienced translational investigator with a research focus on innate immune cell function and models of infection and aging. She has extensive laboratory experience in human studies and has participated in cross-center collaborative projects to reduce variation in assays for Luminex, flow cytometry, and CyTOF. **Dr. Montgomery** directs Yale's core facility for CyTOF2 (medicine.yale.edu/intmed/rheum/cytof/index.aspx), which is in the building where the investigators of Projects 1 and 2 have their labs. Her experience with CyTOF includes a limiting dilution method and studies of Natural Killer cell function in viral immunity (5, 12). In the Core, she will be joined by **Dr. Shrikant Mane**, the Director of the Yale Center for Genome Analysis (YCGA), who has particular expertise in transcriptomics. Core C studies will be conducted by Montgomery lab members **Dr. Yi Yao**, who is well versed in CyTOF methods (5, 12), and **Ms. Xiaomei Wang,** who has >20 years experience with molecular and biochemical assays. Transcriptional studies will be conducted in coordination with **Dr. Mane** and the YCGA. There will be close communication and flow of biologic samples from **Core B, Clinical Recruitment and Biostatistics**, to Core C. Dr. Montgomery works closely with Drs. Chupp and Cohn (Core B Lead). We are located on the same lab floor and have an integrated workflow to conduct these novel assays (13).

Technological Advances: CyTOF provides unprecedented multidimensional immune cell profiling. Flow cytometry has been a powerful tool for analysis of the immune system on a cellular level but it is limited by the number of markers that can be combined and the overlap of emission spectra of the fluorescent reporters. Mass cytometry or CyTOF (Cytometry by Time-Of-Flight) is a new technology for multiparameter single cell analysis which uses heavy metal ions as antibody labels. By using heavy metal ions as labels and mass spectrometry as a readout, many more markers can be combined, providing unprecedented multidimensional immune cell profiling. An important advantage of the CyTOF technology is that the metal-conjugates have little/no background, and no overlap between channels, thus removing compensation issues that can be challenging and may reduce sensitivity in FACS. The result is that experiments combining 40 or so antibody specificities can be routine with tremendous detail for cellular analysis (14, 15). CyTOF has already been applied to characterizing peripheral blood cells, γδ cells in Celiac disease, and signatures that correlate with parameters of surgical recovery, rheumatic or infectious disease (16-21), and even holds the promise of examination of solid tumors (22, 23). However single cell functional diversity has not yet been employed for airway cells. The multiparameter antibody panels proposed here represent the most in depth characterization of airway samples to date. This collection of high-quality data will be important to define mechanisms of severe asthma.

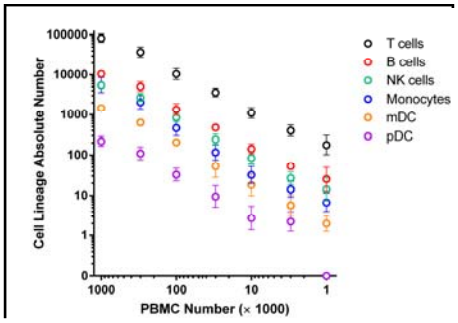


Fig. 1. CyTOF Detection of distinct cell lineages from serially diluted PBMCs. Normalized to detection of 1 x 10^6 PBMCs. N=4. (5)

Preliminary Studies. CyTOF supports detection of immune cell subsets and function from sputum samples. The Montgomery lab recently demonstrated efficient detection of immune cells by CyTOF with as few as 10^4 (and in some cases 10^3) PBMCs (**Fig. 1**)(5), especially relevant for limited airway cells in the proposed research. To demonstrate the feasibility of the proposed studies, we collected induced sputum samples from asthmatic subjects (4-26 x 10^6 total cells, n=6) following the YCAAD hypertonic saline protocol (see **Core B, Clinical Recruitment and Biostatistics**). Using CyTOF, we detected distinct viable cell subsets (24, 25) , which is important as viability can be an issue in cells of induced sputum. Labeling quantifies non-immune bronchial epithelial cells and the majority of neutrophil and monocyte/macrophage markers as

expected. Airway inflammation in severe asthmatics is associated with increased eosinophilic and neutrophilic airway inflammation and cytokine expression (26-28). Notably, SPADE analysis, where each circular node is a similar population, node color indicates intensity, and adjacent nodes are most similar (29), captures the heterogeneity of the sample and distinguishes clusters of cells within each subset in sputum (**Fig. 2**).

We have also developed a system to evaluate the functional response of mixed inflammatory cell populations isolated from the sputum to achieve the aims of Projects 1, 2, and 3. Sputum cells were acquired from PI Chupp through YCAAD and processed for CyTOF on the same day. To examine the functional status of immune cells in sputum, we designed an antibody panel to identify multiple cell lineages and production of cytokines. Mixed sputum cells were stimulated to gauge functional status. The results show isolation of sufficient cells from induced sputum and the ability to detect both the variation between subjects and stimulated production of the pro-inflammatory cytokine TNF following treatment of sputum with LPS (**Fig. 3**)(30). These preliminary studies demonstrate the feasibility of the proposed studies for cell yield, phenotypic and functional characterization of cells from sputum, and support our goal to employ CyTOF to profile phenotypic and functional diversity of airway cells.

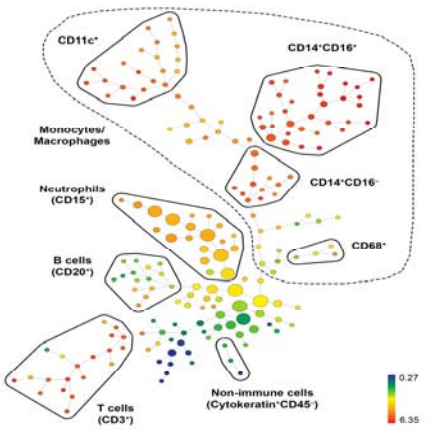


Fig 2. Cell subsets from sputum. SPADE analysis captures the heterogeneity of clusters of cells within sputum.

Airway Transcriptional studies and Single Cell RNA-Seq on airway cells. PI Chupp has analyzed gene expression in induced sputum within a heterogeneous cohort of individuals with asthma and defined 3 **t**ranscriptomic **e**ndophenotypes of **a**sthma (TEA clusters) that correlate with clinical asthma(11) and identified a biomarker associated with severe asthma, CHI3L1/YKL-40 (see **Project 1**). Further, Dr. Bothwell (**Project 2**) has identified that Dickkopf (Dkk)-1 is involved in a chronic asthma response. We conducted preliminary studies using Fluidigm C1 integrated fluidics circuits for efficient capture of single cells from sputum for RNA-seq (**Fig 4**). Using high quality total RNA (RIN >7) prepared using mRNA Seq Kit (Illumina), we converted polyA+ RNA into full-length cDNA, and performed universal amplification of the cDNA transcripts. The preliminary single cell RNA sequencing data detected 89 out of the 96 wells on the Fluidigm C1 capturing plate as single cell wells. These 90 single cell wells provided 327,381,652 reads in pairs with read length of 101bps. On average, each cell has 3,678,446 reads and around 53.96% of these reads can be mapped to the human genome (UCSC hg19). Thus we confirm the suitability of this platform for the proposed analysis of primary airway cells.

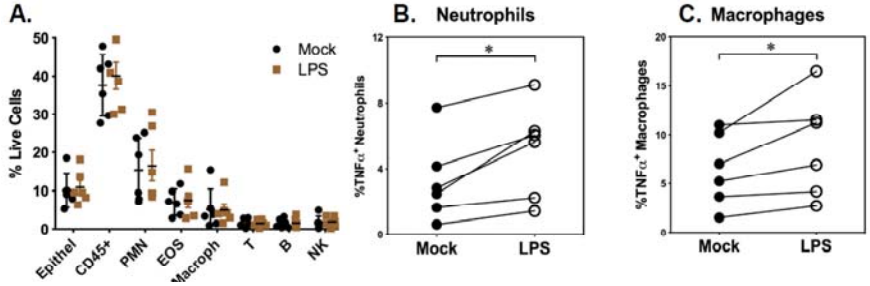


Fig. 3. CyTOF detection of multiple lineages and cell activation in sputum. Cells from induced sputum (1-3 x 10^6 /condition) were labeled for lineage markers (A): epithelial cells (Cytokeratin$^+$ CD45$^-$); Total immune cells (CD45$^+$); T cells (CD3$^+$); B cells (CD20$^+$); PMN (CD66b$^+$CD15$^+$CD16$^+$); Eos (CD66b$^+$CD15$^+$CD16$^-$); macrophages (CD14$^+$/CD11c$^+$/CD163$^+$); NK cells (CD56$^+$CD3$^-$). Production of TNF +/- LPS (0.5 µg/ml for 6 hr) for PMN (B) and macrophages (C). N =6; * p<0.02.

Single cell capture is particularly attractive for the analysis of sputum as it provides an opportunity to select cells of a certain size for capture. Buccal squamous cells often contaminant the mucus plugs of induced sputum, and if >25% squamous cells, we exclude samples from analysis. However, since these cells are much larger than inflammatory cells, they are easily excluded from capture using C1 fluidics. This single cell capture method will significantly increase our yield of useable sputum samples, expected to increase from approximately 50-60% to 80-90%. This transformative approach allows single cell transcriptomic data for detailed analysis of functional status of cells in sputum of patients with a range of severity of asthma and holds the promise of distinguishing critical gene pathways relevant to disease severity. Single cell RNA-Seq data will be incorporated by the driving projects to discriminate the next generation endotypes of disease associated with the established molecular pathways.

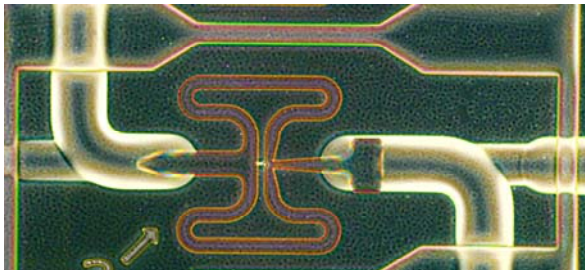


Fig. 4. Fluidigm C1 integrated fluidic circuit. A single cell from a sputum cell suspension is captured for analysis. The captured inflammatory cell is the small white spot centered in the red flow cell circuit.

C. Approach. General strategy for multivariate signatures to define asthma severity. The goal of this Core is to provide validated in depth multiparameter measurements of samples for the driving Projects as outlined in Table 1. Core C will use multidimensional CyTOF to broadly quantify airway and immune cell status for baseline phenotyping, including single-cell phenotyping for samples with limited numbers of cells (**Aim 1**); quantify levels of cytokines by multiplex ELISA (MSD) (**Aim 2**); and generate quantitative transcriptional profiles of samples by RNA-Seq (bulk and single cell) (**Aim 3**). The phenotypic and functional data will be provided to Project 3 investigators through web interface for high-dimensional computational analysis and integrated with clinical status to inform the Asthma MAP in-depth model of asthma severity. Through unified performance of shared protocols, the data produced can be employed to investigate mechanisms of severe asthma and directed therapeutic strategies.

Clinical sample processing and timeline. The types of samples to be used in the Projects includes airway cells from induced sputum, peripheral blood cells, serum, and tonsils from mild and severe asthmatic subjects (**Table 1**). Some samples may be obtained at up to three time points as detailed in Core B. Sample processing will depend on the sample type and analysis. Airway samples are acquired by sputum induction with hypertonic saline as detailed in Core B (11), and enriched cells from the mucus plugs will be provided to Core C. Our labs are in the same hallway and this is a rapid workflow already established for our team (13). The cell pellet is resuspended for analysis by CyTOF. Previously, samples with >25% squamous cells were considered contaminated and have not been analyzed by bulk RNA-seq. However, since CyTOF and single cell capture can exclude these cells, a higher proportion of sputum samples (80-90%) will be considered high quality. Samples will be assessed as received throughout Years 1-4 of the award period and assays conducted on groups of samples (or bar-coded samples) to reduce batch variation.

Specific Aim 1. Multiparameter Immune phenotyping and functional measurements.

Aim 1A. **CyTOF identification of airway cell subsets and frequencies.** We will perform multiparameter profiling of blood and airway cells received from Core B for baseline measurements, or from Projects 1 and 2 following stimulation. Through use of the CyTOF technology, we will collect unprecedented detail of phenotypic and functional parameters of lung and immune cells. In our facility, we have developed validated phenotypic and functional panels for CyTOF characterization of cells using 35-marker antibody panels specific for human innate and adaptive cells (medicine.yale.edu/intmed/rheum/cytof/index.aspx). These panels serve as the baseline phenotyping to produce a quantitative measure of immune cell subsets, numbers, and cell-associated surface markers for each subset. We have shown in preliminary studies that the cells recovered from sputum are sufficient for CyTOF and are readily activated and suitable for analysis of cell function (**Fig. 3**)(30).

We will assess freshly harvested airway cells from sputum with an initial panel of 40 marker CyTOF Airway Panel (**Table 2**) comprised of a combination of available metal-conjugated antibodies from commercial sources (DVS Sciences) and through consortium relationship with the Longwood Medical Area CyTOF facility, metal conjugated-anti-fluorescent antibodies, and custom metal conjugations performed in-house. Samples of airway cell suspensions or PBMCs will be labeled in deep well plates according to our established conditions for CyTOF (5, 12). We use 'metal-minus-one' techniques to assess issues of cross-talk between channels. Iridium-191/193 and cisplatin (DNA intercalators) are added to identify nucleated cells and viability, respectively, and a 4-bead calibrator (EQ4) is included with each sample to track instrument variation. Cells will be labeled, washed, fixed and permeabilized. The CyTOF antibody panel will be titrated and optimized on sputum (not PBMCs) to define concentrations for detection of rare cells as disease severity may lead to variability in sputum cell number. In addition, through the use of barcoding, it is possible to physically combine individual samples for assay on CyTOF and then acquire distinct data sets. We are adopting barcoding this year to minimize sample variability as well as costs, and to screen out cell doublets.

The CyTOF airway phenotyping panel was developed with each Research Project for detection of key markers relevant to distinguish cell status (**Table 2**). Panels include human immunophenotyping labels already in use in our CyTOF facility, with markers for major immune cell subsets, e.g., B cells, $CD4^+$ and $CD8^+$ T, $CD16^{+/-}$NK, and CD123/CD11c dendritic cells (5, 31), markers that distinguish structural lung cells from immune cells (i.e., cytokeratin), as well as additional markers for monocytes (CD14, CD16) reflecting the many classes of monocytes in circulation (32). As macrophages constitute a large proportion of sputum cells in asthmatics (**Fig. 2**), and are important in disease pathogenesis and progression (33), multiple subsets of macrophages in sputum will be refined using HLA-DR, CD14, CD16, CD11c, CD61, CD68 (24, 34, 35), in addition CD15 for neutrophils (PMN), NK markers CD56, and $CD15^+/CD16^-$ for Eosinophils, which accumulate in severe asthma (10, 25). Recent reports have noted subgroups of immature, pro-inflammatory low-density PMN in autoimmune and asthmatic subjects with more severe disease activity (36). Low-density PMN express canonical PMN makers with elevated levels of CD66b and CD11b (36), which are included in our panel, and

can be identified as a branch in SPADE plots. Other markers included for the Airway Panel are TLR4, a key component of the innate immune system that detects LPS and triggers host defenses (37, 38), and the chitinase-like protein YKL-40 (See Project 1), which PI Chupp has shown is elevated in circulation and in the cytoplasm of macrophages and neutrophils in severe asthmatics (6, 7). As even 40 markers may not provide comprehensive phenotyping, we will add more panels and/or substitute other markers as studies progress. Newly recognized biomarkers may be included, such as the sialic acid binding lectin, Siglec-8, an innate immune receptor expressed on Eos in sputum (39). Gating of subsets follows exclusion of debris (Iridium-; DNA^-), cell doublets (Iridium high; DNA^{hi}) and dead cells ($cisplatin^+$).

Aim 1B. Detection of cell functional efficiency. Building on the foundation of phenotyping panels, we will quantify the functional efficiency of the cells from Projects 1 and 2 by detection of surface lineage markers and intracellular staining of cytokine, chemokine, and inflammatory mediators relevant to each study population. Customized in depth CyTOF antibody panels will be developed with Projects 1 and 2 to assess cell functions reflecting the pathways under study as outlined in Project proposals. Our preliminary YCAAD findings reveal that severe asthmatics have a higher percentage of PMN and Eos in the sputum, accompanied by higher levels of IL-5. The advantage of the multidimensional platform is the ability to detect each cytokine in each lineage simultaneously, e.g., the frequency of $CD4^+$ T cells producing IL-2, IFNγ (Th1), IL-4 (Th2), and co-stimulatory molecules CD80, CD86 and CD40 on monocyte and DC subsets. For samples with sufficient cell number, we will use multiple antibody panels to detect determinants of functional status following stimulation as outlined in Projects 1 and 2. Examples include markers of inflammatory macrophages (IL-6, IFNγ, CXCL10) and cytokines specific for each Project quantified from each lineage in one sample tube (16, 40) (**Table 3**). These studies will identify cellular mechanisms through quantifying signaling mediators and phosphorylation intermediates by CyTOF.

Aim 1C. Multidimensional analysis of airway cells. Core C will conduct quality control for instrument performance and data generated. Initial assessments of variability in sputum samples, cell lineage and subset frequencies will be conducted to validate data quality, as well as bead normalization (41) and de-barcoding (barcodes to be adopted soon). CyTOF FCS files will be uploaded to Project 3 for analysis using established programs such as SPADE or vi-SNE within Cytobank (29, 42, 43), algorithms such as Phenograph (44) or new dimensionality reduction with unsupervised clustering by Dr. Krishnaswamy (45) in **Project 3**.

Aim 2. Cytokine Production by ELISA/MSD. We will determine the concentration of secreted mediators to assess differential cytokine production, as mRNA may not reflect the protein changes. Cytokine levels in the serum and sputum supernatant will be measured using Meso Scale ELISA technology (MSD) according to the manufacturer's instructions and will parallel the immunophenotyping studies described in **Aim 1**. Bead array multiplex assays based on the MSD technology represent a major resource for immune phenotyping, and have enhanced sensitivity and reduced background signals based on electro-chemoluminescent detection. MSD can measure multiple analytes in a drop of blood. For immunophenotyping, plasma proteins are of particular interest – 51 of those can be measured simultaneously in one assay well. For a broad panel of cytokines, we will quantify cytokine production using the MSD Human High Sensitivity Cytokine/Chemokine multi-analyte ELISA kit according to the manufacturer's instructions and normalizing using batched control sera in duplicate in each plate according to our

Project	Markers for CyTOF Panel 2
1	STAT-1, CHI3L1, IL-13ra2, CRTH2, pERK, AKT, WNT
2	IL-4, IL5, Il-13, Dkk-1, IL-17A, PSGL-1, CD62P, CD25, CD41, STAT-1, pERK

Table 3. Markers for customized CyTOF panels

Metal label	Marker
Qdot-Cd	HLA-DR
89Y	CD45
141Pr	CCR6
142Nd	IL-4
143Nd	IL-5
144Nd	FITC α-IFNβ
145Nd	YKL-40
146Nd	CD8a
147Sm	CD20
148Nd	CD16
149Sm	CCR4
150Nd	MIP1β
151Eu	CD123
152Sm	TNFα
153Eu	CD61
154Sm	CD15
155Gd	CD4
156Gd	IL-6
158Gd	CD11b
159Tb	CD11c
160Gd	CD14
161Dy	Cytokeratin
162Dy	CXCR3
163Dy	IL-8
164Dy	IL-13
165Ho	IFNγ
166Er	CD80
167Er	CD66b
168Er	IL-12
169Tm	CD45RA
170Er	CD3
171Yb	CD68
172Yb	CD38
173Yb	TLR4
174Yb	CD62L
175Lu	CD56
176Yb	IL-10
195Pt	Cisplatin
Iridium	DNA

Table 2. Initial CyTOF panels.

established protocols (46). The concentration levels of all samples will be mean normalized by a plate specific normalization factor calculated by taking the mean across all cytokines. The cytokines to be tested include the TH1/TH2 panel and innate cytokines and chemokine mediators that are relevant to lung function (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12 (p40), IL-13, IL-17, IL-18, IL-23, IFNα/β, IFN-γ, TNFα, TGF-β, CCL2 (MCP-1), CCL8 (MCP-2), CXCL10 (IP-10), CCL5 (MIP2), and CCL3 (MIP-1α)). Supernatants may be tested at dilutions up to 1:200, an indication of the sensitivity of the MSD system.

Aim 3. Transcriptional Profiles of asthma heterogeneity. We will quantify gene expression patterns in batched analysis of human airway cells by bulk and single cell RNA-Seq to probe the intrinsic differences between cells of interest and to define the molecular signatures of airway cells and identify unique states implicated in heterogeneity of asthma. We have previously employed RNA-Seq with Project 3 Lead Gerstein to show differential responses to infection with West Nile virus (47). For single cell RNA-Seq, we use Fluidigm C1 integrated fluidic circuits (IFC) to capture individual cells (**Fig. 4**). Throughout the study, the expertise of Dr. Mane will position us to deploy cutting edge novel technologies as they become available.

Aim 3A. Transcription profiling of airway cells to identify signatures of severe asthma. The transcriptional studies of baseline (Core B) and stimulated samples (Projects 1, 2) will provide differential expression data for Project 3**.** Data will be background corrected and normalized (48) to identify genes that are differentially expressed between cohorts. Expression patterns from different patient clusters are expected to reveal determinants of disease pathogenesis. For bulk RNA-seq of sputum samples, cells will be deposited into lysis buffer for processing. Total RNA will be isolated from using miRNeasy mini kit (Qiagen, CA), which includes isolation of non-coding RNAs. Total RNA quality will be determined by OD ratio (A_{260}/A_{280} and A_{260}/A_{230}) by nanodrop; RNA integrity >7 (Agilent Bioanalyzer gel) is required for further processing.

RNA-Seq Library Preparation. For bulk RNA-Seq samples, low abundance RNA species such as LncRNA will be enriched by depletion of ribosomal RNA using a hybridization/bead capture procedure, with total RNA input requirement of 500ng (Ribo-Zero Gold Kit, Epicentre). mRNA is purified from approximately 500pg-1ng of total RNA using the Clontech SMARTer Ultra Low RNA Kit. The kit utilizes an oligo(dT) primer, primes the first-strand synthesis reaction, SPRI beads are used to selectively bind first-strand cDNA. ss cDNA is then amplified by LD PCR to make ds cDNA and is again purified using SPRI beads. During amplification the poly A sequence serves as the universal priming site for end-to-end cDNA amplification. Thus, cDNA without these sequences, such as prematurely terminated cDNAs, containing genomic DNA, or cDNA transcribed from Poly A minus RNA, are not exponentially amplified. The beads are washed with 80% ethanol, eluted, and sheared using the Covaris, which results in 200-500bp DNA fragments. The fragmented cDNA library is end-repaired, A-tailed, and adapters are ligated. Indexed libraries that meet appropriate cut-offs for both are quantified by qRT-PCR using a commercially available kit (KAPA Biosystems) and insert size distribution determined with the LabChip GX. Samples yielding of ≥0.5 ng/μl are sequenced..

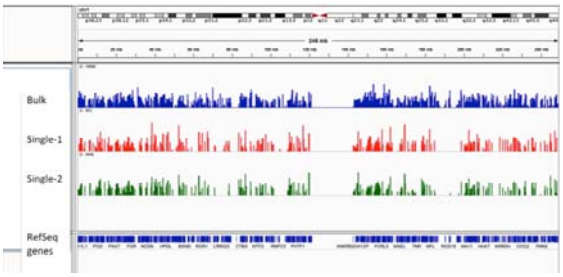


Figure 5. Results from paired RNA-Seq studies shows reads mapped to Chr1 for two single cell RNA-seq and one bulk RNA-seq sample.

Illumina HiSeq Sequencing. Sample concentrations are normalized to 2 nM and loaded onto Illumina Rapid version 1 flow cells at a concentration that yields 140-170 million passing filter clusters per lane. Samples are sequenced using 75 bp, paired-end sequencing on an Illumina HiSeq 2500. The 6 bp index is read during an additional sequencing read that automatically follows the completion of read 1. Data generated during sequencing runs are simultaneously transferred to the YCGA high-performance computing cluster. A positive control (prepared bacteriophage Phi X library) provided by Illumina is spiked into every lane at a concentration of 0.3% to monitor sequencing quality in real time. Gene expression in samples from batched analysis allows us to probe the mechanisms underlying differential transcriptional program related to asthma severity.

Data Analysis and Storage: The sequencing data will be transferred and stored in Yale HPC server Bulldog-N for coordinated analysis with **Project 3, Asthma MAP**, who will create a workflow in a uniform fashion using the RSEQtools pipeline to quantify transcript abundances, identify splicing and modifications, annotate transcripts to portions of the genome, and identify non-coding RNAs and transcribed pseudogenes. The statistical expertise of Dr. Yan (Core B), and her knowledge of asthmatic transcriptional patterns, will be especially valuable for coordinating analytic approaches. Signal intensities are converted to individual base calls during a run using the system's Real Time Analysis (RTA) software. Base calls are transferred from the machine's dedicated personal computer to the Yale High Performance Computing cluster via a 1 Gigabit network mount for downstream analysis. Primary analysis - sample de-multiplexing and alignment to the

human genome - is performed using Illumina's CASAVA 1.8.2 software suite. Only data with sample error rate is less than 2% will be submitted to the analytic team in Project 3. Technical noise and dynamic range will be assessed by using RNA control spike-in standards and by comparing single cells with the bulk samples.

Aim 3B. Single Cell RNA-Seq. Recent advances in analysis of cell transcription at the single cell level should allow distinguishing critical gene pathways relevant to disease severity. Single cell RNA-Seq complements bulk RNA-seq in characterizing the heterogeneity among different cell types (**Fig. 5**), circumventing any issues of mRNA abundance, and supporting assignment of particular transcripts to individual cell types as part of distinguishing heterogeneity of asthma endotypes.

Cells are separated as single cell and captured using the Fluidigm C1 integrated fluidics circuits (**Fig. 4**)(IFC). Our protocols follow manufacturer's instruction with strict RNase free working conditions in consideration of the RNA starting material. A suspension of single cells (200 – 1000 cells) is introduced in the cell input well of the C1 IFC leading to ~75% of wells having a single cell. Selection of nest size (10-17 μM) in the IFC allows the exclusion of squamous cells from the preparation. After confirmation that the cells are present, the prepared lysis, reverse transcription, and PCR reagents are added to the IFC to convert polyA+ RNA into full-length cDNA with SMARTer Ultra Low Input RNA Kit (Clontech), and perform universal amplification of the cDNA transcripts producing single-stranded cDNA that contains the SMARTer universal tag sequence. Prematurely terminated cDNAs, contaminating genomic DNA, or cDNA transcribed from RNA without polyA tail will not contain universal tag at both ends and will not be exponentially amplified. However, degraded RNAs present in low-quality RNA that still have polyA tails may be amplified, yielding shorter cDNA fragments with incomplete coverage at the 5′ end of the transcript. Full-length transcripts are enriched during PCR, because of pairing of the SMARTer tag at the 5′ end of the cDNA with its own reverse complement at the 3′ end. The mRNA library prep script on the Fluidigm C1 instrument runs for ~8 hours. Amplicons (~3.5 μl) are harvested from the C1 plate, diluted for library preparation (Illumin's Nextera XT DNA protocol).

Single cell RNA-Seq Library Preparation and Sequencing. Concentrations of the cDNAs (PicoGreen assay) are normalized (0.10– 0.3 ng/ul) using the C1 Harvest reagent and then Tagmentation, which employs in vitro transposition to simultaneously fragment and tag DNA in a single-well reaction. Tagmentation is followed by a limited-cycle (12 cycles) PCR reaction, which ligates the Illumina adaptors and dual indexes the samples. Purification using AMPure XP bead purification (0.7x) and quantifying recovered DNA by qRT-PCR using a commercially available kit (KAPA Biosystems). Insert size distribution is determined with the LabChip GX or Bioanalyzer. Samples with a yield of ≥0.5 ng/ul are used for sequencing. Each RNA-Seq library is layered on the Illumina flow cell, bridge amplified to get around 40- 45 million raw reads. Saturation analysis confirmed that this sequencing depth is sufficient to detect most genes expressed by single cells. The DNA reads on the flow cell will be sequenced on Genome Analyzer IIx using appropriate base pair sequencing recipe. At the current rate, on an average 33-37 million passing filter reads per lane are obtained on the genome Analyzer IIx, which yield 2.3 to 3.7 billion sequenced bases using 100 bp single end sequencing recipe (~1.2 h/base).

Data Analysis and Storage: Data will be processed by **Project 3, Asthma MAP** (see above).

Expected results, **and alternate approaches.** Our collaborative multidisciplinary team has developed the infrastructure and workflow to ensure success for Core C in conducting the high dimensional measurements outlined above. The key technologies are already in use and we do not anticipate any significant experimental difficulties (5, 46, 47). Important resources for the current project are our established channels of communication and data sharing, and track record and infrastructure for translational research.

- **Variation of assays.** We incorporate safeguards to minimize variation including rigorous quality control of cell viability, constant lots of reagents, and 4 markers (EQ™ Calibration Beads) for CyTOF normalization.
- **Limited cell number.** CyTOF can take most advantage of available cells and we have shown detection from only 10,000 cells. If needed, we can also collect samples from bronchoalveolar lavage (7).
- **Add Alternative assays** as needed in the Projects, e.g., microRNAs, using a specific enrichment.

Protection of Human Subjects

Risks to Human Subjects

a. Human Subjects Involvement and Characteristics. Over the course of this project, we plan to recruit 200 asthmatic and 50 non-asthmatic subjects through the Yale Center for Asthma and Airway Diseases (YCAAD). Each subject will return approximately every 9 months and complete 3 visits over the 5-year grant period. At each of the 3 visits the subject will be administered a questionnaire and perform physiological testing, and blood and sputum samples will be obtained. Asthmatic and non-asthmatic subjects recruited for these studies will be 12 years old or greater. Through a distinct HIC protocol, for these studies we will also recruit asthmatic and non-asthmatic children (age 7-17 years old) undergoing adenotonsillectomy, to donate tonsillar tissue, blood samples, and nasal brushings to the YCAAD biorepository.

Drs. Cohn and Chupp have recruited subjects for the past 15 years through the *Mechanisms and Mediators of Asthma and Chronic Obstructive Pulmonary Disease (COPD) human investigation* protocol (Yale HIC 0102012268, PI Chupp, Co-I Cohn). Over 800 subjects have been recruited to date. Tonsillar tissue will be obtained as part of the *Correlation of Gene Expression Profile with Clinical Response to Adenotonsillectomy* protocol (Yale HIC 1009007345, PI Karas, Co-I Chupp). This is a repository is to investigate the relationship between adenotonsillar hypertrophy and asthma and airway diseases, and identify genes, molecules and pathways that are associated with disease development, progression, and severity.

YCAAD Study Protocol: Exclusions will be based on health considerations. We will exclude any patient with a contraindication to any of the required testing including sputum induction, spirometry, or phlebotomy. These exclusions include severe cardiovascular, renal, or liver disease, bleeding diathesis, or advanced neuromuscular disease. A centerpiece of the YCAAD translational research program and this proposal is the airways disease phenotyping protocol developed to study human asthma. This protocol has been developed to acquire the full complement of clinical information, physiologic measurements and biologic samples to perform genotype-phenotype studies. Following informed consent, subjects are administered a questionnaire by the study coordinator. This includes a detailed asthma and medical history, asthma activity questionnaire (Asthma Control Test), Asthma Quality of Life Questionnaire, and details about medications, histories of smoking, occupational and environmental exposures, history of allergy, sinus disease, gastroesophageal reflux, obesity, and medication compliance. All data is uploaded into a HIPAA compliant web-based database developed by YCAAD. Blood is drawn and processed for the isolation of DNA, RNA, serum and plasma. Samples are aliquoted and banked for future studies. Blood is sent to the clinical laboratory for CBC and differential, serum IgE level, ImmunoCap testing for 22 common aeroallergens and routine chemistries. Following phlebotomy, patients undergo FeNO testing and spirometry (in adherence with ATS guidelines) before and after short acting bronchodilator administration. Sputum induction is done using hypertonic saline and immediately processed. Mucus plugs are removed using a dissecting microscope to remove squamous cell contamination, the cellular and aqueous compartments are separated, cell counts and differentials are quantified, and aliquots of supernatants, cell protein, and cellular RNA are stored for later analysis. The protocol is practical, efficient, and enrolls large numbers of subjects in a timely fashion. We will phenotype up to 5 subjects weekly. Subjects will be scheduled to return for repeat sampling as described, which is important for determination of the stability of their disease and their biological samples.

Asthma Inclusion criteria. Recruitment through YCAAD is designed to enroll a wide-range of asthmatic subjects in terms of age, disease severity and ongoing treatment. Inclusion criteria for asthmatic subjects are: (1) ≥ 12 years of age; (2) < 10 pack years of tobacco, and have not smoked for ≥ 1 year. Asthmatic subjects must have a diagnosis of asthma defined by the following criteria (based on the National Asthma and Education and Prevention Panel Guidelines): (1) a history of chronic symptoms (>2 years) compatible with asthma (determined by a study investigator/pulmonary physician specializing in asthma); and (2) historical evidence of variable airflow obstruction determine by: (a) > 12% improvement in FEV_1 after an inhaled $beta_2$ agonist, inhaled corticosteroids, or an oral prednisone burst; (b) methacholine reactivity with a provocative concentration causing a 20% decrease in FEV_1(PC20) of <8 mg/ml; or (c) 20% diurnal variation of peak expiratory flow rates on 2 days over a 2-3 week period. Patients will be excluded if they cannot safely undergo the studies required for participation or have other chronic lung disease or asthma variant disease (ABPA, Churg-Strauss Syndrome, vocal cord dysfunction, etc.). Normal subjects must have no history of any chronic lung disease. Non-asthmatic control subjects will be excluded if they have a diagnosis of any ongoing acute or chronic lung disease, have smoked ≥10 pack-years or have abnormal spirometry.

Adenotonsillectomy Study Protocol. Parents and patients identified by providers as potential subjects will be

invited to participate in the research study after they have been consented independently for their planned procedure of adenotonsillectomy. The parent and child will complete a YCAAD pediatric questionnaire and an asthma activity score, and spirometry will be done for those children aged six years or older who are capable of completing the test. Intraoperatively, a venous blood sample (5-10ml) and nasal swab sample will be obtained. After a portion of the tissue is sent to surgical pathology, as is standard for adenotonsillectomy, a portion of the removed tonsils and/or adenoid tissue will be taken for study purposes. An additional specimen will be held in the pathology department, flash frozen in OCT in the event more tissue is needed for analysis. Samples will be processed and banked with the assigned de-identified study subject number. A second study visit will take place six months following surgery and includes an interim history using the follow-up questionnaire focused on stability of upper airway and asthma symptoms. Repeat PFT testing will be performed in children aged six years or older, if possible. An additional venous blood sample will be obtained for the repository. Subjects may decline any of the testing at any of these visits.

b. Sources of Materials. In this proposal, the following materials will be collected from human subjects: clinical data (questionnaire, history and physical examination data, lung function data and relevant laboratory test results), blood, sputum, nasal brushings and tonsils/adenoids. Clinical data will be identified with the study subject's name. This information will be maintained in a locked file and these records are kept separate from other clinical records to assure confidentiality. Only Drs. Cohn, Chupp, Niu, and study coordinators will have access to these confidential records. All research specimens, including blood, and sputum specimens are identified by a patient code for correlation purposes. All samples and datasets will be de-identified and only known to study investigators involved in consent and sample acquisition. Investigators and staff will receive only de-identified data and samples for their studies and analyses. All data sets uploaded and samples delivered to Core C and the Yale Center for Genomic Analysis will be de-identified.

The studies in this proposal will adhere to the U.S. Department of Health and Human Services Office for the Protection from Research Risk (OPRR). All information relating to participating subjects will be de-identified. Specimens may only be used in accordance with the conditions stipulated in the Yale Human Investigation Committee (HIC) (see approval letters in Appendix).

c. Potential Risks. Risks associated with the YCAAD study (HIC 0102012268) including pulmonary function tests, blood draw and sputum induction are minimal (adults) and greater than minimal (children 12-17 years old) and are explained to subjects in detail at the time of consent (see consent forms in Appendix). Bronchospasm is a risk of sputum induction, but the safety of sputum induction in asthmatic subjects, even those with severe asthma has been well documented in several studies (ref). Spirometry is repeatedly tested during the procedure to monitor for bronchospasm, and if wheezing or a significant drop in FEV1 occurs, subjects are given bronchodilators. Wheezing, if it occurs, is usually transient and treatable with bronchodilators. A Data and Safety Monitoring (DSM) Plan and DSM Committee are in place to assess adverse events (see Appendix). To date, we have had no serious adverse events in our laboratory, even in severe asthma subjects undergoing sputum induction. Clinical Recruitment and Biostatistics Core Lead (Dr. Cohn) or a research coordinator will monitor all procedures performed. After any procedure performed in YCAAD, subjects are instructed to contact the YCAAD on-call physician if any problem occurs. YCAAD is covered 24 x 7 by pulmonary physicians who are familiar with the management of acute bronchospasm in asthmatic patients. Risks associated with the adenotonsillectomy study (Yale HIC 1009007345) are minimal for the blood draw and there is no added risk of apportioning adenoid and tonsil tissue to the biorepository. Emergency equipment and personnel are immediately available in the event of an adverse reaction at all times during these study visits.

Adequacy of Protection Against Risks

a. Recruitment and Informed Consent. Subjects will be recruited from YCAAD and from the pediatric ENT offices of Dr. Karas. Once a subject is deemed eligible for this study, informed consent will be obtained. The study procedures, risks and benefits will be clearly explained to each subject. All questions will be answered, and time will be allowed for the subject to make an informed decision before signing the consent form. If the subject agrees to enroll, a consent form (see Appendix) will be reviewed and explained, and the subject or consenting adult will be asked to sign it.

b. Protections Against Risk. Risks will be minimized by appropriate subject exclusions, close medical supervision throughout the protocol, and adaptation of testing to identify patients who might be at higher risk. Subjects experiencing adverse events will have access to full inpatient medical facilities of the Yale-New

Haven Hospital if more intensive treatment is necessary to reverse an asthma attack or complication during the study. All patients will be under full-time coordinator supervision throughout every phase of the YCAAD procedures. In the adenotonsillectomy protocol, at least one physician and/or nurse will be present throughout the study visits and procedures. Adverse events will be reported to the Yale IRB (Human Investigation Committee) and the DSMC.

Potential Benefits of the Proposed Research to Human Subjects and Importance of the Knowledge to be Gained

These studies are not designed to provide direct benefit to the participants. However, the knowledge gained from this work will enhance information about the pathogenesis of asthma and may lead to improved diagnosis, treatment and prevention of asthma in the future. All subjects receive asthma education and copies of blood work and lung function tests as part of their visit, and have full access to a YCAAD physician, if desired. If any major test abnormalities are encountered, YCAAD physicians counsel the subjects and provide referrals to appropriate specialists as needed.

Inclusion of Women and Minorities

Subjects will be recruited through the Clinical Recruitment and Biostatistics Core (Core B) and through the office of Dr. Karas for adenotonsillectomy without any bias of race or gender. The YCAAD asthma and control populations and the children undergoing adenotonsillectomy are racially diverse and reflect the population in New Haven and surrounding communities. All minority groups already take part in these research programs and we will continue to actively recruit women and minorities as these studies go forward. Based on demographics from past studies, the asthmatic population in YCAAD is approximately 71% female and 31% Black and Hispanic. The demographics of the adenotonsillectomy population is approximately 37% female, 14% Black and 36% Hispanic.

OMB Number: 0925-0002

Planned Enrollment Report

Study Title: NextGen Biorepository Study

Domestic/Foreign: Domestic

Comments:

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/Alaska Native	0	0	0	0	**0**
Asian	3	2	0	0	**5**
Native Hawaiian or Other Pacific Islander	0	0	0	0	**0**
Black or African American	20	18	8	8	**54**
White	85	84	12	10	**191**
More than One Race	0	0	0	0	**0**
Total	**108**	**104**	**20**	**18**	**250**

Study 1 of 2

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

OMB Number: 0925-0002

Planned Enrollment Report

Study Title: Adenotonsillectomy Biorepository Study

Domestic/Foreign: Domestic

Comments:

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/Alaska Native	0	0	0	0	**0**
Asian	2	2	0	0	**4**
Native Hawaiian or Other Pacific Islander	0	0	0	0	**0**
Black or African American	12	10	6	4	**32**
White	28	22	8	6	**64**
More than One Race	0	0	0	0	**0**
Total	**42**	**34**	**14**	**10**	**100**

Study 2 of 2

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

Inclusion of Children

The proposal will recruit children between the ages of 2 and 21 years (consent and assent form are included in Appendix 1 and 2). The investigative team for Yale #HIC0102012268 will request that the participants (12-21 years old) undergo phlebotomy, sputum induction, lung function testing and complete an asthma questionnaire with the subject and his/her parent/legal guardian. Venipuncture and pulmonary function testing and questioning are part of the routine care of children with asthma. Sputum induction is a low-risk procedure and is outlined in the Appendix. All interviews and procedures will be performed in the Yale Center for Asthma and Airways Disease. Overall, the study poses more than a minimal risk to minor subjects, but its benefits outweigh these risks. The investigative team for Yale # HIC1009007345 will request that the participant (2-18 years old) who was previously scheduled to undergo adenotonsillectomy, consent to have blood drawn intraoperatively, perform spirometry before surgery, if capable, and agree to donate excess tonsil tissue to the biorepository for analyses. These procedures are assessed to be of minimal risk to child subjects. The investigative team has experience caring for children should any adverse events arise during sample acquisition.

Inclusion of children in this study is important since we are interested in the relationship of transcriptomic endophenotypes across the spectrum of asthma. Our studies thus far suggest that there are stable Transcriptional Endophenotypes of Asthma (TEA) in children and adults with severe asthma and, in particular, near fatal asthma. Endotypes seen in adult asthmatics may be evident in young subjects, and this may help us predict the course of disease as they age. Further, we may be able to discern which patients will benefit from more aggressive care and elucidate molecular pathways involved in the pathogenesis of severe asthma. We will follow subjects longitudinally and will define how transcriptomic signatures relate with progression of this disease.

Literature Cited

1. Akinbami, L. J., J. E. Moorman, and X. Liu. 2011. Asthma prevalence, health care use, and mortality: United States, 2005-2009. *Natl Health Stat Report*: 1-14.
2. Cohn, L., J. A. Elias, and G. L. Chupp. 2004. Asthma: mechanisms of disease persistence and progression. *Annu Rev Immunol* 22: 789-815.
3. Moore, W. C., D. A. Meyers, S. E. Wenzel, W. G. Teague, H. Li, X. Li, R. D'Agostino, Jr., M. Castro, D. Curran-Everett, A. M. Fitzpatrick, B. Gaston, N. N. Jarjour, R. Sorkness, W. J. Calhoun, K. F. Chung, S. A. Comhair, R. A. Dweik, E. Israel, S. P. Peters, W. W. Busse, S. C. Erzurum, E. R. Bleecker, L. National Heart, and P. Blood Institute's Severe Asthma Research. 2010. Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. *Am J Respir Crit Care Med* 181: 315-323.
4. Woodruff, P. G., B. Modrek, D. F. Choy, G. Jia, A. R. Abbas, A. Ellwanger, L. L. Koth, J. R. Arron, and J. V. Fahy. 2009. T-helper type 2-driven inflammation defines major subphenotypes of asthma. *Am J Respir Crit Care Med* 180: 388-395.
5. Yao, Y., R. Liu, M. S. Shin, M. Trentalange, H. Allore, A. Nassar, I. Kang, J. Pober, and R. R. Montgomery. 2014. CyTOF supports efficient detection of immune cell subsets from small samples. *J. Immunol. Methods.* 415: 1-5.
6. Chupp, G. L., C. G. Lee, N. Jarjour, Y. M. Shim, C. T. Holm, S. He, J. D. Dziura, J. Reed, A. J. Coyle, P. Kiener, M. Cullen, M. Grandsaigne, M. C. Dombret, M. Aubier, M. Pretolani, and J. A. Elias. 2007. A chitinase-like protein in the lung and circulation of patients with severe asthma. *N Engl J Med* 357: 2016-2027.
7. Ober, C., Z. Tan, Y. Sun, J. D. Possick, L. Pan, R. Nicolae, S. Radford, R. R. Parry, A. Heinzmann, K. A. Deichmann, L. A. Lester, J. E. Gern, R. F. Lemanske, Jr., D. L. Nicolae, J. A. Elias, and G. L. Chupp. 2008. Effect of variation in CHI3L1 on serum YKL-40 level, risk of asthma, and lung function. *N Engl J Med* 358: 1682-1691.
8. Ober, C., and G. L. Chupp. 2009. The chitinase and chitinase-like proteins: a review of genetic and functional studies in asthma and immune-mediated diseases. *Curr Opin Allergy Clin Immunol* 9: 401-408.
9. Levin, J. C., L. Gagnon, X. He, E. D. Baum, D. E. Karas, and G. L. Chupp. 2014. Improvement in asthma control and inflammation in children undergoing adenotonsillectomy. *Pediatr Res* 75: 403-408.
10. Ortega, H., G. Chupp, P. Bardin, A. Bourdin, G. Garcia, B. Hartley, S. Yancey, and M. Humbert. 2014. The role of mepolizumab in atopic and nonatopic severe asthma with persistent eosinophilia. *Eur Respir J* 44: 239-241.
11. Yan, X., J. Chu, J. Gomez, M. Koenigs, C. Holm, X. He, M. F. Perez, H. Zhao, S. Mane, F. D. Martinez, C. Ober, D. L. Nicolae, K. C. Barnes, S. J. London, F. Gilliland, S. Weiss, T., B. A. Raby, L. Cohn, and G. L. Chupp. 2015. Non-invasive analysis of the sputum transcriptome discriminates clinical phenotypes of asthma. *Amer. J. Respir. Crit. Care Med.* 191: 1116-1125.
12. Strauss-Albee, D. M., J. Fukuyama, E. C. Liang, Y. Yao, J. A. Jarrell, A. L. Drake, J. Kinuthia, R. R. Montgomery, G. John-Stewart, S. Holmes, and C. A. Blish. 2015. NK cell repertoire diversity reflects immune experience and predicts viral susceptibility. *Sci Trans Med* 7: 297ra115.
13. Lee, N., S. You, M. S. Shin, W. W. Lee, K. S. Kang, S. H. Kim, W. U. Kim, R. J. Homer, M. J. Kang, R. R. Montgomery, C. S. Dela Cruz, A. C. Shaw, P. J. Lee, G. L. Chupp, D. D. Hwang, and I. Kang. 2014. IL-6 receptor alpha defines effector memory CD8+ T cells producing Th2 cytokines and expanding in asthma. *Am J Resp Crit Care Med* 190: 1383-1394.
14. Bandura, D. R., V. I. Baranov, O. I. Ornatsky, A. Antonov, R. Kinach, X. Lou, S. Pavlov, S. Vorobiev, J. E. Dick, and S. D. Tanner. 2009. Mass cytometry: technique for real time single cell multitarget immunoassay based on inductively coupled plasma time-of-flight mass spectrometry. *Anal Chem* 81: 6813-6822.
15. Ornatsky, O., D. Bandura, V. Baranov, M. Nitz, M. A. Winnik, and S. Tanner. 2010. Highly multiparametric analysis by mass cytometry. *J Immunol Methods* 361: 1-20.
16. Bendall, S. C., E. F. Simonds, P. Qiu, A. D. Amir el, P. O. Krutzik, R. Finck, R. V. Bruggner, R. Melamed, A. Trejo, O. I. Ornatsky, R. S. Balderas, S. K. Plevritis, K. Sachs, D. Pe'er, S. D. Tanner, and G. P. Nolan. 2011. Single-cell mass cytometry of differential immune and drug responses across a human hematopoietic continuum. *Science* 332: 687-696.

17. Han, A., E. W. Newell, J. Glanville, N. Fernandez-Becker, C. Khosla, Y. H. Chien, and M. M. Davis. 2013. Dietary gluten triggers concomitant activation of CD4+ and CD8+ alphabeta T cells and gammadelta T cells in celiac disease. *Proc Natl Acad Sci U S A* 110: 13073-13078.
18. Gaudilliere, B., G. K. Fragiadakis, R. V. Bruggner, M. Nicolau, R. Finck, M. Tingle, J. Silva, E. A. Ganio, C. G. Yeh, W. J. Maloney, J. I. Huddleston, S. B. Goodman, M. M. Davis, S. C. Bendall, W. J. Fantl, M. S. Angst, and G. P. Nolan. 2014. Clinical recovery from surgery correlates with single-cell immune signatures. *Sci Transl Med* 6: 255ra131.
19. Nair, N., H. E. Mei, S. Y. Chen, M. Hale, G. P. Nolan, H. T. Maecker, M. Genovese, C. G. Fathman, and C. C. Whiting. 2015. Mass cytometry as a platform for the discovery of cellular biomarkers to guide effective rheumatic disease therapy. *Arthritis Res Ther* 17: 127.
20. Nair, N., E. W. Newell, C. Vollmers, S. R. Quake, J. M. Morton, M. M. Davis, X. S. He, and H. B. Greenberg. 2015. High-dimensional immune profiling of total and rotavirus VP6-specific intestinal and circulating B cells by mass cytometry. *Mucosal Immunol*.
21. O'Gorman, W. E., E. W. Hsieh, E. S. Savig, P. F. Gherardini, J. D. Hernandez, L. Hansmann, I. M. Balboni, P. J. Utz, S. C. Bendall, W. J. Fantl, D. B. Lewis, G. P. Nolan, and M. M. Davis. 2015. Single-cell systems-level analysis of human Toll-like receptor activation defines a chemokine signature in patients with systemic lupus erythematosus. *J Allergy Clin Immunol* in press.
22. Giesen, C., H. A. Wang, D. Schapiro, N. Zivanovic, A. Jacobs, B. Hattendorf, P. J. Schuffler, D. Grolimund, J. M. Buhmann, S. Brandt, Z. Varga, P. J. Wild, D. Gunther, and B. Bodenmiller. 2014. Highly multiplexed imaging of tumor tissues with subcellular resolution by mass cytometry. *Nat Methods* 11: 417-422.
23. Angelo, M., S. C. Bendall, R. Finck, M. B. Hale, C. Hitzman, A. D. Borowsky, R. M. Levenson, J. B. Lowe, S. D. Liu, S. Zhao, Y. Natkunam, and G. P. Nolan. 2014. Multiplexed ion beam imaging of human breast tumors. *Nat Med* 20: 436-442.
24. Vidal, S., J. Bellido-Casado, C. Granel, A. Crespo, V. Plaza, and C. Juarez. 2012. Flow cytometry analysis of leukocytes in induced sputum from asthmatic patients. *Immunobiology* 217: 692-697.
25. Schleich, F. N., M. Manise, J. Sele, M. Henket, L. Seidel, and R. Louis. 2013. Distribution of sputum cellular phenotype in a large asthma cohort: predicting factors for eosinophilic vs neutrophilic inflammation. *BMC Pulm Med* 13: 11.
26. Hanania, N. A., M. J. King, S. S. Braman, C. Saltoun, R. A. Wise, P. Enright, A. R. Falsey, S. K. Mathur, J. W. Ramsdell, L. Rogers, D. A. Stempel, J. J. Lima, J. E. Fish, S. R. Wilson, C. Boyd, K. V. Patel, C. G. Irvin, B. P. Yawn, E. A. Halm, S. I. Wasserman, M. F. Sands, W. B. Ershler, and D. K. Ledford. 2011. Asthma in the elderly: Current understanding and future research needs--a report of a National Institute on Aging (NIA) workshop. *J Allergy Clin Immunol* 128: S4-24.
27. Moore, W. C., A. T. Hastie, X. Li, H. Li, W. W. Busse, N. N. Jarjour, S. E. Wenzel, S. P. Peters, D. A. Meyers, E. R. Bleecker, L. National Heart, and P. Blood Institute's Severe Asthma Research. 2014. Sputum neutrophil counts are associated with more severe asthma phenotypes using cluster analysis. *J Allergy Clin Immunol* 133: 1557-1563.
28. Ungurs, M. J., N. J. Sinden, and R. A. Stockley. 2014. Progranulin is a substrate for neutrophil-elastase and proteinase-3 in the airway and its concentration correlates with mediators of airway inflammation in COPD. *Am J Physiol Lung Cell Mol Physiol* 306: L80-87.
29. Qiu, P., E. F. Simonds, S. C. Bendall, K. D. Gibbs, Jr., R. V. Bruggner, M. D. Linderman, K. Sachs, G. P. Nolan, and S. K. Plevritis. 2011. Extracting a cellular hierarchy from high-dimensional cytometry data with SPADE. *Nat Biotechnol* 29: 886-891.
30. Yao, Y., Q. Liu, N. Niu, X. Yan, L. Cohn, G. L. Chupp, and R. R. Montgomery. 2015. Multiparameter functional single cell profiling of airway immunity. *in preparation*.
31. Strauss-Albee, D. M., J. Fukuyama, E. C. Liang, Y. Yao, J. A. Jarrell, A. L. Drake, J. Kinuthia, R. R. Montgomery, G. John-Stewart, S. Holmes, and C. A. Blish. 2015. NK cell repertoire diversity reflects immune experience and predicts viral susceptibility. *Sci Trans Med* in press.
32. Wong, K. L., J. J. Tai, W. C. Wong, H. Han, X. Sem, W. H. Yeap, P. Kourilsky, and S. C. Wong. 2011. Gene expression profiling reveals the defining features of the classical, intermediate, and nonclassical human monocyte subsets. *Blood* 118: e16-31.
33. Yang, M., R. K. Kumar, P. M. Hansbro, and P. S. Foster. 2012. Emerging roles of pulmonary macrophages in driving the development of severe asthma. *J Leukoc Biol* 91: 557-569.

34. Dudley, D., D. M. Baker, M. J. Hickey, and D. D. Hickstein. 1989. Expression of surface antigen and mRNA for the CD11c (alpha X, p150) subunit of the human leukocyte adherence receptor family in hematopoietic cells. *Biochem Biophys Res Commun* 160: 346-353.
35. Pons, A. R., A. Noguera, D. Blanquer, J. Sauleda, J. Pons, and A. G. Agusti. 2005. Phenotypic characterisation of alveolar macrophages and peripheral blood monocytes in COPD. *Eur Respir J* 25: 647-652.
36. Fu, J., M. C. Tobin, and L. L. Thomas. 2014. Neutrophil-like low-density granulocytes are elevated in patients with moderate to severe persistent asthma. *Ann Allergy Asthma Immunol* 113: 635-640 e632.
37. Akira, S., K. Takeda, and T. Kaisho. 2001. Toll-like receptors: critical proteins linking innate and acquired immunity. *Nat Immunol* 2: 675-680.
38. Chun, E., S. H. Lee, S. Y. Lee, E. J. Shim, S. H. Cho, K. U. Min, Y. Y. Kim, and H. W. Park. 2010. Toll-like receptor expression on peripheral blood mononuclear cells in asthmatics; implications for asthma management. *J Clin Immunol* 30: 459-464.
39. Mroz, R. M., A. Holownia, P. Wielgat, A. Sitko, T. Skopinski, E. Chyczewska, and J. J. Braszko. 2013. Siglec-8 in induced sputum of COPD patients. *Adv Exp Med Biol* 788: 19-23.
40. Bodenmiller, B., E. R. Zunder, R. Finck, T. J. Chen, E. S. Savig, R. V. Bruggner, E. F. Simonds, S. C. Bendall, K. Sachs, P. O. Krutzik, and G. P. Nolan. 2012. Multiplexed mass cytometry profiling of cellular states perturbed by small-molecule regulators. *Nat Biotechnol* 30: 858-867.
41. Finck, R., E. F. Simonds, A. Jager, S. Krishnaswamy, K. Sachs, W. Fantl, D. Pe'er, G. P. Nolan, and S. C. Bendall. 2013. Normalization of mass cytometry data with bead standards. *Cytometry A* 83: 483-494.
42. Amir el, A. D., K. L. Davis, M. D. Tadmor, E. F. Simonds, J. H. Levine, S. C. Bendall, D. K. Shenfeld, S. Krishnaswamy, G. P. Nolan, and D. Pe'er. 2013. viSNE enables visualization of high dimensional single-cell data and reveals phenotypic heterogeneity of leukemia. *Nat Biotechnol* 31: 545-552.
43. Chen, T. J., and N. Kotecha. 2014. Cytobank: providing an analytics platform for community cytometry data analysis and collaboration. *Curr Top Microbiol Immunol* 377: 127-157.
44. Levine, J. H., E. F. Simonds, S. C. Bendall, K. L. Davis, A. D. Amir el, M. D. Tadmor, O. Litvin, H. G. Fienberg, A. Jager, E. R. Zunder, R. Finck, A. L. Gedman, I. Radtke, J. R. Downing, D. Pe'er, and G. P. Nolan. 2015. Data-Driven Phenotypic Dissection of AML Reveals Progenitor-like Cells that Correlate with Prognosis. *Cell* 162: 184-197.
45. Krishnaswamy, S., M. H. Spitzer, M. Mingueneau, S. C. Bendall, O. Litvin, E. Stone, D. Pe'er, and G. P. Nolan. 2014. Systems biology. Conditional density-based analysis of T cell signaling in single-cell data. *Science* 346: 1250689.
46. Qian, F., J. Thakar, X. Yuan, M. Nolan, K. O. Murray, W. T. Lee, S. J. Wong, H. Meng, E. Fikrig, S. H. Kleinstein, and R. R. Montgomery. 2014. Immune markers associated with host susceptibility to infection with West Nile virus. *Viral Immunology* 27: 39-47.
47. Qian, F., L. Chung, W. Zheng, V. M. Bruno, R. P. Alexander, Z. Wang, X. Wang, S. Kurscheid, H. Zhao, E. Fikrig, M. Gerstein, M. Snyder, and R. R. Montgomery. 2013. Identification of genes critical for resistance to infection by West Nile virus using RNA-Seq analysis. *Viruses* 5: 1664-1681.
48. Qian, F., G. Goel, H. Meng, X. Wang, F. You, L. Devine, K. Raddassi, M. N. Garcia, K. O. Murray, C. R. Bolen, R. Gaujoux, S. S. Shen-Orr, D. Hafler, E. Fikrig, R. J. Xavier, S. H. Kleinstein, and R. R. Montgomery. 2015. Systems Immunology reveals markers of susceptibility to West Nile virus infection. *Clin. Vacc. Immunol.* 22: 6-16.

Resource Sharing Plan

Data generated in these studies will be freely available to members of the research community with a goal to enable dissemination of data, while also protecting the privacy of the participants and the utility of the data, by de-identifying and masking potentially sensitive data elements in compliance with the NIH public data sharing policy. All other resources developed in the course of the proposed studies will be available by request to qualified academic investigators for non-commercial research.

For all studies, we will follow the National Institute of Health's Genomic Data Sharing Policy. The raw datasets corresponding to expression, genomic, and genetic data generated by these studies will be submitted to Gene Expression Omnibus (GEO) or the Sequence Read Archive (SRA) for use by other investigators. As datasets are analyzed, then validated, we will proceed with deposition in ImmPort according to a timeline negotiated with the Program Officer. Sample data in the YCAAD biorepository are, and will continue to be, available on the internet through the YCCI research accelerator, a publicly accessible platform for scientific collaboration (ycci.researchaccelerator.org). In addition, tools, pipelines, derived datasets and analyses will be made available through the website (asthmaMAP.gersteinlab.org) which will serve as an organizational tool for the participants in this cooperative proposal as well as a repository and resource for the greater research community. Details of the contents and construction of the asthma MAP website are in Project 3, Research Proposal Aim 3.

Yale University School of Medicine and all investigators will adhere to the NIH Grants Policy on Sharing of Unique Research Resources including the “Sharing of Biomedical Research Resources: Principles and Guidelines for Recipients of NIH Grants and Contracts” issued in December 1999. Specifically, material transfers would be made with no more restrictive terms than in the Simple Letter Agreement or the UBMTA and without reach through requirements. Should any intellectual property arise which requires a patent, we would ensure that the technology remains widely available to the research community in accordance with the NIH Principles and Guidelines document. In addition, we will provide relevant protocols and published data upon request. Accepted versions of final, peer-reviewed manuscripts emanating from this research will be deposited on-line to PubMed Central in accord with NIH Public Access.

OMB Number: 4040-0001
Expiration Date: 06/30/2016

APPLICATION FOR FEDERAL ASSISTANCE

SF 424 (R&R)

5. APPLICANT INFORMATION **Organizational DUNS*:** 0432075620000

Legal Name*: YALE UNIVERSITY
Department:
Division:
Street1*: OFFICE OF SPONSORED PROJECTS
Street2: 25 Science Park
City*: NEW HAVEN
County:
State*: CT: Connecticut
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 065208237

Person to be contacted on matters involving this application

Prefix:	First Name*:	Middle Name:	Last Name*:	Suffix:
	Marybeth		Brandi	

Position/Title: Proposal Manager
Street1*: 25 Science Park
Street2: 150 Munson Street
City*: New Haven
County:
State*: CT: Connecticut
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 06520-8237
Phone Number*: 203-737-3495 Fax Number: Email: marybeth.brandi@yale.edu

7. TYPE OF APPLICANT*

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
IOF Management Core

12. PROPOSED PROJECT

Start Date*	Ending Date*
07/01/2016	06/30/2021

OMB Number: 4040-0010
Expiration Date: 06/30/2016

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	YALE UNIVERSITY
Duns Number:	0432075620000
Street1*:	300 Cedar St
Street2:	TAC S440
City*:	NEW HAVEN
County:	
State*:	CT: Connecticut
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	065208237
Project/Performance Site Congressional District*:	CT-003

Project/Performance Site Location 1

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	YALE UNIVERSITY
DUNS Number:	0432075620000
Street1*:	300 Cedar St
Street2:	TAC S441
City*:	NEW HAVEN
County:	
State*:	CT: Connecticut
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	065208237
Project/Performance Site Congressional District*:	CT-003

File Name

Additional Location(s)

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ❍ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ❍ Yes ❍ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6

If NO, is the IRB review Pending? ❍ **Yes** ❍ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ❍ **Yes** ● **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ❍ Yes ❍ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. Is proprietary/privileged information included in the application?* ❍ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ❍ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ❍ Yes ❍ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ❍ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ❍ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Project_Summary_Core_D-IOF.pdf
8. Project Narrative*	
9. Bibliography & References Cited	Core_A_and_D_References_Cited.pdf
10.Facilities & Other Resources	
11.Equipment	

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

Project Summary

The objective of the IOF Core is to provide initial pilot support for research to develop new assays or to refine existing technologies for better defining mechanisms underlying the onset and progression of asthma and allergic diseases, as well as improving standardization across network members. The IOF Core also will seek to promote inter-disciplinary investigations and patient-oriented research, including the necessary infrastructure for cross-AADCRC analysis such as common data standards and validated assays for use by all network investigators. We recognize that there are few funding opportunities to support the cost of novel investigations in this area. An additional aim of this Core will be to attract new investigators to investigation of the immunopathophysiology of asthma or allergic diseases.

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: RUTH Middle Name R Last Name*: MONTGOMERY Suffix:

Position/Title*: Associate Professor
Organization Name*: YALE UNIVERSITY
Department:
Division:
Street1*: YALE UNIVERSITY SCH OF MEDICINE
Street2: DEPT OF INTERNAL MEDICINE
City*: NEW HAVEN
County:
State*: CT: Connecticut
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 065200000

Phone Number*: (203) 785-7039 Fax Number: (203) 785-7053 E-Mail*: RUTH.MONTGOMERY@YALE.EDU

Credential, e.g., agency login: RMONTGOMERY

Project Role*: Other (Specify) Other Project Role Category: Project Lead

Degree Type: Degree Year:

File Name

Attach Biographical Sketch*:

Attach Current & Pending Support:

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2016 **End Date*:** 06-30-2017 **Budget Period: 1**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	RUTH	R	MONTGOMERY		Project Lead		0.6			0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
1	Secretarial/Clerical	2.4			0.00	0.00	0.00
1	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2016 **End Date*:** 06-30-2017 **Budget Period: 1**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2016 **End Date*:** 06-30-2017 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**0.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**0.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**0.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: Core_D_IOF_Budget_Justification_Asthma_U19.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12004239 Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2017 **End Date*:** 06-30-2018 **Budget Period: 2**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		RUTH	R	MONTGOMERY		Project Lead		0.6			0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
1	Secretarial/Clerical	2.4			0.00	0.00	0.00
1	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2017 **End Date*:** 06-30-2018 **Budget Period: 2**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2017 **End Date*:** 06-30-2018 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**0.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**0.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**0.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name:
Core_D_IOF_Budget_Justification_Asthma_U19.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12004239 Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2018 **End Date*:** 06-30-2019 **Budget Period: 3**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	RUTH	R	MONTGOMERY		Project Lead		0.6			0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
1	Secretarial/Clerical	2.4			0.00	0.00	0.00
1	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2018 **End Date*:** 06-30-2019 **Budget Period: 3**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2018 **End Date*:** 06-30-2019 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**0.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**0.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**0.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: Core_D_IOF_Budget_Justification_Asthma_U19.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2019 **End Date*:** 06-30-2020 **Budget Period: 4**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		RUTH	R	MONTGOMERY		Project Lead		0.6			0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
1	Secretarial/Clerical	2.4			0.00	0.00	0.00
1	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2019 **End Date*:** 06-30-2020 **Budget Period: 4**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2019 **End Date*:** 06-30-2020 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**0.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**0.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**0.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: Core_D_IOF_Budget_Justification_Asthma_U19.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2020 **End Date*:** 06-30-2021 **Budget Period: 5**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	RUTH	R	MONTGOMERY		Project Lead		0.6			0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
1	Secretarial/Clerical	2.4			0.00	0.00	0.00
1	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2020 **End Date*:** 06-30-2021 **Budget Period: 5**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2020 **End Date*:** 06-30-2021 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**0.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**0.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**0.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: Core_D_IOF_Budget_Justification_Asthma_U19.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

CORE D Infrastructure and Opportunity Fund Management Core (IOF)

NOTE: If the IOF Core is funded, then the following will be requested:

BUDGET JUSTIFICATION

Key Personnel

Ruth Montgomery, PhD, (IOF Core Lead). Dr. Montgomery is an Associate Professor in Internal Medicine and a cell biologist with extensive experience with innate immune cells in translational studies. She is senior author on many recent publications defining immune status in human cohorts, such as age-related deficiencies in immune responses of macrophages, dendritic cells, and toll-like receptor pathways, and markers of individual susceptibility to viral infections. She has a track record of basic and translational research, and is the Director of the Yale CyTOF facility, a key resource in this proposal. As co-PI with Dr. Erol Fikrig of an NIH Biodefense contract HHS N272201100019C “Innate Immune Pathways in Elderly and Immunosuppressed Populations,” she has overseen enrollment of >1500 healthy individuals for studies of differential innate immune responsiveness in human cohorts. Dr. Montgomery has managed the successful launch of the CyTOF facility and is senior author on a recent manuscript demonstrating efficient detection of cell subsets by CyTOF starting with as few as 10,000 cells.

Dr. Montgomery will oversee the planning and execution of all phases of the IOF Pilot Core in coordination with NIAID staff including the timely announcement and dissemination of information about the program, management of awarded Pilot Projects, and the follow-up assessment of the success of each awarded project. Awarded proposals will be monitored via brief biannual progress reports and by face-to-face meetings. She will meet regularly with the Administrative Core and Executive Committee to review the status of the Pilot projects and address any issues that arise. Dr. Montgomery has a track record of experience in these functions over several years through the IOF for the NIAID-supported U19 program in Human Immunology (HIPC), Pilot Project Program for the Yale Rheumatic Disease Research Core (P30 AR053495), and participation in two NIH T32-supported training grants. She is well accustomed to mentoring junior investigators and research progress. Finally, she will oversee financial management and reporting requirements of the IOF Core. This is distinct from her duties as Core C Lead. Dr. Montgomery’s effort as IOF Core Lead will be 0.6 calendar months.

Non-Key Personnel

Susan Ardito, (Senior Administrative Assistant). Ms. Ardito is a Senior Administrative Assistant in the Section of Pulmonary, Critical Care and Sleep Medicine. She will be responsible for coordinating the Pilot Project proposals led by early stage investigators within the AADCRC, as well as for the development of resources that the AADCRC Steering Committee may deem necessary. If Yale University is selected by NIAID to manage the IOF for the entire AADCRC program, Ms. Ardito will assist the IOF Core Lead in this regard, including fund disbursement, administration, and reports. Ms. Ardito will meet regularly with the Core Lead to support efficient operation of the Core. Her effort in this Core will be 2.4 calendar months annually.

Other Expenses

Travel
Travel resources are requested for the Core Leader and Yale Asthma U19 Administrator to attend meetings with the U19 NIAID Program staff as needed.

Supplies and Other Expenses
Funds are requested in this Core to facilitate communication of the Core Lead with Pilot awardees, including web-based conferencing.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		0.00
Section B, Other Personnel		0.00
Total Number Other Personnel	5	
Total Salary, Wages and Fringe Benefits (A+B)		0.00
Section C, Equipment		0.00
Section D, Travel		0.00
1. Domestic	0.00	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		0.00
1. Materials and Supplies	0.00	
2. Publication Costs	0.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	0.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	0.00	
9. Other 2	0.00	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		0.00
Section H, Indirect Costs		0.00
Section I, Total Direct and Indirect Costs (G + H)		0.00
Section J, Fee		0.00

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

1. Project Director / Principal Investigator (PD/PI)

Prefix:
First Name*: GEOFFREY
Middle Name: L
Last Name*: CHUPP
Suffix:

2. Human Subjects

Clinical Trial? ● No ○ Yes
Agency-Defined Phase III Clinical Trial?* ○ No ○ Yes

3. Permission Statement*

If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?

○ Yes ● No

4. Program Income*

Is program income anticipated during the periods for which the grant support is requested? ○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

Budget Period*	Anticipated Amount ($)*	Source(s)*

PHS 398 Cover Page Supplement

5. Human Embryonic Stem Cells

Does the proposed project involve human embryonic stem cells?* ● No ❍ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s): ❑ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

6. Inventions and Patents (For renewal applications only)

Inventions and Patents*: ❍ Yes ● No

If the answer is "Yes" then please answer the following:

Previously Reported*: ❍ Yes ❍ No

7. Change of Investigator / Change of Institution Questions

❑ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:

First Name*:

Middle Name:

Last Name*:

Suffix:

❑ Change of Grantee Institution

Name of former institution*:

PHS 398 Research Plan

Please attach applicable sections of the research plan, below.

OMB Number: 0925-0001

1. Introduction to Application (for RESUBMISSION or REVISION only)	
2. Specific Aims	Core_D_IOF_Specific_aims.pdf
3. Research Strategy*	Core_D_IOF_Res_Strat_Plan_Asthma_U19.pdf
4. Progress Report Publication List	

Human Subjects Sections

5. Protection of Human Subjects
6. Inclusion of Women and Minorities
7. Inclusion of Children

Other Research Plan Sections

8. Vertebrate Animals
9. Select Agent Research
10. Multiple PD/PI Leadership Plan
11. Consortium/Contractual Arrangements
12. Letters of Support
13. Resource Sharing Plan(s)

Appendix (if applicable)

14. Appendix

Tracking Number: GRANT12004239 Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

Specific Aims

The objective of the IOF Core is to provide initial pilot support for research to develop new assays or to refine existing technologies for better defining mechanisms underlying the onset and progression of asthma and allergic diseases, as well as improving standardization across network members. The IOF Core also will seek to promote inter-disciplinary investigations and patient-oriented research, including the necessary infrastructure for cross-AADCRC analysis such as common data standards and validated assays for use by all network investigators. We recognize that there are few funding opportunities to support the cost of novel investigations in this area. An additional aim of this Core will be to attract new investigators to investigation of the immunopathophysiology of asthma or allergic diseases.

Administrative Structure of the IOF Management Core

A. Pilot Project and Investigator Eligibility

Project Eligibility. IOF pilot awards will be for investigators who wish to initiate novel studies and develop preliminary data necessary for longer term support, such as by the NIH R01 mechanism. Research considered suitable for IOF applications can include the pursuit of hypotheses generated as a result of work under the funded Asthma and Allergic Diseases Cooperative Research Centers (AADCRC), and it may be led by scientists outside the U19 funded group. Ideas for collaborative investigations with U19 scientists, especially across disciplines and involving either basic or clinical researchers will be welcome. We also expect IOF proposals from investigators seeking to develop new assays or to refine existing technologies for better defining mechanisms underlying the onset and progression of asthma and allergic diseases, as well as improving standardization across network members.

Investigator Eligibility. Investigators who will be eligible for IOF awards include:
1) Established investigators in asthma/allergic/immunologic diseases who seek to explore a novel concept or investigate a novel human cohort by bioinformatics, multiplex, or systems biology approaches. Such studies would not be part of their ongoing research.
2) Established investigators in related disciplines who have novel ideas for investigation of the immunopathophysiology of asthma or allergic diseases. The information gained would augment the data being collected by the U19 investigators.
3) New investigators, without current or past NIH support as independent investigators, who seek funds to support a project that will eventually lead to R01 or other investigator-initiated grant support in the area of asthma or allergic diseases.

Applicants will be faculty members associated with a component AADCRC institution who are at a rank higher than Postdoctoral Fellow or Associate Research Scientist. It is expected that IOF proposals will promote collaborative efforts with other investigators in the U19 program; however, project eligibility will hinge primarily upon the quality of the science in the proposal and the potential to develop into longer term project support.

B. Pilot Project Director and AADCRC Steering Committee

Ruth Montgomery PhD will serve as Director of the IOF Core. In coordination with NIAID Program staff and the AADCRC Steering Committee, she will ensure that all aspects of the program are conducted efficiently. This effort will include the timely announcement and dissemination of information about the program, and working with NIAID Program staff for the assignment of reviewers, the distribution of the proposals and their reviews to the Steering Committee for decision regarding funding (*described further below*). Dr. Montgomery will discuss the eligibility and the scope of work with potential applicants in order to optimize the scientific value of the projects. She also will provide general oversight of each IOF award recipient, monitor subcontract awards and reporting, ensure that scientific progress is being made, and conduct ongoing discussions with each recipient. Dr. Montgomery has a track record of experience in these functions as she was awarded several of the IOF pilot projects through the Yale U19 in the Human Immunophenotyping Consortium. Dr. Montgomery also participates in two NIH T32-supported training grants and is well accustomed to mentoring junior investigators and research progress.

C. Structure of the Pilot Project Proposals

Grants will be funded for up to $100,000 per year for no more than two years, and project funds will be disbursed annually. Support for supplies and for technical assistance will be included; however, major equipment purchases and clinical trials will not be permitted except with previous approval from NIAID Program Staff. Support will typically be for one year but grantees may be funded for an additional year pending satisfactory progress.

All grantees will provide a brief annual progress report. These written reports will be collated by NIAID Program Staff in coordination with Dr. Montgomery, supplemented with information from a formal face-to-face at the AADCRC annual meeting, and discussed by the AADCRC Steering Committee at its meetings. The Committee then will make a recommendation about continuation of funding based upon progress toward completion of specific aims, or a rational justification with alternative approaches in cases where progress toward completion of aims has not been made.

D. Plan for Solicitation of Pilot Project Proposals

The announcement of each annual IOF award funding cycle will be made three months prior to each submission deadline via (niaid.nih.gov/topics/asthma/research/Pages/AADCRCs) the AADCRC website and additional forms of communication. Complete application information, including availability of funds, eligibility and guidelines for preparation of applications, will be widely distributed at the component institutions using existing email groups and "listserves" for faculty and research staffs. The plan for announcement will follow on-line advertisement procedures already in use for the AADCRC. In addition, Core Leader Montgomery will promote discussion in the regularly scheduled meeting of the Steering Committee of emerging scientific areas or interested individuals or that might be suitable for new IOF project proposals. This will serve to promote interest in the IOF program to obtain input on novel ideas that would increase the overall scientific value of the U19. Announcements also will be sent to all Departmental Chairs and Section Chiefs within the component institutions with the request that an announcement of the IOF award program be made at Faculty Meetings. The email announcement will include a link to the AADCRC website listing full details of the program. This information also will request that potential applicants contact the Core Director for additional information, particularly regarding eligibility.

E. Submission and Review of Pilot Project Proposals

Applications will include biosketches, budgets, and a research plan of no more than 5 pages in length and will be submitted through the AADCRC web site. Upon receipt of applications, NIAID Program Staff in coordination with the Core Leader will contact prospective reviewers to ensure their availability and a timely turnaround in receipt of reviews. Each application will be reviewed by 2-3 experts, one of whom may be internal to the participating institutions and one external, who will be asked to provide written comments on the proposal within 3 weeks. Applications will be scored on a numerical score of 1-10, and specifically focus upon the criteria of scientific impact and likelihood of future, independent funding. Once the reviews are completed, NIAID Program staff will distribute the proposals and reviewers' reports to the members of the Steering Committee, who will deliberate on the proposals and make a decision regarding funding. We anticipate the first receipt deadline of October 1, 2016, and that successful applicants will be notified in mid-January for funding to begin ~ February 1, 2017.

F. Management of the Pilot Project Program

The IOF Core Director will be in regular contact with NIAID Program Staff to review progress and discuss any management or scientific issues that arise from the IOF pilots. After funding decisions take place, the Core Project Leader will review funded IOF projects with the component AADCRC groups to ensure efficient utilization of U19 resources by the funded IOF awardees and to promote collaborative interactions.

Upon selection for funding, IOF proposals will be initiated for funding through the IOF Management Core. Dr. Montgomery will be aided by an administrative assistant (Ms. Susan Ardito) and an accountant within the Internal Medicine business office (Ms. Alli Saravanam). Yale School of Medicine has a centralized Office of Grants and Contracts Financial Administration providing oversight to all activities related to extramural research funding with management and accounting of all grants handled by each administrative unit. For a letter of institutional commitment to administration of this project, please refer to the Overall component. Approval of release of funds will require compliance with all applicable Federal regulations, policies, and guidelines for research involving human subjects and will be confirmed with input from NIAID Program staff. As part of Yale University, the business office is obligated to identify and comply with applicable governmental laws and regulations, and sponsor or donor requirements or restrictions. Departments, business support centers and institutional offices are responsible for assuring a sound business and control environment. In addition, the Office of University Auditing reviews business practices through a program of internal audits as well as experience with A-133 federal audits. Management of the IOF funding will be coordinated through the Internal Medicine business office. A dedicated accountant from our department, Ms. Alli Saravanam, will monitor the disbursement and tracking of IOF funds in coordination with NIAID Program staff. She will ensure regulatory compliance, financial oversight, and the follow-up assessment of the success of each project. The IOF Director will call *ad hoc* meetings as needed to address emergent issues; for example, if an IOF award recipient leaves the institution or receives extramural support for their project.

After selection for funding, projects will be monitored via brief biannual progress reports and by oral input from the grantee to Dr. Montgomery. Termination of grants prior to completion of one year of funding will be

considered if the project receives outside support, if the investigator leaves the institution, or if the funds are discovered to have been inappropriately used (*i.e*. not for the project proposed). Funds remaining then will be earmarked for the next funding cycle and used to support additional proposals. The IOF program will disburse up to $100,000 annually per each award, drawing on funds from the Infrastructure and Opportunities Fund as prioritized by the Steering Committee.

In addition to research progress, the biannual reports will include information about publications and grants submitted and received. Data and resource sharing plans will be developed for each funded IOF and the Core will oversee compliance with the proposed timetable. The IOF Core Director (Montgomery) participates in two NIH T32-supported training grants and is well accustomed to mentoring junior investigators and research progress. With our administrative assistant Ms. Ardito, they have experience and an electronic template for this level of record keeping. We anticipate no difficulties in documenting the progress, successes, and shortcomings of the particular IOF projects, or in monitoring resource sharing, publication and grant milestones going forward. As this information accrues, it will be included in the in the annual progress reports and presented to the Steering Committee and External Advisory Board.

References Cited

None

OMB Number: 4040-0001
Expiration Date: 06/30/2016

APPLICATION FOR FEDERAL ASSISTANCE

SF 424 (R&R)

5. APPLICANT INFORMATION **Organizational DUNS*:** 0432075620000

Legal Name*: YALE UNIVERSITY
Department:
Division:
Street1*: OFFICE OF SPONSORED PROJECTS
Street2: 25 Science Park
City*: NEW HAVEN
County:
State*: CT: Connecticut
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 065208237

Person to be contacted on matters involving this application

Prefix:	First Name*:	Middle Name:	Last Name*:	Suffix:
	Marybeth		Brandi	

Position/Title: Proposal Manager
Street1*: 25 Science Park
Street2: 150 Munson Street
City*: New Haven
County:
State*: CT: Connecticut
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 06520-8237
Phone Number*: 203-737-3495 Fax Number: Email: marybeth.brandi@yale.edu

7. TYPE OF APPLICANT*

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Single Cell Mechanisms of YKL-40 and Transcriptomic Endotypes of Asthma

12. PROPOSED PROJECT

Start Date*	Ending Date*
07/01/2016	06/30/2021

OMB Number: 4040-0010
Expiration Date: 06/30/2016

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	YALE UNIVERSITY
Duns Number:	0432075620000
Street1*:	300 Cedar St
Street2:	TAC S440
City*:	NEW HAVEN
County:	
State*:	CT: Connecticut
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	065208237
Project/Performance Site Congressional District*:	CT-003

Project/Performance Site Location 1

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	YALE UNIVERSITY
DUNS Number:	0432075620000
Street1*:	300 Cedar St
Street2:	TAC S440
City*:	NEW HAVEN
County:	
State*:	CT: Connecticut
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	065208237
Project/Performance Site Congressional District*:	CT-003

File Name

Additional Location(s)

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ● **Yes** ❍ **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ❍ Yes ● No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6

If NO, is the IRB review Pending? ❍ **Yes** ❍ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ❍ **Yes** ● **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ❍ Yes ❍ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. Is proprietary/privileged information included in the application?* ❍ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ❍ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ❍ Yes ❍ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ❍ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ❍ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Project_1_Project_Summary.pdf
8. Project Narrative*	
9. Bibliography & References Cited	Project_1_References_Cited.pdf
10.Facilities & Other Resources	Facilities_Project_1_Asthma_U19.pdf
11.Equipment	Project_1_Equipment.pdf

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

Project Summary

Scientific research over the last four decades has identified Type 2 inflammation as the driving immunologic response in many individuals with asthma. These efforts also demonstrated that many patients have low or no evidence of Type 2 inflammation and that the complex pathophysiology seen among patients with asthma is due to differential contributions related to genetic susceptibility and environmental exposures that include diet, air quality, geography, and the airway microbiome. This is especially true in individuals with severe or refractory disease (5-10% of asthmatics or 25-30 million in the U.S), underscoring the fact that the current paradigm of asthma severity used in the clinic and for research is inadequate because it combines patients with different inflammatory endotypes and obscures many molecular networks that contribute to disease pathobiology. Thus, new tools and approaches are needed to identify subgroups of disease to progress from the traditional approach of defining severity as a continuum from mild to severe, to the model that defines distinct endotypes of disease driven by different patterns of dysregulated biologic pathways that will respond to a precisely determined therapeutic approach. To this end, we have discovered two endotypes that are associated with severe asthma. The first was based on the discovery that CHI3L1/YKL-40 is elevated in a subgroup of patients with severe asthma and correlates with airway remodeling, genetic polymorphisms in *CHI3L1*, and the levels of YKL-40 in the airway. The second endotype was discovered using a novel pathway-based, unsupervised cluster analysis of sputum gene expression that identified 3 “transcriptional endotypes” of asthma (TEA clusters): one subgroup with a history of near fatal attacks, one subgroup with a high rate of hospitalizations for asthma, and a third subgroup with milder disease. While we have identified a number of genes and pathways associated with each TEA cluster, a detailed understanding regarding of the targets that drive these endotypes has not been reached. We hypothesize the existence of unique cell populations, transcriptomes, and functional responses in the airway that are associated with endotypes of disease. To prove this hypothesis, we will determine the single cell signatures and functional responses associated with YKL-40 endotypes, TEA clusters, and integrated clusters of asthma in subjects enrolled in the NextGen Study. We will determine the immunophenotype of the cell populations in the airway by single cell CyTOF analysis of sputum cells associated with these endotypes, characterize the cell expression signatures associated with the endotypes, and define the functional responses of cells associated with YKL-40 endotypes, TEA clusters, and integrated asthma endotypes. Ultimately, we will develop an integrated model of asthma using single cell data and clustering results. We contribute the pipelines and tools developed in the course of these studies and develop a web-based application for interrogation of the model for asthma research—Asthma MAP—for public access.

Facilities and Other Resources - Project 1

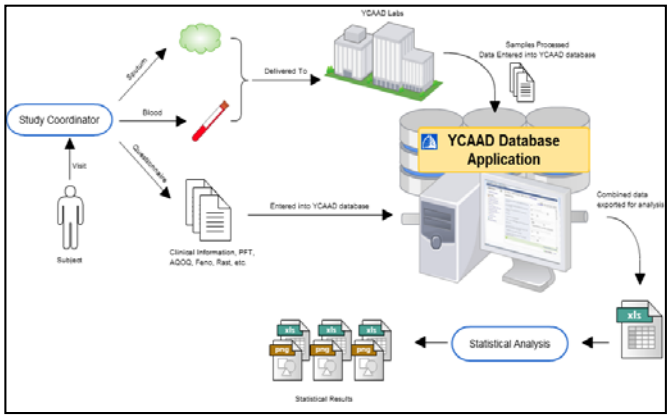
Unique features of the Yale research environment contributing to the probability of success

Yale provides unique opportunities for the successful achievements of the proposed work on defining asthma endotypes using next generation technologies. The academic office and laboratory of the PI, Dr. Chupp, are located on the fourth and fifth floor of The Anlyan Center (TAC) on the Yale School of Medicine campus – a new center designed to facilitate transdisciplinary collaboration for the advancement of science. Almost all of the Investigators on the Yale Asthma U19 team are located in TAC or nearby on the Yale Campus, further enabling the collaborations which have led to the transcriptomic studies outlined in this proposal. The following describe the facilities and resources the PI, Leads and Co-Leads will use for the studies defined herein.

Research Facilities

Research Laboratories. Dr. Chupp has a research laboratory in the Division of Pulmonary and Critical Care Medicine on the 4th floor of the TAC building. This laboratory occupies 1500 square feet of space and is stocked and equipped for the cell and molecular investigations described in this application. The laboratory is a Level II Biosafety facility and contains tissue culture, cell biology, molecular biology, darkroom, and computing facilities. The Chupp lab resides in a large, open laboratory structure with shared equipment and resources and is situated close to YCAAD, the Bronchoscopy Suite, The Yale Center for Statistical Genomics and Proteomics and the The Yale Center for Clinical Investigation (YCCI). The floor also houses the Yale CyTOF facility and **Yale Center for Precision Pulmonary Medicine (P^2MED)**, that Dr. Chupp co-directs. Blood and airway samples from subjects participating in the NextGen study will be processed and distributed to the other laboratories as outlined in the Clinical Recruitment and Biostatistics Core B.

The YCAAD Informatics System: An important feature of the YCAAD research program is the web-based informatics platform that was constructed to handle the high flow of subjects and data required for these studies (see figure). Custom built, in collaboration with the Yale Center for Medical Informatics (YCMI) (which serves as a focus for training, research, and institutional computer systems development and supports a range of computing and informatics projects), YCMI provided the infrastructure for the development of the YCAAD web-based database platform which is central to the PI's translational asthma research program. This secure, HIPAA-compliant, web-based portal allows program personnel, including research coordinators, laboratory technicians, genomics specialists, and biostatisicians, the ability to upload and download de-identified clinical, laboratory, and genomic data from different locations. Clinical data is directly entered into the system at the time of the phenotyping visit from a HIPAA-compliant, protected and encrypted iPad. Experimental results are uploaded by laboratory scientists, and a customized query application easily downloads data files with the requisite clinical phenotype and experimental data in any desired file format. This system greatly increases the pace of scientific discovery and resource sharing. As described in the Core B proposal, this system is now being integrated into ONCORE, and Yale School of Medicine database application that is integrated with the Yale New Haven Hospital electronic medical record (EPIC). This will significantly enhance research efforts in the future toward our goal of precision medicine for patients with airway disease.



Yale FACS facility is located in the TAC building, close to all the laboratories of the investigators and a short indoor walk from the YCAAD clinical research center. There is a second facility a short walk down the street in the Amistad Building. These facilities will be used for the immunophenotyping described in aim 2. In The Anlyan Center, facilities include 3 FACSCalibur instruments (detecting three colors), 4 Stratedigm analyzers (2 with 8-color detection, and 2 with 13-color detection capability), and three multilaser LSRII instruments that can each detect 12 colors. An additional LSRII and FACSCalibur are located in the Amistad Building, and an LSRII, Fortessa and FACSCalibur are located nearby at 300 George Street. Additional analytic capabilities in The Anlyan Center core include a Bio Rad Bio-Plex multi-analyte detection system and Amnis Imagestream-X flow cytometer. Cell sorting facilities in TAC include 4 high-speed sorters: a BD FACSVantage SE, a BD Aria, a DakoCytomation MoFlo, and Sony iCyt Reflection with 8, 10, 8, and 12 color capability, respectively. This

resource is available to the PIs for flow cytometric analyses and cell sorting. This facility has recently expanded to include an AMNIS ImageStream Imaging Flow Cytometer. (info.med.yale.edu/immuno/cytometry).

CyTOF Mass Spectometry Facility (Directot, Dr. Ruth Montgomery). CyTOF2 with inductively coupled plasma ion source with free running RF generator and a balanced load coil interface. The CyTOF2 includes a 5-stage vacuum system with 2-stage plasma-vacuum interface and TOF turbo-pump; ion-neutral decoupling ion deflector, low-mass cut-off quadrupole and point-to-parallel focusing lens system; Orthogonal TOF analyzer operated at >75000 spectra/second TOF cycle frequency; Discrete dynode ion detector. The CyTOF2 detection system is based on 1 GS/s 8-bit signal digitizing boards; Direct sampling spray chamber for individual cell assays, Auto fill Syringe pump, and microconcentric nebulizer. In addition, our instrument uses CyTOF Autosampler Model AS5 that handles up to three 96-well plates, a 2-position 4-port automated valve for simultaneous flush/sample, and adjustable injection volume up to 0.95 ml. We have established network services for Apple or Intel processor computers including Instrument control and data handling computer system with CyTOF MS-1 software. Computers are available in the facility for data analysis use by the staff and users.

Yale Center for Statistical Genomics and Proteomics. Dr. Xiting Yan, a computational biologist and biostatistician, is a member of the YCAAD research group and has computational workstations in both the TAC building and the Yale Center for Statistical Genomics and Proteomics. Dr. Yan is also the Director of the **P^{2}MED** data analysis hub. The Center is part of the Yale School of Medicine West Campus and is located across the street from Dr. Chupp's laboratory and on Yale's West Campus in West Haven. The Yale Center for Statistical Genomics and Proteomics has extensive computing facilities including a 20-node Linux cluster, 20 Dell PC desktops, 2 LINUX workstations, and 4 laser printers. All of these computers have direct internet connections. Dr. Hongyu Zhao, a co-Investigator on this proposal, is the Director of this Center and has collaborated with Dr. Chupp on *CHI3L1* genomics for several years.

Yale Center for Genome Analysis. This is a full service facility dedicated to providing RNA expression profiling, DNA genotyping, high-throughput DNA sequencing, whole transcriptome profiling, microRNA analysis services and equipped with 12 Illumina Genome Analyzers, 1 454 sequencer, Affymetrix, NimbleGen, Solexa, Sequenom, Applied Biosystems 7900 systems, as well as in-house spotted arrays. Co-Core Lead Shrikant Mane, PhD, is the director and founder of this resource and has 20 years experience in molecular and cell biology. In addition to Dr. Mane, the Yale Center for Genome Analysis has 20 full time staff members. The Center contains Class 100 and Class 1000 clean rooms for printing and slide processing. The services offered by the Center have contributed to over 200 publications since its inception. This resource has emerged as one of the leaders in the field of identification of disease-causing genetic factors, as evidenced by two recent articles in *Science* that identified genes associated with age-related macular degeneration as well as coronary disease and metabolic risk factors. This center is Dr. Mane's laboratory, where RNA sequencing studies for this proposal will be completed. This is outlined in detail in the Precision Profiling Core C proposal.

Clinical Research Facilities

Yale Center for Asthma and Airways Disease (YCAAD). Yale-New Haven Hospital is the largest referral center in the state, and YCAAD is the only dedicated adult asthma center in the region. This active, rapidly growing center receives over 3000 visits a year and is the hub of the asthma clinical/translational research program at Yale. For the last 15 years, the PI of this proposal, Geoffrey Chupp MD, has built a center and infrastructure to phenotype and endotype human asthma in a longitudinal, high throughput fashion. This protocol (See appendices) has enrolled over 800 subjects over the last 15 years. Six years ago, the protocol was expanded to include a 2 hour study visit that includes an extensive coordinator-administered asthma questionnaire, lung function testing, hypertonic saline sputum induction and blood drawing for genomic level analyses of RNA isolated from the blood and sputum. We now enroll new asthma subjects at a rate of approximately 100 per year and evaluate existing subjects at follow up visits. This infrastructure is central to the studies outlined in this proposal.

Yale Center for Clinical Investigation. The Yale Center for Clinical Investigation (YCCI) is a multidisciplinary resource funded through a Clinical Translational Science Award (CTSA) from the National Institutes of Health. Launched in January 2006, the YCCI supports and facilitates clinical and translational research and training across the entire medical campus. This collaborative was designed to foster and support the development of clinical and translational research at Yale. Through YCCI funding, Dr. Chupp developed the YCAAD research

program and continues to be closely associated with the YCCI, taking advantage of the Biostatistics/Informatics and Genomic/Proteomic resources. The YCCI is integrated with all aspects of human-based research at Yale, and the interactive environment at the Medical School encourages involvement in YCCI.

Pulmonary Function Laboratory. The adult Pulmonary Function Testing (PFT) Laboratory consists of over 2000 square feet, located in Yale-New Haven Hospital and adjacent to YCAAD. The PFT Lab is directed by Dr. Chupp and staffed by six certified PFT technicians who are expert in performing all routine and many specialized pulmonary function tests, including methacholine challenge and exhaled nitric oxide measurements. The PFT lab has 6 Collins DS560 systems for performing spirometry, static lung volume measurements and diffusing capacity measurements. In addition, two Collins body plethysmograph, Medgraphics computerized dry rolling spirometer, computerized Stedwell survey plus wet spirometer, and a custom-built dry pneumotech for methacholine challenge testing. This equipment is all networked through a file server to a Dek 386 450-D2LP-based database. The laboratory performs over 8000 tests a year and has extensive experience in the performance of pulmonary function tests for clinical and translational research studies. All studies are done in accordance with ATS guidelines. A renovation and further expansion of this facility is scheduled to take place in 2016 for the purpose of streamlining procedures and increasing the capacity of the lab.

Computers

The PI and team members on this grant have individual desktop computers (Apple or IBM-compatible), with all necessary computer peripherals attached to color printers and scanners in their offices. E-mail and internet access are provided via the Yale University Information Technology Network with connections to the University's mainframe system, impressive biostatistical support, access to computer-focused literature evaluation, and genetic searches. Dr. Chupp's laboratory has 3 genomic analytical work stations, two in the TAC building and one other nearby in the Medical Informatics section at 300 George Street.

Offices

The PI and the co-Investigators have academic research offices on the 4th and 5th floor of The Anlyan Center, equipped with a full complement of administrative equipment. The PI and the project leaders have full-time administrative assistance. The offices are convienently located near Dr. Chupp's laboratory, YCAAD, and the bronchoscopy facilities.

Animal

Not applicable.

Equipment - Project 1

Chupp Laboratory

Dr. Chupp's laboratory occupies 600 square feet in The Anlyan Center at Yale School of Medicine. It contains a Dako Auto stainer (Dako) with the capability to do automated immunohistochemistry, a DNA cycler (Perkin Elmer), microfuge, microwave, Gel apparatus and power supply, and a 3 Dell computers for YCAAD database management and computational analysis of genomic data. It also contains a chemical hood, -20° C freezers and a 4° C refrigerator. There is a -80° freezer dedicated to human samples; this is locked and has a remote sensing alarm to notify an on-call member of the research team if there is a temperature abnormality. Three -80° C freezers are located next to Dr. Chupp's lab. The laboratory is fully equipped for tissue culture, cell biology and molecular biology, with a darkroom and computing facilities. There is a coulter counter, laminar flow hoods, incubators, chemical hood, walk-in cold room, liquid scintillation counter, gamma counter, pH meters, balances, refrigerators, liquid nitrogen freezing unit, -70° C freezer, fast protein liquid chromatography apparatus (FPLC, Pharmacia), HPLC (Waters), computers, vertical SDS gels (Hoeffer), 2-D gels, UV gel viewer, Polaroid camera system, submerged gel system, dot-blot apparatus, hot wire bag sealer, Geiger counter, baking oven, freeze-dryer, Microfuge, 37° C rotating incubator, DNA thermal cycler (Perkin Elmer), speed vac concentrator, ultracentrifuge, super speed centrifuge, sequencing gels, power supply, laser densitometer, fluorescent microscope (Nikon Microphot-FXA) and luminometer, and ELISA plate reader. There is also an Applied Biosystems 7800 Fast Real Time PCR system for genotyping and quantitative PCR, Luminex bead reader, fluorescent microscope and a FACS facility with six FACS machines and cell sorting capability, confocal microscopy and animal irradiators which are easily accessible in the building. Glass washing and autoclave facilities are available on the same floor.

Nanostring nCounter Analysis System utilizes a digital color-coded barcode technology that is based on direct multiplexed measurement of gene expression and offers high levels of precision and sensitivity (>1 copy per cell). The technology uses molecular "barcodes" and single molecule imaging to detect and count hundreds of unique transcripts in a single reaction. Each color-coded barcode is attached to a single target-specific probe corresponding to a gene of interest. Mixed together with controls, they form a multiplexed CodeSet.

MesoScale Discovery System. A Meso Scale (MSD) QuickPlex SQ 120 is in place in PI Chupp's lab for sensitive detection of multiple analytes in a 96 well format. Features include electrochemoluminescence detection by CCD camera, plate motion control, and a plate barcode reader. MSD reads wells in ~ 2 min and supports immunoassay in a dynamic range > 5 logs. MSD technology has enhanced sensitivity (< 1.0 pg/ml) and reduced background signals compared to standard colorimetric or fluorescent ELISA kits. This system will be in use for YKL-40 measurements from blood and airway samples collected from subjects enrolled in the NextGen study.

Computer and YCAAD GenEx Database

Dr. Chupp and the other investigators have individual desktop computers (Apple or IBM-compatible), with all requisite computer peripherals along with color printers and scanners in their offices. E-mail and internet access are provided via the Yale University Information Technology Network. This provides access also to the University's mainframe system, impressive biostatistical support, access to computer focused literature evaluation and genetic searches. In addition, the YCAAD GenEx Database is on a server maintained by the Yale School of Medicine and accessible through the Yale intranet. This database was custom built for characterizing asthma severity and is a secure, HIPAA compliant, password protected database that can be used by multiple investigators simultaneously from multiple sites. This database efficiently integrates clinical data and genomic expression data generated by the Center for Genome Analysis. Combined, this informatic hardware infrastructure will facilitate the success of these studies.

Yale Center for Genomics and Proteomics

The Yale Center for Genome Analysis, directed by Dr. Shrikant Mane (Co-Lead, Core C, in this proposal), is a full service facility dedicated to providing RNA expression profiling, DNA genotyping, high-throughput DNA sequencing, whole transcriptome profiling, microRNA analysis services and is equipped with 12 Illumina Genome Analyzers, 1-454 sequencer, Affymetrix, NimbleGen, Solexa, Sequenom, Applied Biosystems 7900 systems, as well as in-house spotted arrays. In addition to Dr. Mane, the Yale Center for Genome Analysis has

twenty full time staff members. It occupies 5,350 square feet of space and contains Class 100 and Class 1000 clean rooms for printing and slide processing. Genotyping for *CHI3L1* will be conducted at YCGAP

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: GEOFFREY Middle Name L Last Name*: CHUPP Suffix:

Position/Title*: Associate Professor
Organization Name*: YALE UNIVERSITY
Department:
Division:
Street1*: 300 Cedar Street, TAC S441B
Street2: PO Box 208057
City*: New Haven
County:
State*: CT: Connecticut
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 065208057

Phone Number*: 203-785-3627 Fax Number: 203-785-3826 E-Mail*: geoffrey.chupp@yale.edu

Credential, e.g., agency login: GCHUPP

Project Role*: Other (Specify) Other Project Role Category: Project Lead

Degree Type: Degree Year:

File Name

Attach Biographical Sketch*:

Attach Current & Pending Support:

PROFILE - Senior/Key Person

Prefix:	First Name*: Jose	Middle Name Luis	Last Name*: Gomez-Villalobos	Suffix:

Position/Title*:	Instructor
Organization Name*:	YALE UNIVERSITY
Department:	
Division:	
Street1*:	300 Cedar Street TAC?441 South
Street2:	P.O. Box 208057
City*:	New Haven
County:	
State*:	CT: Connecticut
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	065200000

Phone Number*: 203.785.4162 Fax Number: E-Mail*: jose.gomez-villalobos@yale.edu

Credential, e.g., agency login: JGVILLALOBOS

Project Role*: Other (Specify) Other Project Role Category: Co-Lead

Degree Type: MD,INT MED Degree Year:

	File Name
Attach Biographical Sketch*:	Gomez_Bio_Asthma_U19.pdf
Attach Current & Pending Support:	

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2016 **End Date*:** 06-30-2017 **Budget Period: 1**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	GEOFFREY	L	CHUPP		Project Lead		2.4			36,660.00	11,365.00	48,025.00
2.	Jose		Gomez-Villalobos		Co-Investigator		1.2			14,935.00	4,630.00	19,565.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **67,590.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	4.8			18,173.00	5,633.00	23,806.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Technician	3.6			21,221.00	6,578.00	27,799.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**51,605.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**119,195.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2016 **End Date*:** 06-30-2017 **Budget Period: 1**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2016 **End Date*:** 06-30-2017 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	49,805.00
2. Publication Costs	3,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**52,805.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**175,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Modified total direct cost	66.5	175,000.00	116,375.00
		Total Indirect Costs	**116,375.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**291,375.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: Project_1_Budget_Justification_asthma_U19.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2017 **End Date*:** 06-30-2018 **Budget Period: 2**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	GEOFFREY	L	CHUPP		Project Lead		2.4			36,660.00	11,365.00	48,025.00
2.	Jose		Gomez-Villalobos		Co-Investigator		1.2			15,383.00	4,769.00	20,152.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **68,177.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	4.8			18,718.00	5,802.00	24,520.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Technician	3.6			21,857.00	6,776.00	28,633.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**53,153.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**121,330.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2017 **End Date*:** 06-30-2018 **Budget Period: 2**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2017 **End Date*:** 06-30-2018 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	47,670.00
2. Publication Costs	3,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**50,670.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**175,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Modified total direct cost	66.5	175,000.00	116,375.00
		Total Indirect Costs	**116,375.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**291,375.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name:
Project_1_Budget_Justification_asthma_U19.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2018 **End Date*:** 06-30-2019 **Budget Period: 3**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	GEOFFREY	L	CHUPP		Project Lead		2.4			36,660.00	11,365.00	48,025.00
2.	Jose		Gomez-Villalobos		Co-Investigator		1.2			15,845.00	4,912.00	20,757.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **68,782.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	4.8			19,280.00	5,976.00	25,256.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Technician	3.6			22,513.00	6,978.00	29,491.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**54,747.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**123,529.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2018 **End Date*:** 06-30-2019 **Budget Period: 3**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2018 **End Date*:** 06-30-2019 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	45,471.00
2. Publication Costs	3,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**48,471.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**175,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Modified total direct cost	66.5	175,000.00	116,375.00
		Total Indirect Costs	**116,375.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**291,375.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: Project_1_Budget_Justification_asthma_U19.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2019 **End Date*:** 06-30-2020 **Budget Period: 4**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	GEOFFREY	L	CHUPP		Project Lead		2.4			36,660.00	11,365.00	48,025.00
2.	Jose		Gomez-Villalobos		Co-Investigator		1.2			16,320.00	5,059.00	21,379.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **69,404.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	4.8			19,858.00	6,156.00	26,014.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Technician	3.6			23,188.00	7,188.00	30,376.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**56,390.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**125,794.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2019 **End Date*:** 06-30-2020 **Budget Period: 4**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2019 **End Date*:** 06-30-2020 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	43,206.00
2. Publication Costs	3,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**46,206.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**175,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Modified total direct cost	66.5	175,000.00	116,375.00
		Total Indirect Costs	**116,375.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**291,375.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: Project_1_Budget_Justification_asthma_U19.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2020 **End Date*:** 06-30-2021 **Budget Period: 5**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	GEOFFREY	L	CHUPP		Project Lead		2.4			36,660.00	11,365.00	48,025.00
2.	Jose		Gomez-Villalobos		Co-Investigator		1.2			16,809.00	5,211.00	22,020.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **70,045.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	4.8			20,454.00	6,341.00	26,795.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Technician	3.6			23,884.00	7,403.00	31,287.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**58,082.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**128,127.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2020 **End Date*:** 06-30-2021 **Budget Period: 5**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2020 **End Date*:** 06-30-2021 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	40,873.00
2. Publication Costs	3,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**43,873.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**175,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Modified total direct cost	66.5	175,000.00	116,375.00
		Total Indirect Costs	**116,375.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**291,375.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: Project_1_Budget_Justification_asthma_U19.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

PROJECT 1

BUDGET JUSTIFICATION

Key Personnel

Geoffrey Chupp, M.D., (Principal Investigator and Project 1 Lead). Dr. Chupp is Professor of Medicine, Pulmonary and Critical Care Medicine, Yale University School of Medicine. He is the founder and Director of the Yale Center for Asthma and Airways Disease (YCAAD) and Cardiopulmonary Function Laboratory at Yale New Haven Hospital. He is an experienced clinical investigator who has developed novel approaches to genomic profiling of the blood and sputum in airway diseases including asthma and emphysema. He has developed an interdisciplinary, collaborative team (including Dr. Rajeevan and Dr. Yan) of researchers to develop novel approaches to evaluate genomic data acquired from individuals with asthma. His responsibility as PI is to oversee all aspects of these studies and to ensure the overall success of this project, from coordinating analyses with the research team to publishing the results. Dr. Chupp will dedicate 2.4 calendar months annually to this project.

Jose-Gomez-Villalobos, M.D., M.S., (Co-Lead). Dr. Gomez is an Assistant Professor of Medicine, Pulmonary and Critical Care Medicine, Yale University School of Medicine. Over the last 5 years, his research has focused on the effect of genetic variation in the *CHI3L1* gene in asthma, the effect of secondhand cigarette smoke in asthma, computational biology and bioinformatics of asthma and airways disease. Dr. Gomez developed the clustering algorithm that defined the YKL-40 endotypes. Dr. Gomez's effort on this grant will be 1.2 calendar months per year.

Non-Key Personnel

Qing Liu, M.D., Ph.D. (Technician). Dr. Liu is an Associate Research Scientist, Pulmonary, Critical Care, and Sleep Medicine, Yale University School of Medicine. Dr. Liu is an accomplished cell and molecular biologist who has been a member of the Pulmonary Section for over 12 years. He has extensive experience in all the technical aspects required for these studies and has been processing samples for YCAAD for several years. Dr. Liu will work closely with Dr. Chupp on these studies and will be responsible for the processing of all biologic samples and isolation of RNA for the assays defined in Project 1 define YKL-40 and Transcriptomic Endotypes of asthma. Dr. Liu will also perform stimulation experiments outlined as outlined in Project 1. Dr. Liu's active participation of 3.6 calendar months annually is required for the successful accomplishment of these studies.

To Be Named (Postdoctoral Associate). This individual will work in the Chupp lab to develop stimulation experiments and follow-up studies based on the results of baseline studies. This will include close interactions with the Precision Profiling Core C, and Project 3 investigators. The annual effort for this individual will be 4.8 calendar months.

Other Expenses

Laboratory Materials and Supplies
The budget for reagents including molecular biology reagents, solutions, MSD reagents, nanostring assay supplies, cell culture supplies, chemicals, genotyping supplies, and general lab supplies is as follows:
Year 1: $49,805
Year 2: $47,670
Year 3: $45,471
Year 4: $43,206
Year 5: $40,873

Domestic Travel
$3,000 per year is budgeted to defray travel costs for the Lead and Co-Lead to attend meetings at NIH and other national meetings.

Publications

$3,000 annually is budgeted to defray costs of publications of papers written by the Dr. Chupp and his research team from the results of these studies.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		343,998.00
Section B, Other Personnel		273,977.00
Total Number Other Personnel	10	
Total Salary, Wages and Fringe Benefits (A+B)		617,975.00
Section C, Equipment		0.00
Section D, Travel		15,000.00
1. Domestic	15,000.00	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		242,025.00
1. Materials and Supplies	227,025.00	
2. Publication Costs	15,000.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	0.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	0.00	
9. Other 2	0.00	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		875,000.00
Section H, Indirect Costs		581,875.00
Section I, Total Direct and Indirect Costs (G + H)		1,456,875.00
Section J, Fee		0.00

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

1. Project Director / Principal Investigator (PD/PI)

Prefix:
First Name*: GEOFFREY
Middle Name: L
Last Name*: CHUPP
Suffix:

2. Human Subjects

Clinical Trial? ● No ○ Yes
Agency-Defined Phase III Clinical Trial?* ○ No ○ Yes

3. Permission Statement*

If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?

○ Yes ● No

4. Program Income*

Is program income anticipated during the periods for which the grant support is requested? ○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

Budget Period*	Anticipated Amount ($)*	Source(s)*

PHS 398 Cover Page Supplement

5. Human Embryonic Stem Cells

Does the proposed project involve human embryonic stem cells?* ● No ❍ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s): ❑ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

6. Inventions and Patents (For renewal applications only)

Inventions and Patents*: ❍ Yes ❍ No

If the answer is "Yes" then please answer the following:

Previously Reported*: ❍ Yes ❍ No

7. Change of Investigator / Change of Institution Questions

❑ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:

First Name*:

Middle Name:

Last Name*:

Suffix:

❑ Change of Grantee Institution

Name of former institution*:

PHS 398 Research Plan

Please attach applicable sections of the research plan, below.

OMB Number: 0925-0001

1. Introduction to Application (for RESUBMISSION or REVISION only)	
2. Specific Aims	Project_1_Specific_Aims.pdf
3. Research Strategy*	Project_1_Research_Strategy.pdf
4. Progress Report Publication List	
Human Subjects Sections	
5. Protection of Human Subjects	Protection-HumanSubjects_Asthma_U19.pdf
6. Inclusion of Women and Minorities	Inclusion-Women_Minorities_Asthma_U19.pdf
7. Inclusion of Children	Inclusion-Children_Asthma_U19.pdf
Other Research Plan Sections	
8. Vertebrate Animals	
9. Select Agent Research	
10. Multiple PD/PI Leadership Plan	
11. Consortium/Contractual Arrangements	
12. Letters of Support	
13. Resource Sharing Plan(s)	ResourceSharingPlan_Asthma_U19.pdf
Appendix (if applicable)	
14. Appendix	

Tracking Number: GRANT12004239 Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

Specific Aims

As the most common chronic inflammatory disease of the lung, asthma will likely afflict over 10% of the U.S. population by the end of this decade.[1] Scientific efforts over the last forty years have identified Type 2 inflammation as the driving immunologic response in many individuals with asthma.[2] These efforts also demonstrated that many patients have low or no evidence of Type 2 inflammation and that the complex pathophysiology seen among patients with asthma is due to differential contributions from genetic susceptibility and environmental exposures that include diet, air quality, geography, and the endogenous microbiome.[3,4] This is especially true in individuals with severe or refractory disease (5-10% of asthmatics or 25-30 million in the U.S) and underscores the fact that the current paradigm of asthma severity, used in the clinic and for research, is inadequate because it combines patients with different inflammatory endotypes and obscures many molecular networks that contribute to disease pathobiology.[5] New tools and approaches are needed to identify subgroups of disease so we can move from the traditional approach of defining severity as a continuum from mild to severe, to a model that defines distinct endotypes of disease driven by different patterns of dysregulated biologic pathways that will respond to a precisely determined therapeutic approach.[6] Up to now, most efforts to identify asthma subgroups have used clustering algorithms of clinical variables, validating lung function and age of disease onset as key determinants of disease phenotypes.[7] But, a true understanding of disease endotypes requires the examination of the molecular perturbations associated with the disease and/or clusters already established. Our translational research program is focused on such efforts through the study of a well characterized heterogeneous cohort of individuals with asthma. We have identified two endotypes of disease by: 1) the identification of a molecule that is associated with subgroups of patients with refractory asthma, Chitinase-3-Like-1(CHI3L1)/YKL-40 and 2) unsupervised cluster analysis of sputum transcriptomes that identified transcriptomic endotypes of asthma (TEA) clusters associated with severe disease.[8-10] The first discovery was that circulating levels of CHI3L1/YKL-40 are elevated in a subgroup of patients with severe asthma, and correlates with airway remodeling, genetic polymorphisms in *CHI3L1*, and the levels of YKL-40 in the airway. The second discovery was made with a novel pathway-based, unsupervised cluster analysis of sputum gene expression that identified 3 “transcriptional endotypes” of asthma (TEA clusters): one subgroup with a history of near fatal attacks, one with a high rate of hospitalizations for asthma, and a subgroup with milder disease. However, while we have established these clinically meaningful endotypes of disease and validated them in other cohorts, a detailed understanding of the genes, pathways, and networks that drive the YKL-40 endotypes and TEA clusters has not been determined. **We hypothesize the existence of unique cell populations, transcriptomes, and functional responses that are associated with YKL-40 endotypes and TEA clusters of asthma.** Our multidisciplinary team will determine the cellular immunophenotypes, transcriptomes, gene pathways and networks that are associated with the YKL-40 endotypes, TEA clusters and integrated endotypes that will be identified in Project 3, in a heterogeneous cohort of individuals with asthma enrolled in the NextGen study**.** Correlations with T follicular helper (Tfh) cells, Dickkopf (Dkk-1), and immunogenic microbiota will also be characterized (Project 2). We will pursue the following aims:

Aim 1: Determine the single cell signatures and functional responses associated with YKL-40 endotypes of asthma from baseline and follow-up samples from the NextGen Study (Core B). Studies will be conducted on NextGen study samples to determine: a) the immunophenotype of the cell populations that produce YKL-40 in the airway using single cell CyTOF analysis of sputum cells at baseline, b) single cell signatures and networks associated with YKL-40 endotypes of asthma, and c) the functional responses of the cell populations defined in Aim 1a and 1b to cytokines including IL-13 and YKL-40.

Aim 2: Determine the single cell signatures and functional responses associated with TEA clusters of asthma from baseline and follow-up samples from the NextGen Study (Core B). Studies will be conducted on NextGen study samples to determine: a) the cell populations associated with TEA clusters of asthma determined by single cell CyTOF analysis of sputum cell populations at baseline, b) the single cell transcriptomic signatures and networks associated with TEA clusters and c) the functional responses of the cell populations defined in Aim 1a and 1b to cytokines including TNF, IL-13 and YKL-40.

Aim 3: Define the functional responses associated with endotypes of asthma identified in Projects 2 and 3 compared to YKL-40 endotypes and TEA clusters. Studies will be conducted on samples collected from subjects enrolled in the NextGen study at follow-up visits to determine: a) the functional responses of cell populations associated with novel asthma clusters of disease identified in Project 3 by integrated modeling, b) how the cellular immunophenotypes and cellular responses differ among different endotypes compared to the integrated clusters, and c) the relationship between YKL-40 endotypes and TEA clusters with Tfh cells, Dkk-1, and immunogenic microbiota endotypes defined in Project 2.

Significance: In the case of asthma, we can only achieve true precision medicine if we understand the molecular mechanisms underlying the endotypes of disease. We believe that defining these mechanisms at the single cell level that are associated with YKL-40 endotypes and TEA clusters will be a significant step forward towards reaching this ambitious goal with implications for development of therapeutics and a deeper understanding of asthma pathogenesis.

In most individuals with asthma, symptoms are easily controlled by treatment with bronchodilators and low doses of inhaled corticosteroids. However, a significant proportion of asthmatics have persistent symptoms despite receiving high doses of inhaled corticosteroids. Compared to controlled asthmatics, these "refractory" asthmatics have medical costs that are 6 to 7-fold higher and require frequent hospitalization.[11] Studies over the last two decades have defined several molecular targets in the Type 2 inflammatory pathway that contribute to refractory asthma, leading multiple pharmaceutical companies to develop therapeutic monoclonal antibodies against targets such as IL-5 and IL-13.[12-14] Since as many as 30% of severe asthmatics are not allergic and have no evidence of eosinophilia, but have significant airway obstruction, there remains a significant unmet need for therapeutics in severe asthma.[5] Thus, despite being a small percentage of all asthmatics, these subgroups constitute a large number of patients (millions in the U.S.) whose disease is unlikely to be controlled with these new biologic therapies. Since current animal models of asthma only recapitulate adaptive Th2 driven disease (most commonly to ovalbumin or house dust mite antigen), at this point, in time research on asthma heterogeneity can only be accomplished through the study of asthma in humans.[15,16] *We have established a cohort of individuals with asthma to study asthma heterogeneity and resolve endotypes of disease. A major goal of this U19 is to define the pathobiology of asthma endotypes in humans by examining cellular phenomena in the sputum.*

Although differences in environmental exposures and compliance may explain a significant proportion of asthma heterogeneity, it is clear that the biologic networks that drive asthmatic inflammation are pathobiologically diverse across the spectrum of disease. This concept supports the growing belief that asthma is actually a collection of several different airway diseases, each driven by a different set of gene networks with similar and overlapping inflammatory, physiologic, and clinical features. Research efforts in asthma are now focused on defining asthma subgroups or phenotypes and endotypes to improve the development and targeting of therapeutics to improve healthcare outcomes, especially for patients with the most severe asthma. The best example of novel phenotypes in asthma are the SARP clusters.[7,17,18] These subgroups have validated the importance of lung function and age of disease onset as critical discriminating features that underlie asthma heterogeneity. The best example of an asthma endotype is the Th2 high definition of disease that is strongly linked to exaggerated IL-13 expression.[12] Importantly, while both endotypes and phenotypes reduce the heterogeneity of the population being examined, there remains significant heterogeneity within each subgroup identified. This is true of the SARP clusters, Th2 high/low defined disease, as well as the endotypes that we have established including YKL-40 endotypes, and the TEA clusters. This highlights the need for further study of the pathobiologic mechanisms associated within these endotypes. Only then will we know which subgroups are most generalizable, robust and relevant to pathobiology and clinical care. *To do this properly, we need to employ a combination of methods that include, leveraging existing clusters of asthma with novel biologic measurements, examining the pathobiology of the endotypes at the single cell level, and defining new endotypes as higher resolution datasets and computational approaches become available.*

Innovation. There are several innovative aspects to this proposal that warrant discussion:

Novel models of asthma heterogeneity. A central theme of this U19 proposal is to develop novel, clinically and pathobiologically meaningful ways to deconstruct the complex disease of asthma and make these tools publicly accessible. The YKL-40 endotypes and TEA clusters we have identified provide two complementary and innovative approaches to define subgroups of asthma. The studies outlined herein will significantly enhance our understanding of the biologic drivers of these endotypes at an unprecedented level of resolution. Combined with our goal to define novel asthma clusters through integrated analysis of multidimensional data, these studies will move our understanding of asthma heterogeneity significantly forward.

Single cell study of airway cells from asthmatics. In addition to the novel endotypes of disease which the Yale U19 team has described to date, our multidisciplinary team is pioneering the use of single cell technologies on sputum derived inflammatory cells including CyTOF and single cell RNA-seq (technical aspects and preliminary data are described in Core C and computational approaches are described in Project 3). This provides an opportunity to move beyond Gaussian based statistics to a transformative approach to precisely determine cellular lineage and immunophenotypes of cell populations from a large population of cells which contribute to disease. We will also characterize cell populations in the airway using single cell transcriptomes as opposed to our current approach of staining cells with a Wright stain, or immunophenotyping by FACS. These are truly innovative and transformative concepts that will move the field of asthma heterogeneity forward.

Research Strategy

Aim 1: Determine the single cell signatures and functional responses associated with YKL-40 endotypes of asthma from baseline and follow-up samples from the NextGen Study (Core B). *Studies will be conducted on NextGen study samples to determine: a) the immunophenotype of the cell populations that produce YKL-40 in the airway using single cell CyTOF analysis of sputum cells at baseline, b) single cell signatures and networks associated with YKL-40 endotypes of asthma, and c) the functional responses of the cell populations defined in Aim 1a and 1b to cytokines including IL-13 and YKL-40.*

Rationale. The driving goal of this project is to characterize the cell populations and transcriptomic signatures in the airway that we have established are associated with two endotypes of severe asthma. We will also determine the functional responses of cells in the airway associated with these endotypes, after stimulation with cytokines associated with asthmatic airway inflammation. We will use this information to identify the cellular populations and expression signatures linked to each endotype, ultimately defining molecular pathways associated with CHI3L1/YKL-40 and the TEA clusters. We will also interrogate the functional cellular responses associated with the integrated endotypes identified in project 3. We will begin these studies in aim 1, by defining the cell populations and cellular responses associated with CHI3L1/YKL-40, a chitinase-like-molecule that we discovered is elevated in blood and airway of a subgroup of severe asthmatics and a critical mediator of inflammation and remodeling responses in asthma.

Preliminary studies. Chitinases are a family of evolutionarily conserved hydrolases that belong to the 18-glycosyl hydrolase (GH 18) gene family.[19] These proteins were characterized by their ability to cleave chitin, the second most common naturally occurring polysaccharide (cellulose is the most common). Chitinases have been studied extensively in lower life forms where they function to control chitin homeostasis and degrade chitin in the surrounding environment. Mammals do not produce chitin, but have been shown to have genes for enzymatically active chitinases and several chitinase-like proteins (CLP) that bind to chitin, but do not catalyze it.[20] Evidence in animals and humans suggests that both chitinases and chitinase-like proteins are potent regulators of inflammatory responses. In the airway, chitinases and CLP are juxtaposed between the environment and the host by controlling exposure to chitin inhaled from the environment by degrading it and modulating innate and adaptive immune responses to it.[19] The mammalian chitinases that have been studied and implicated in the pathogenesis of asthma are the true chitinases, chitotriosidase (CHIT1) and acidic mammalian chitinase (AMCase), and the CLP, Chitinase-3-Like-1 (CHI3L1)/YKL-40 (Breast Regression Protein 39 (BRP-39) in mice). In humans, YKL-40 regulates homeostasis of many organs and tissues. YKL-40 is produced by many cell types including monocytes, macrophages, neutrophils, bronchial epithelial cells, chondrocytes and synovial cells, indicating its widespread importance.[19] In addition, YKL-40 controls cell proliferation, survival and stimulates human lung fibroblasts and airway smooth muscle cells to produce cytokines. Recently, co-immunoprecipitation studies identified IL-13 receptor alpha 2 (IL-13Rα2) as a receptor for YKL-40. YKL-40 binds to IL-13Rα2 in a complex with IL-13 that leads to activation of ERK, mitogen-activated protein kinase (MAPK), protein kinase B/AKT, followed by Wnt/β-catenin signaling. This cascade of events regulates oxidant injury, apoptosis, pyroptosis, inflammasome activation, antibacterial responses, melanoma metastasis, and TGF-β production *in vivo*.[21,22] *Our research in human asthma demonstrates a strong link between YKL-40 and severe asthma and, more recently, specific endotypes of asthma (outlined below).*

The importance of CHI3L1/YKL-40 in asthma pathogenesis was recognized from studies in transgenic (TG) mice that overexpress IL-13 in the airway.[23] These IL-13 TG animals develop intense peri-bronchial eosinophilic inflammation, airway remodeling and airway hyperresponsiveness, with histopathology reminiscent of the lungs from autopsies of severe asthmatics.[24] Microarray analysis of whole lung lysates from IL-13 TG compared to wild-type mice revealed marked elevations of mRNA encoding the murine homologue of YKL-40, BRP-39. These studies demonstrated that IL-13 stimulates expression of BRP-39 mRNA and protein in the murine lung and suggested this CLP might contribute to allergic airway inflammation. Additional studies in knockout mice that lack BRP-39 demonstrated that eosinophilic inflammation and Th2 cytokine induction was decreased by $\geq$ 85% after standard OVA sensitization and challenge compared to WT mice.[25] To gain insights into the mechanisms by which YKL-40/BRP-39 contribute to Th2 inflammation, we characterized the recovery and survival of T cells in OVA sensitized and challenged WT and BRP-39 knockout mice and demonstrated increased percentages of cells undergoing cell death with accumulation of apoptotic cells compared to WT mice.[15,25,26] *These studies demonstrate that CHI3L1/YKL-40/BRP-39 is an important modulator of Th2 inflammation and regulates apoptosis. This supports our hypothesis that YKL-40 is associated with an asthma endotype and that individuals with asthma with elevated levels of YKL-40 have increased cell survival airway inflammation compared to individuals with lower levels of YKL-40 expression.*

YKL-40 is associated with severe asthma in humans.[10,20,27] Based on the animal studies described above, we hypothesized that YKL-40 would be increased in asthmatics compared to normal individuals. Although asthma is traditionally considered an organ-specific disease, we believed that YKL-40 levels in the blood could discriminate asthma severity. To test this, we performed a cross-sectional analysis of serum samples from our cohort of asthmatic subjects from the Yale Center for Asthma and Airway Disease (YCAAD). YKL-40 was readily appreciated in the serum of normal volunteers and was significantly higher in the serum of asthmatics (P=0.02). YKL-40 increased with asthma severity, with the highest levels being observed in refractory asthmatics, compared to moderate and mild asthmatics (P for trend = 0.003, Figure 1). Circulating YKL-40 levels also correlated with asthma severity in the two other cohorts examined from the University of Wisconsin and the University of Paris (data not shown).

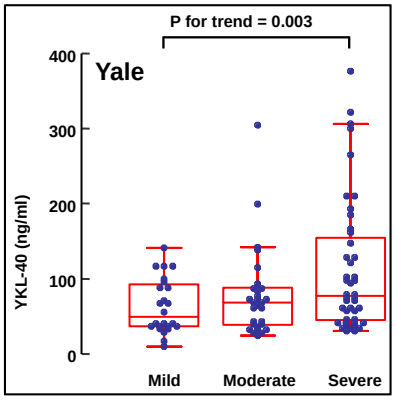


Figure 1. YKL-40 is increased in the blood in asthma**.**

We then performed immunohistochemistry for CHI3L1/YKL-40 on bronchial biopsies from the University of Paris cohort (Figure 2). The numbers of YKL-40-positive staining cells in asthmatics was significantly increased over control subjects that exhibited rare YKL-40 expressing cells (Figure 2, panel A). As shown in Figure 2, YKL-40 staining was seen in subepithelial cells from the majority of asthmatics (panels B-E). In severe asthmatics, the number of YKL-40 staining subepithelial cells was increased, and staining of the bronchial epithelium was also evident (Figure 2, panels D and E). In BAL, cytospin preparations showed that YKL-40 was found in the cytoplasm of macrophages and neutrophils (Figure 2, panel F). Importantly, in asthmatics, YKL-40 staining cells in the airway wall correlated with asthma severity and serum YKL-40 levels ($r = 0.55$, $p < 0.001$) (Figures 3). Serum YKL-40 levels also correlated inversely with FEV_1 in all three cohorts (Yale, $r = -0.22$, $P = 0.01$; Wisconsin, $r = -0.33$, $P = 0.009$ and Paris, $r = -0.21$, $P = 0.005$; data not shown). There was a significant correlation between sub-basement membrane (SBM) thickness and the serum YKL-40 levels in this population ($r = 0.51$, $P = 0.003$, Figure 4). In addition to these seminal observations, in more recent studies we have demonstrated that YKL-40 levels in the sputum correlate with asthma severity and that YKL-40 levels rise significantly in bronchoalveolar lavage fluid of atopic asthmatics after segmental antigen challenge (Figure 5).[9,28]

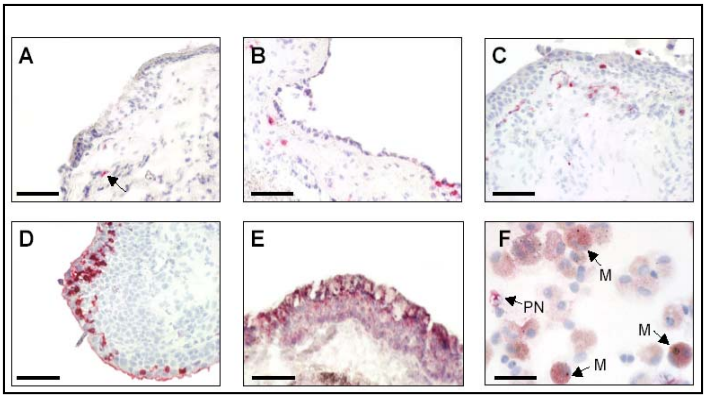


Figure 2. Immunohistochemical analysis of YKL-40 in the airway and BAL in asthma.

Finally, to further understand the patients with high levels of circulating YKL-40, a post-hoc analysis correlating serum YKL-40 levels and asthma characteristics in the Yale cohort demonstrated that YKL-40 levels correlated positively with the number of corticosteroid tapers in the last year, the dose of oral corticosteroids and the frequency of rescue inhaler use, and negatively with the percent predicted FEV_1. YKL-40 was not associated with history of atopy or IgE level. Multivariable analysis of the data was undertaken to determine if the correlation between YKL-40 and asthma severity persisted after adjustments for confounders, including age, race, gender, history of atopy, BMI, and levels of serum IgE. In accord with our initial observations, this analysis demonstrated that asthma severity was associated with YKL-40 levels (adjusted P for trend = 0.02, data not shown) after adjustment for these factors. *These findings indicate that circulating YKL-40 is elevated in severe asthma, independent of IgE, atopy, and eosinophilic inflammation, and negatively correlates with lung function and airway remodeling. Although YKL-40 was initially identified downstream of IL-13, these studies show that in human asthma, YKL-40 is not specific to Th2 inflammatory responses, and is most strongly linked to severe phenotypes of disease. These data support our contention that YKL-40 is a marker of a specific endotype of asthma.*

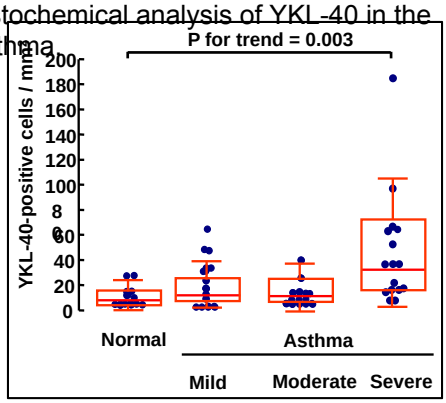


Figure 3. YKL-40 levels in the airway correlate with asthma severity.

CHI3L1/YKL-40 polymorphisms and asthma severity. We have also identified genetic polymorphisms in *CHI3L1* that suggest this molecule defines an endotype of asthma, in particular, severe asthma.[9,29] We conducted studies to determine if there are genetic polymorphisms in *CHI3L1* that are associated with asthma and asthma severity. In collaboration with Dr. Carole Ober at the University of Chicago, three asthma populations were examined; a well characterized founder population of European descent--the Hutterites (a related 13-generation, 1,623-person pedigree), 3 outbred Caucasian populations from the Childhood Origins of ASThma (COAST) birth cohort study, and 2 outbred asthma case-control populations from Freiberg Germany, and Chicago. A genome-wide association study was performed using the ~ 500,000 affymetrix SNP chip for YKL-40 levels. Four SNP with locus P values $\leq 10^{-7}$, meeting the criteria for genome-wide significance ($P < 0.05$) were identified. All 4 SNP were in the *CHI3L1* gene, and one SNP was in the promoter (rs4950928) that is known to influence *CHI3L1* transcription. In all 3 populations, several of these SNP were associated with asthma prevalence, lung function, and bronchial hyperresponsiveness. Since the rs4950928 -131C/G SNP had been shown to interact with MYC/MAX and increase YKL-40 levels, it was considered the functional polymorphism. A second population-based study of 6,514 Caucasian individuals also found an association between the rs4950928 SNP and asthma, however, this study did not examine serum YKL-40 levels.[30] *These studies indicate that genetic variation in the promoter region of CHI3L1 is associated with asthma, but severe asthma was not examined.*

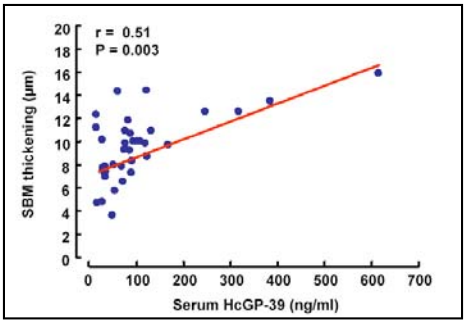


Figure 4. Serum YKL-40 levels correlate with airway remodeling (sub-basement membrane thickness). HcGP-39=YKL-40

The studies detailed above clearly demonstrated a strong link of the promoter SNP in *CHI3L1* to asthma prevalence and lung function, however, the populations examined were of mild severity. Therefore, we pursued studies to determine if genetic polymorphisms in *CHI3L1* are associated with asthma severity and airway levels of CHI3L1/YKL-40.[9,31,32] We genotyped multiple SNP spanning the *CHI3L1* promoter and coding regions and correlated these SNP with asthma severity (based on the SARP clustering algorithm) and the levels of CHI3L1/YKL-40 in the serum and sputum in 261 YCAAD and 919 SARP subjects.[17] Association and haplotype analyses were conducted to identify effects on airflow obstruction, serum and sputum YKL-40 levels. Fifteen SNPs in *CHI3L1* were associated with FEV1 and serum YKL-40 levels. Importantly, rs12141494, residing in intron 6, was most significantly associated with serum YKL-40 levels and post-bronchodilator FEV1 in both the YCAAD and SARP cohorts, in contrast to the promoter SNP, rs4950928, which did not correlate. In asthmatics, rs12141494 was the only SNP associated with both serum YKL-40 levels and asthma severity, an effect that is independent of the previously defined promoter SNP, rs4950928, in both study populations. Haplotype analysis in all European ancestry individuals demonstrated that the combination of the G allele at rs12141494 and the minor allele (G) at rs4950928 were associated with lower YKL-40 levels and higher FEV1 percent predicted. Subsequent analysis of sputum supernatants demonstrated that individuals with asthma and the risk allele A at rs12141494 are associated with higher levels of YKL-40 in the airway ($P = <0.05$, Figure 6). Therefore, genetic abnormalities in *CHI3L1* are also associated with asthma severity, airway remodeling, and airway levels of YKL-40. This suggests that mutations in the *CHI3L1* coding region affect YKL-40 expression in the airway and contribute to airway remodeling in asthma severity. *These discoveries further support our hypothesis that YKL-40 is a marker of a disease endotype characterized by the activation of specific cell populations and pathways that contribute to severe features of disease.*

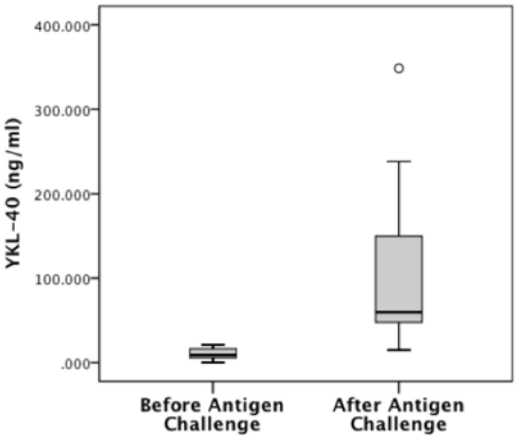


Figure 5. Meso Scale-measured YKL-40 levels in BAL before and after segmental antigen challenge ($P<0.001$).

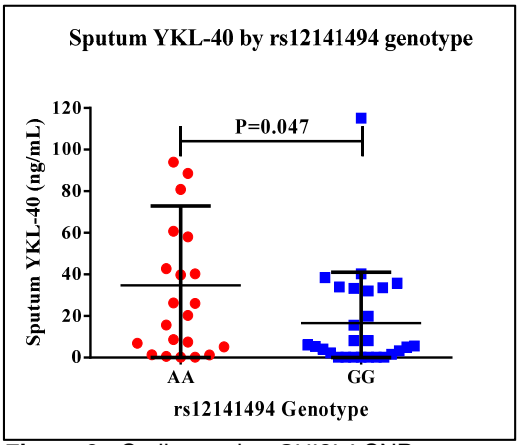


Figure 6: Coding region *CHI3L1* SNP associated with sputum YKL-40 levels.

Since we determined that YKL-40 in the circulation and genetic polymorphisms correlate with asthma severity, we hypothesized that combining YKL-40 levels in the circulation with clinical characteristics in a clustering algorithm would identify pathobiologically and clinically meaningful CHI3L1/YKL-40 endotypes. For this analysis, Dr. Gomez, Co-lead of this project, conducted an unsupervised cluster analysis of 156 individuals with asthma in YCAAD to identify the YKL-40 endotypes. Then, recursive partitioning was used to develop a classifier for cross-validation of the YKL-40 clusters in 167 individuals from the Severe Asthma Research Program (SARP). We also analyzed total sputum cell transcriptome measured by Affymetrix ST 1.0 Gene microarrays to identify pathways that are associated with the resulting endotype groups. Clustering analysis revealed that 4 clusters provided the lowest connectivity in the YCAAD cohort. Two YKL-40 subgroups had higher sputum and serum YKL-40 levels, compared to the other YKL-40 subgroups. YKL-40 endotype group 3 (E3) was associated with severe airflow obstruction, early life onset of disease, low FeNO levels, and a history of near fatal asthma attacks, while YKL-40 endotype 4 (E4) was characterized by the highest BMI and significantly higher serum YKL-40 levels, and preserved lung function (Figure 7 and data not shown). Evaluation of the YKL-40 endotype groups in the SARP cohort showed similar differences in age of onset, lung function, and YKL-40 level among groups E3 and E4 (Figure 8). Analysis of the E3 transcriptome showed down-regulation of apoptotic pathways consistent with findings in mouse models of asthma outlined above.[25] *These studies demonstrate the utilization of YKL-40 levels and clinical features of asthma in a clustering analysis that identified two YKL-40 endotypes of disease characterized by severe persistent airflow obstruction, near-fatal asthma and obesity. These clusters have distinct transcriptomic profiles in the airway. We will use the YKL-40 endotype algorithm in patient subgroups and determine cellular immunotypes and transcriptomes associated with YKL-40 endotype.*

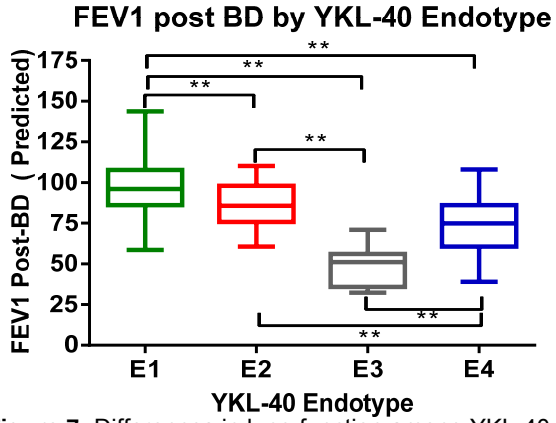


Figure 7: Differences in lung function among YKL-40 Endotypes (P < 0.05 for bars with asterisks)

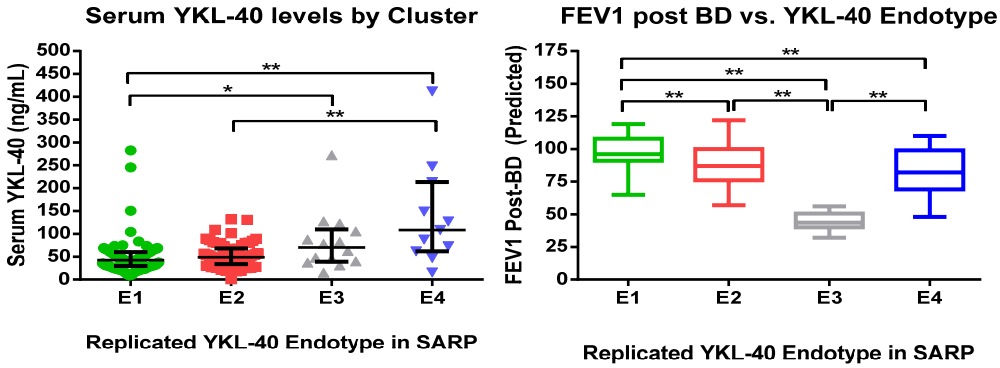


Figure 8: YKL-40 Endotypes are replicated in SARP. P < 0.05 for bars with asterisks

Summary:

A. CHI3L1/YKL-40 drives pathobiology in mouse models of asthma. (1) YKL-40 is expressed in an exaggerated manner in Th2 inflammation in asthma models. 2) In YKL-40-deficient mice the biologic effects of IL-13 and ovalbumin-driven inflammation are significantly inhibited.

B. Human studies show that YKL-40 is important to the pathogenesis of asthma. (1) YKL-40 is expressed in exaggerated quantities in the serum of asthmatics and correlates positively with asthma severity and airway remodeling, but inversely with lung function in multiple asthma populations. (2) YKL-40 levels are associated with polymorphisms in *CHI3L1* that correlate with asthma prevalence, severity, bronchial hyperresponsiveness, and inversely with lung function. These findings have been validated in multiple studies indicating that YKL-40 is important to the pathogenesis of asthma.

C. YKL-40 is associated with a disease endotype of asthma in humans. (1) Clustering analysis that includes YKL-40 and SARP clustering characteristics identifies 4 subgroups of patients and 2 YKL-40 endotype clusters with elevated levels of YKL-40 in the blood and airway (YKL-40 E3 an E4). YKL-40 E3 is associated with early life onset of disease and airway remodeling, while YKL-40 E4 is associated with higher BMI, adult onset disease, and near normal lung function, compared to the other subgroups. (2) YKL-40 binds to IL-13Rα2 and activates AKT, MAPK, and Wnt/β catenin.

Approach. We will conduct studies using single cell technologies to determine the single cell signatures and functional responses associated with YKL-40 endotypes. Baseline sputum cell samples collected in the NextGen study will be evaluated by CyTOF by the Precision Profiling Core C. Immunophenotyping will be conducted using a customized panel of markers developed in collaboration with Dr. Montgomery, Lead of the Precision Profiling Core C to identify M1/M2 macrophages, dendritic cells, NK, Th1, Th2, Th17, Treg, CD8, B cells, Tfh cells as well as YKL-40 pathway signaling molecules including YKL-40, MAPK, AKT, Wnt/βCatenin, IL-13Rα2, and CRTH2, IL-8. SPADE will be used to identify subpopulations of YKL-40 producing cells within each cell lineage.[33] The levels of the YKL-40 producing cells will be compared by the different YKL-40 endotypic markers that we have outlined above including: a) blood YKL-40 level, sputum YKL-40 level, *CHI3L1* haplotype, and YKL-40 endotype group. Each subject's YKL-40 endotype group will be determined using the recursive partitioning algorithm developed by Dr. Gomez as outlined above (See biosketch). Some enrolled subjects will have had *CHI3L1* genotyping done in the past so, when needed, *CHI3L1* haplotype will be determined by sequenom as previously described.[9] These studies will compare the differences in YKL-40 producing cell populations among the different CHI3L1/YKL-40 endotype markers. As outlined in Core C, CyTOF can be done with as little as 10,000 cells per well, so we know there will be an adequate number of usable samples for these studies (~75% or patients enrolled into the YCAAD phenotyping protocol or 150 subjects).[33] Sputum and serum YKL-40 levels will be determined in the Chupp lab using a Meso Scale immunoassay (Meso Scale Discovery, Rockville, MD). Meso Scale technology uses electrochemoluminescence detection and has enhanced sensitivity and reduced background signals compared to standard colorimetric or fluorescent ELISA kits we have used to date.[9,29,34,35] One of the important features of this assay compared to standard ELISA is a significantly increased dynamic range and level of detection for sputum samples. We have determined that YKL-40 was below the level of detection in 70% of BAL fluid samples by standard ELISA, and was 100% detectable by Meso Scale that was sensitive to less than 1.0 pg/ml with a dynamic range of 4 logs (Figure 5). Sputum inflammatory cytokine levels will also be correlated with YKL-40 endotypic markers as described in the Precision Profiling Core C. CyTOF computational analyses using Density Resampled Estimate of Mutual Information DREMI and density Rescaled Visualization DREVI developed by Dr. Smita Krishnaswamy, Co-Lead on Project 3 (See biosketch).[36] These computational tools will be particularly relevant to sputum cell stimulation studies conducted on samples collected at longitudinal visits. These studies will define the cellular immunophenotype of cells producing YKL-40 and how these cellular phenotypes differ amongst different YKL-40 subgroups including genetic haplotype, YKL-40 endotype, and serum and sputum YKL-40 level.

In addition to using CyTOF to characterize the populations that produce YKL-40, we will also determine the lineage of cells that are associated with *CHI3L1* gene expression and YKL-40 synthesis. For these analyses, single cell RNA-seq transcription will be conducted at the Keck sequencing facility under the guidance of Dr. Shrikant Mane who has been part of the YCAAD research team since 2008. Details of single cell sample processing and preliminary data showing single cell transcriptomic profiling of the sputum are described in Precision Profiling Core C. Cellular transcriptomic signatures will be correlated with each subject's YKL-40 classifiers including: YKL-40 endotype, YKL-40 protein levels in the sputum and blood, and *CHI3L1* haplotype. *CHI3L1* gene expression will also be correlated with the YKL-40 classifiers. For these analyses, we will utilize the computational pipelines and tools that will be developed by Project 3, aim 1. These studies will determine how CHI3L1/YKL-40 expression evolves with the shifts in inflammatory populations.[37]

To determine the cellular responses to YKL-40, for aim 1c, mixed cell cultures from the sputum will be stimulated *in vitro* with YKL-40, TNF, and Th1/Th2 inflammatory cytokines including IFN-γ and IL-13 and analyzed by CyTOF.[38] To demonstrate the feasibility of these experiments, mixed culture sputum cells were stimulated with LPS (Figure 10). Using CyTOF, we detected distinct cell subsets by SPADE analysis, where each circular node is a similar population, node color indicates intensity, and adjacent nodes are most similar (Figure 10). A CyTOF antibody panel was designed to identify multiple cell lineages and production of cytokines. The results show isolation of sufficient cells from induced sputum and the ability to detect both the variation between subjects and stimulation-induced production of the pro-inflammatory cytokine TNF in both PMN and monocyte/macrophages from sputum following treatment with LPS. As with all the Projects, we have included in the CyTOF panel surface and intracellular markers of YKL-40 pathway activation and function to best define the baseline level of YKL-40 pathway activation in sputum cells. For stimulation experiments, customized CyTOF panels will be developed in conjunction with Dr. Montgomery on results of baseline analysis. These panels will be used on samples collected from NextGen study at NextGen longitudinal visits.

Statistical considerations. Data analysis will be conducted by YCAAD's computational biologist, Dr. Xiting Yan, Director of Precision Pulmonary Medicine Biostatistics (see biosketch and Core B) and in collaboration with the Gerstein/Krishnaswamy team (Project 3, see biosketch).[39,40] For all aims, standard parametric and non-parametric statistical approaches will be used for cytokine data analysis depending on the distribution of the data. YKL-40 protein levels are usually not normally distributed.[9,41] For the multidimensional data produced by CyTOF, FlowJo is not adequate, and more advanced analysis platforms are required such as SPADE, ViSNE, or Citrus. These clustering programs have been designed for CyTOF datasets to visually and quantitatively

gauge the phenotypic diversity between cell types and donors (see preliminary data generated on a sputum sample in Project 3). These algorithms are in routine use in Core C. Dr. Krishnaswamy has a particular interest in conditional density based analysis of cell signaling in single-cell data and has developed conditional-Density Resampled Estimate of Mutual Information (DREMI) to quantify the strength of the influence that a protein X (in this case YKL-40) has on protein Y and conditional-density Rescaled Visualization (DREVI) to visualize and characterize the edge response function underlying protein interactions (see Krishnaswamy et al, Science 2014).[36] These analytical platforms are outlined in more detail in Project 3, aim 2. For single cell RNAseq data, analytical tools developed in Project 3 will be utilized to identify sputum cell lineages that are associated with *CHI3L1* expression and YKL-40 levels.[42-45]

Power calculation. We calculated power for bulk-RNA-seq gene expression studies because these analyses require the largest sample size to achieve a genome-wide false discovery rate probability <0.05 (Table 1). Assuming that genes less than 2-fold change between two groups are regarded as "no change," Sizepower (R package) was used to calculate the power with various values of the parameters – false discovery rate (FDR) and the anticipated standard deviation (SD) of the difference in log-expression between groups. The SD of sputum gene expression ranges from 0.4-0.6. FDR is controlled by the mean number of false positives fixing the anticipated number of genes in an experiment that are not differently expressed at 10,000. Table 1 demonstrates that we have adequate power for an FDR of 0.05 if we generate bulk RNAseq from 150 sputum samples in the YCAAD biorepository to analyze. Power for single cell RNA-seq studies will be significantly higher compared to bulk RNA-seq as there will be many cells captured from each subject.

Table 1: Power for gene expression For genome-wide FDR

Sample Size	SD	0.05	0.1	0.15	0.2	0.3
50	0.5	100	100	100	100	100
	0.75	99.99	100	100	100	100
	1.00	98.42	99.13	99.41	99.56	99.72
	1.25	87.46	91.56	93.54	94.76	96.21
	1.50	68.49	76.10	80.25	83.02	86.62
75	0.5	100	100	100	100	100
	0.75	100	100	100	100	100
	1.00	99.95	99.98	99.99	99.99	100
	1.25	97.97	98.86	99.22	99.41	99.63
	1.50	89.08	92.77	94.52	95.58	96.84
100	0.5	100	100	100	100	100
	0.75	100	100	100	100	100
	1.00	100	100	100	100	100
	1.25	99.75	99.88	99.92	99.95	99.97
	1.50	98.17	98.17	98.72	99.02	99.36

Deliverables/expected results. The translational infrastructure in place and the preliminary data we have generated demonstrate the feasibility of CyTOF and Single Cell RNA-seq on the sputum. This highlights the strength of YCAAD's multidisciplinary team and shows that we will achieve our aims. Specifically, we will define the cell populations and signatures associated with YKL-40 endotypes. This will significantly enhance our understanding of the role YKL-40 plays in asthma pathogenesis. We expect to find that YKL-40 drives the activation of MAPK, AKT, and Wnt/βcatenin and increases IL-8 and TGF-β expression, primarily in macrophages of M1 lineage.[46] We may also detect novel cell types associated with YKL-40 expression such as cells of mast or dendritic cell lineage. While we know that YKL-40 is expressed in many cell types, CyTOF and single cell transcriptome studies will likely show that cells shifting towards the development of remodeling responses will have the highest level of YKL-40 expression. YKL-40 will be modulated by both Th1 and Th2 cytokines and activation of STAT 6 by IL-13 will drive YKL-40 expression.[47] Single cell analysis will reveal cellular lineages and might show YKL-40 is associated with a specific subset of cells or myofibroblast cell lineage consistent with its pro-fibrotic, tissue remodeling properties.[38,48,49] We suspect the YKL-40 endotype classifier will show that YKL-40 E3, the remodeled endotype, is associated with remodeling responses, while YKL-40 E4 is associated with sirtuin 1 expression and more Th2 inflammation in the airway.[27]

Points of Discussion and Alternative approaches*:*

Adequate sputum samples. The protocols we have developed with CyTOF and single cell RNA-seq are extremely well suited for studies of sputum. CyTOF and single cell capture can be done on as little as 10,000 cells and, with single cell capture, we can exclude buccal squamous cell collection based on their very large size compared to inflammatory and bronchial epithelial cells. This is a major advantage and step forward for our program and will increase our yield of high quality sputum RNA samples at least by a factor of 20%. This suggests there will be at least 150 baseline samples available for RNA-seq analysis. We have also demonstrated the feasibility of low input for RNA-seq and single cell capture from the sputum (detailed in Precision Profiling Core C), so there will be adequate cells for all these studies. In addition, we have patients returning for follow-up visits 2 and 3 as part of the NextGen study protocol, so additional opportunities will present themselves for the acquisition of samples if cell isolation or sample quality is an issue.

Batch effect. Batch effect can be a significant issue, so we try to minimize any alterations in protocols and track if any change in processing could affect results. In addition, when detected, we adjust using several statistical approaches as we've previously reported.[8]

Aim 2: Determine the single cell signatures and functional responses associated with TEA clusters of asthma from baseline and follow-up samples from the NextGen Study (Core B). *Studies will be conducted on NextGen study samples to determine: a) the cell populations associated with TEA clusters of asthma determined by single cell CyTOF analysis of sputum cell populations at baseline, b) the single cell transcriptomic signatures and networks associated with TEA clusters and c) the functional responses of the cell populations defined in Aim 1a and 1b to cytokines including TNF, IL-13 and YKL-40.*

Rationale. We have generated a novel approach to defining endotypes of asthma by conducting unsupervised clustering analysis of microarray measured genome-wide gene expression in bulk RNA isolated from the sputum. These samples were collected from a heterogeneous population of individuals with asthma.[8,50] We have focused on this tissue compartment because it examines gene expression at the site of disease and can be obtained non-invasively. This has allowed for the enrollment of a large number of subjects and captures the "real world" spectrum asthma heterogeneity. This analysis identified 3 transcriptomic endotypes of asthma (TEA), each associated with distinct, clinically meaningful, features of disease and pathways that contribute to the pathophysiology of asthma. In this aim, we will characterize the cell populations and gene expression at the single cell level by CyTOF and single cell RNAseq that are associated with the TEA clusters. Ultimately, these studies will advance our understanding of the TEA clusters we have identified and the pathways and genes that are driving disease pathogenesis in these subgroups of disease and, potentially, asthma in general. Adding single cell analysis to TEA cluster modeling provides the next level of resolution needed to identify specific cell populations and has the capacity to identify novel mechanistic drivers of asthma heterogeneity.

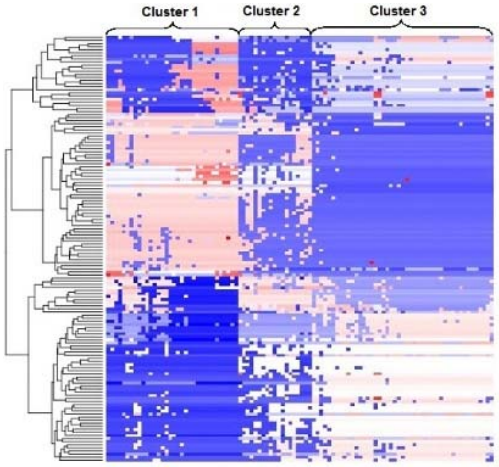


Figure 9: Pathway based unsupervised clustering of sputum gene expression reveals 3 TEA clusters (columns are samples and rows are pathways).

Preliminary Studies. Clustering endotypes using gene expression profiles has been used effectively to sub-classify a number of diseases, in particular malignant diseases such as breast cancer and lymphoma, but to date only a few studies have attempted this in asthma.[51,52] Prior to our description of the TEA clusters, two studies analyzed the sputum transcriptome by microarray analysis: a study of childhood asthma exacerbations and a small adult asthma study that included smokers and did not adjust for batch effect or RNA quality, limiting the conclusions of the study.[51,52] In addition, both studies focused on large differences in gene expression between asthmatics and normals to select genes to cluster, a filtering approach that is commonly used but has the effect of clustering only high abundance genes that may be downstream "danger" signals and not constitutively expressed genes that drive endotypes of disease. In contrast to this computational approach, the novel method that Dr. Yan has developed selects genes for cluster analysis that relies on knowledge-based significance and clusters pathways as opposed to individual genes. This method assumes that genes expressed in a given asthma endotype belong to the same or related biological pathways and has proven to be more robust and less biased than those used previously (Figure 9).[8]

We applied pathway based cluster analysis to microarray measured gene expression from mixed cell sputum RNA collected from subjects enrolled in the YCAAD phenotyping protocol (PI Chupp, R01 HL118346-01, HL095390-01). This included samples that passed quality control criteria for gene expression studies: $\leq$ 20% squamous cells in the sample (indicating oral contamination) and an RNA integrity number (RIN) > 4.0.[8] 112 sputum RNA samples fulfilling the inclusion criteria for asthma had global gene expression measured by microarray analysis using Affymetrix ST 1.0 Gene arrays. For this analysis, the distance between individual samples was calculated based on the expression levels of gene sets defined by publically accessible, annotated pathways and uses a pathway expression metric to cluster samples rather than gene expression. We applied this approach using the KEGG pathways in MsigDB. This reduced the gene number to approximately 5,500 genes (compared to ~8,000) used to redefine the heatmap and distance matrix of sputum gene expression. As can be seen in the figure, this method is more effective than the traditional approach, showing 3 distinct sputum TEA clusters (Figure 9).

Correlations between sputum TEA clusters and the clinical, physiologic and biologic phenotypes of asthma. To understand the pathobiologic relevance of the TEA clusters, we compared the TEA cluster's clinical, physiologic, and biologic characteristics (Table 2, Figure 10, where P values are compared among the TEA clusters by Kruskal-Wallace test). While there were no differences in demographic characteristics of disease, there were significant differences between the clusters in post-bronchodilator (FEV1 P=0.006), bronchodilator response (BDR, P=0.03), FeNO (P = 0.03), IL-13 (P=0.04), and YKL-40 (P=0.04)(data not shown).[8] Importantly, TEA clusters 1 and 2 were associated with an increased likelihood of intubation or hospitalization for asthma, respectively. Therefore, sputum TEA clusters correlate with severe disease characteristics, airway remodeling, FeNO, Th2 cytokines and a biomarker of remodeling independent of a specific cell population in the sputum (figure 10).

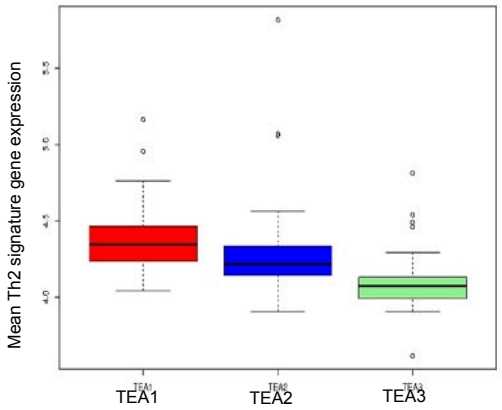


Figure 10: Mean Th2 gene signature (IL-4, 5, 13) among the TEA clusters (P<0.05)

Validation of TEA clusters using a blood derived gene signature. To determine if there is a gene expression signature in the blood that can accurately predict the TEA cluster an individual belongs to, we built a multinomial logistic regression prediction model using gene expression data from matching blood arrays. Genes in the blood that were significantly different between the clusters were selected for this analysis (330 genes). The LASSO algorithm (least squares) was employed to estimate the parameters in the model, which adds a penalty function to the likelihood function to shrink the estimated parameters. The tuning parameter was chosen based on the leave-one-out cross-validation process that provides an estimation for the prediction error. Using this method and gene set, sputum TEA clusters can be predicted with an internal accuracy of 89%. The prediction model was applied to a second set of blood gene expression samples selected from the Asthma BioRepository for Integrative Genomic Exploration (Asthma BRIDGE, N=870) in collaboration with Benj Raby, at the Channing Laboratory at Harvard University School of Medicine. This childhood asthma cohort is 3-4 decades younger than the YCAAD cohort, has a higher percentage of male subjects, and a lower percentage of atopic patients compared to the YCAAD cohort. Gene expression profiling was conducted using HumanHT12 BeadChips (Illumina, San Diego CA), employing a randomized-array allocation strategy to minimize potential confounding by technical batch effects. Quantile normalization and log2 transformation were conducted with the *lumi* package https://biolincc.nhlbi.nih.gov/home/. Cross-platform selection of genes identified 50 out of 53 genes between the Illumina and Affymetrix platforms for the prediction model. Comparison of the TEA clusters in the BRIDGE cohort validated several observations made in the YCAAD cohort (Table 3). First, the prevalence of each of the TEA clusters was similar to the YCAAD cohort, with TEA cluster 2 and 3 being the least and the most prevalent, respectively ($P=2.2x10^{-16}$). Second, also similar to the YCAAD cohort is that TEA cluster 1 was significantly more likely to have a history of intubations ($P=6.0 \times 10^{-6}$), and TEA cluster 2 was more likely to have required hospitalization (P=0.01) compared to the other TEA clusters. TEA cluster 3 was less likely to have been intubated or hospitalized in both cohorts. This data suggests that there are stable transcriptomic profiles in the airway associated with transcriptomic profiles in the blood in

	Cluster 1	Cluster 2	Cluster 3	P Value
Prevalence (N)	34	19	47	0.003
Age at Visit (years)	51 ± 13	49 ±16	45 ± 17.	0.32
Female sex, N (%)	23 (68)	15 (79)	39 (83)	0.26
Race				0.58
White - N (%)	22 (65)	14 (74)	37 (79)	
Black - N (%)	10 (29)	4 (21)	7 (15)	
Other - N (%)	1 (3)	1 (5)	3 (6)	
Hispanic Origin - N (%)	1 (3)	4 (21)	7 (15)	0.04
BMI (Kg/m^2)	30.0 ± 7.2	30 ± 7.3	29.3 ± 8.0	0.84
History of Atopy - N (%)	33 (97)	14 (74)	43 (92)	0.02
Age of Symptom Onset	25.8 ± 19.1	29.3 ± 20.4	20.7 ± 20.9	0.17
Disease Duration (years)	25.2 ± 17.5	20.7 ± 16.9	24.2 ± 17.3	0.70
History of Hospitalization - N (%)	13 (38.1)	13 (68.4)	16 (34.0)	0.03
History of Intubations - N (%)	6.0 (18)	2.0 (11)	2.0 (4)	0.05
OCS tapers in past year- N (%)	19 (55.9)	12 (63.2)	24 (51.1)	0.67
ACT Score	16 ± 6.4	14 ± 6.6	18 ± 5.1	0.22
ICS dose per day (µg)	617 ± 448	530 ± 449	396 ± 356	0.04
Chronic OCS use (%)	4 (11.8)	2 (10.5)	3 (6.4)	0.68

Table 2: Clinical characteristics among the TEA clusters in YCAAD

	Cluster 1 (N=266)	Cluster 2 (N=105)	Cluster 3 (N=499)	P Value
Prevalence in cohort	31%	12%	57%	<2.2x10-16
Age at Visit (years)	14.8 ± 8	10.1 ± 5	12.6 ± 7	3.27 x 10^{-08}
Sex, N (%) Female	128 (48)	45 (43)	212 (43)	0.31
Race				1.68 x 10^{-07}
White - N (%)	102 (38)	79 (75)	284 (57)	
Black - N (%)	105 (40)	9 (9)	124 (25)	
Other - N (%)	29 (11)	7 (7)	37 (7)	
Hispanic Origin - N (%)	30 (11)	10 (10)	54 (11)	0.09
History of Atopy - N (%)	69 (26)	40 (38)	113 (23)	**0.0013**
Age of Symptom Onset	3.50 ± 3.21	3.32 ± 2.89	3.48 ± 2.80	0.86
History of Hospitalization - N (%)	91 (34)	37 (35)	128 (26)	0.011
History of Intubations - N (%)	21 (8)	0 (0)	9 (2)	5.58 x 10^{-06}
ACT Score	14 ± 4	12 ± 3	13 ± 3	8.79 x 10^{-07}

Table 3: Clinical characteristics of TEA clusters in Asthma Bridge cohort

adults and children with asthma that are linked to gene pathway expression in the airway.

Summary. *Our seasoned, multidisciplinary team of experts in asthma, genomics, and computational biology has developed a high throughput system to perform transcriptomic analysis of airway samples collected non-invasively. The data generated using this system has identified TEA clusters that are associated with pathobiologically meaningful features of disease. This demonstrates the utility of sputum transcriptomics to resolve the heterogeneity of asthma. However, to obtain a deeper understanding of the molecular mechanisms that underlie the TEA clusters we will define the cell populations and their functional characteristics using CyTOF and single cell RNA-seq. These studies will provide a critical level of detail that will reveal the pathogenetic underpinnings of the TEA clusters and the genes, pathways, networks, and cellular populations that are contributing to these endotypes.*

Approach. For these studies, total RNA samples from subjects enrolled in the NextGen Endotyping Study will be collected and processed as outlined in the Clinical Recruitment and Biostatistics Core B. The TEA cluster assignment of each subject will be determined by measuring the blood levels of 50 gene signature outlined above using Nanostring nCounter technology (Seattle WA), in the Center for Precision Pulmonary Medicine. The nCounter Analysis System is ideal for this application as it is high throughput, multiplex capable, and utilizes a digital color-coded barcode technology that offers high levels of precision and sensitivity (>1 copy per cell). The technology uses single molecule imaging to detect and count hundreds of unique transcripts in a single reaction. Each color-coded barcode is attached to a single target-specific probe corresponding to a gene of interest. Mixed together with controls, the probes form a multiplexed CodeSet. We will use this system to profile each individual's TEA cluster blood signature. The cellular immunophenotype characterized by CyTOF will be determined as outlined above and in Precision Profiling Core C. Baseline sputum CyTOF immunophenotypes acquired from Precision Profiling Core C and pathophysiologic features of disease acquired from Core B will be compared among the TEA clusters as described in aim 1. As data becomes available from Project 2, we will also examine the relationship between Tfh cell populations, Dkk-1 and IgA-seq results with the TEA clusters. For functional studies of cellular responses, cells will be stimulated as described in aim 1 and customized CyTOF panels will be developed based on findings associated with the TEA clusters at baseline. This will be done in collaboration with Dr. Montgomery and will be used on sputum samples that will be available from NextGen study longitudinal visits (see Precision Profiling Core C for preliminary data on sputum cell stimulation experiments). TNF, Th1/Th2, YKL-40, will be used to determine functional response of cellular subpopulations. Transcriptomic analyses will be conducted on bulk and single cell RNA-seq data made available as outlined in Project 3 to identify the genes that are associated with cellular subpopulations associated with each of the TEA clusters. For these analyses, TEA cluster associated genes in the sputum will be evaluated in both an unsupervised manner and supervised. We will determine if there are cell populations, defined by transcriptomic clustering of single cells, CyTOF immunophenotyping, or traditional methods associated with the TEA clusters. This process will be iterative and in collaboration with the Project 3 team. We will identify the molecular phenotype of cells associated with the TEA clusters, the relevant TEA cluster genes and how they relate to phenotypic features of asthma.

Statistical considerations. The statistical approaches related to this aim are the same as outlined in detail in aim 1 and will be conducted by Dr. Yan, lead of the Data Management and Biostatistics sub-Core and in Collaboration with Project 3 investigators Drs. Krishnaswamy and Gerstein. Gene expression difference among the TEA clusters will be compared on bulk RNA-seq and SC RNA-seq samples. Most of the measures made in human asthma demonstrate a non-parametric distribution, so we routinely use non-parametric tests and control for multiple comparisons to be statistically robust even when in combination with normalized gene expression data. This will be the case for endpoints that compare phenotypic differences among the TEA clusters such as cell concentration in the airway, cell viability, post-bronchodilator FEV1/FVC ratio, bronchodilator response, and sputum cytokine levels. Multiple comparisons will be controlled using the Hochberg method.[53-55] For the multidimensional nature of CyTOF, we are fortunate to have the expertise of Dr. Krishnaswamy to develop novel tools for the CyTOF analysis. For sputum cell stimulation experiments fold change compared to control will be utilized so that data from different subjects can be combined.

Power calculation. Power considerations are identical to aim 1 for gene expression studies. Since we know the distribution of the TEA clusters in 2 populations of asthmatics are similar, we can assume a similar distribution for these studies. Based on our preliminary data; the accuracy of the nanostring TEA cluster classification tool will be approximately 85%, the predicted prevalence of each TEA cluster, and a difference of 20% for any endpoint, we will have a power of 0.90 to show a significant (p=0.05) difference in the endpoints between the TEA clusters.

Deliverables/expected results. We have demonstrated the feasibility of single cell RNA and CyTOF on sputum cell populations and have shown the ability of a blood signature to determine the TEA cluster of enrolled subjects, so these studies will provide important details on the cell populations that are associated with the TEA clusters and CyTOF studies will likely demonstrate specific subpopulations of cells that are associated with each of the TEA clusters. Since TEA cluster 1 is associated with differential expression of L-histidine decarboxylase (HDC), an enzyme in the histamine metabolism pathway that converts histidine to histamine, we may detect

subpopulations of cells with mast cell lineage markers and transcriptional signatures that include C-kit. We anticipate increased responses to Th2 cytokines in these individuals, linking this endotype to higher levels inflammation and severe disease. Since Dr. Bothwell's laboratory has detected higher levels of Dkk-1 in TEA 1, we expect protein levels to be higher in this cluster (see Project 2, aim 2 for preliminary data). TEA cluster 2 is the most heterogeneous cluster with elevated levels of YKL-40 in the sputum, so we anticipate some overlap between this cluster and YKL-40 endotypes. In TEA cluster 3, the largest, least heterogeneous cluster, with the mildest disease, had the most significant differentially expressed genes compared to control subjects including Defensin β□□□DEFB1), an antimicrobial peptide.

Points of Discussion and Alternative approaches. In addition to the discussion points outlined in aim 1 that apply to this aim additional points include:

Generalizability of the TEA clusters to airway biology. We have identified and validated the TEA clusters in adults and children with asthma. One interpretation of these results is that there are common phenomena in the lung amongst individuals that have broader implications to endotypes of other lung diseases. To determine this, we will examine the TEA clusters in publicly accessible datasets from GEO and the Lung Genomics Research Consortium (LGRC). The latter consortium provides access to data from a comprehensive genomic analysis of lung tissue samples from 400 patients with chronic obstructive pulmonary disease (COPD) or interstitial lung disease (ILD), as well as detailed clinical information about those patients. In addition, Next Generation endotypes identified in Project 3 will be compared to the TEA cluster assignments and YKL-40 endotypes, as all of these will be defined in the recruited cohort.

Aim 3: Define the functional responses associated with endotypes of asthma identified in Projects 2 and 3 compared to YKL-40 endotypes and TEA clusters. *Studies will be conducted on samples collected from subjects enrolled in the NextGen study at follow-up visits to determine: a) the functional responses of cell populations associated with novel asthma clusters of disease identified in Project 3 by integrated modeling, b) how the cellular immunophenotypes and cellular responses differ among different endotypes compared to the integrated clusters, and c) the relationship between YKL-40 endotypes and TEA clusters with Tfh cells, Dkk-1, and immunogenic microbiota endotypes defined in Project 2.*

Rationale. The clustering efforts to date, of clinical variables and the sputum transcriptome, have generated clear evidence that machine learning improves our resolution of asthma heterogeneity and indicates that more asthma specific computational tools are needed to define the full breadth of endotypes that are related to this complex disease. This approach provides us an opportunity to redefine how to sub-classify individuals with asthma. Only then will we be able to truly achieve precision medicine for this disease and achieve curative treatments. The evolution of high throughput sequencing, mass spectrometry analytical devices, and single capture technologies provide an opportunity to define individual responses as opposed to averaging across cell populations and individuals. By examining cellular expression and responses to stimuli at the single cell level, we have the capacity to clearly define the cell populations driving the clinical phenotypes that we currently consider as severity. We are clearly on the precipice of a paradigm shift in how we will look at and study asthma, from a single disease defined by variable airflow obstruction and chronic symptoms to a complex physio-immunologic inflammatory disease juxtaposed between the environment and the genome consisting of numerous endotypes. In this aim, we will leverage to combine expertise of our multidisciplinary team of scientists to characterize the pathophysiologic features and cellular functional responses of the asthma subgroups determined by the integrative clustering outlined in Project 3 as well as endpoints measured in Project 2. In contrast to the clustering approaches we used for identification of the TEA clusters, these modeling approaches will be developed using single cell transcriptomic data (SC clusters, see project 3, Table 1) and models that include both clinical features (such as those used to identify the YKL-40 endotypes), cell based signals (protein or transcription), and by gene (logic modeling of disease mechanism). This will entail the integration data described in Aims 1 and 2 of Project 3 to generate integrative clusters of asthma based on clinical data, single cell data, CyTOF and endotype data from Projects 1 and 2 including biomarker and immunogenic microbiome data. This will include integration with clinical and external datasets and use logical modeling to define the regulatory differences between asthma endotypes. Cell-type signatures defined in Aim 2 will be used to deconvolve the bulk-cell RNA-seq data to its component cell transcripts, increasing the effective dynamic range of the cell-specific transcriptional data and facilitating integration with the abundance of bulk-RNA-seq datasets as described in Project 3. These external datasets and the SCyG clusters from Aim 2 will be used to build regulatory network logical models. Each of these clusters, networks and models will be evaluated in the context of established clinical measurements (e.g. FEV1 and FeNO) to identify effective measures to stratify patients and how they might give insight to the mechanisms of asthma disease and heterogeneity. In this aim, we will evaluate the resulting clusters and compare these clusters to YKL-40 endotypes and TEA clusters of asthma.

Furthermore, using the iterative approach, we will develop customized experiments and, using samples collected from follow-up visits from the NextGen study, functional studies will be conducted to define the inflammatory response of the integrated clusters. All of these clusters and analyses will be provided to the community on a publicly accessible, searchable, integrated asthma MAP website dedicated to this effort.

Approach. Experimental approaches will be similar to those described in aims 1 and 2. Cluster determination of each individual enrolled in the NextGen study will be determined as described in Project 3. There will be several different types of clusters identified depending on the type of data clustered (clinical, CyTOF, or RNAseq, or integrated cluster). Customized CyTOF panels will be developed in conjunction with Precision Profiling Core C and stimulation experiments will be designed accordingly. Analyses of cellular sub-populations and transcriptomic signatures will be characterized by CyTOF analyses as described in the Precision Profiling Core C proposal and Project 3, aim 3. Cellular stimulation studies will be conducted on samples collected at NextGen longitudinal visits and customized CyTOF panels will be developed based on the results of the baseline analyses. Stimulation experiments will include Th1/Th2 cytokines, YKL-40, and microbiota antigens identified in project 2. Clinical phenotypes associated with the ISC Clusters will be compared to those associated with YKL-40 endotypes and TEA clusters. Tfh cells, Dkk-1, and IgA-seq measurements will also be integrated into the modeling process as data is generated.

Statistical considerations. Statistical approaches will be similar to those outlined above in aim 1 and 2. We will rely on the expertise of the investigators of all the Projects to enhance these analyses and modeling. The conversations will occur at weekly and ad hoc lab meetings as described in Core A.

Power calculation. Power for these studies is outlined in Aim 1 above.

Deliverables/expected results. These studies will define the clinical phenotypes of the next generation clusters and in comparison to the YKL-40 endotypes and TEA clusters as well as their relationship to Dkk-1, Tfh cells, and immunogenic microbiota. The functional response of airway inflammatory cells among these cells will be defined and used to enhance the specificity of the clustering algorithm and definition of the integrated clusters. We anticipate that there will be some overlap among the various clusters, but anticipate that the integrated clusters will show specific associations to clinical phenotypes such as remodeling and history of flares and possible near fatal asthma. We anticipate that integrated clusters may be associated with specific blood biomarkers and can be tracked from the blood. The next generation clusters will add but not replace other clusters of asthma such as the SARP clusters by providing subgroups based on cellular mechanics rather than clinical variables.

Points of Discussion and Alternative approaches. The points of discussion that are associated with this aim are the same as outlined above in aims 1 and 2.

Interactions with Cores and other driving projects. Project 1 will have extensive interaction with all Next Generation Endotyping of Asthma Heterogeneity Cores and Projects. This speaks to the synergy of the multidisciplinary team and transformative technological and computational approaches outlined in the Yale Asthma U19 proposal.

Timeline. The timeline for Project 1 is outlined in Table 4.

Table 4: Timeline for Project 1	Yea	Year 2	Year 3	Year 4	Year 5
Aim 1: Mechanistic Studies of YKL-40 Endotypes					
a. Baseline CyTOF studies, YKL-40 assays	XXXXX	XXXXX	XXXXX		
b. Bulk and single cell (SC) RNAseq and CyTOF	XXXXX	XXXXX	XXXX	XXXX	XXXXX
c. CyTOF and SC RNAseq Stimulation studies		XXXXX	XXXX	XXXX	
Aim 2: Mechanistic Studies of TEA clusters					
a. Baseline CyTOF studies, Nanostring TEA cluster assay	XXXXX	XXXXX	XXXXX		
b. Bulk and single cell (SC) RNAseq studies CyTOF	XXXXX	XXXXX	XXXXX	XXXXX	XXXXX
c. CyTOF and SC RNAseq Stimulation studies	XXXX	XXXX	XXXXX	XXXXX	
Aim 3: Mechanistic Studies of Integrated SC clusters					
a. Baseline CyTOF studies, identification of ISC clusters	XXXX	XXXX	XXXXX	XXXXX	XXXXX
b. Bulk and single cell (SC) RNAseq studies CyTOF	XXXX	XXXX	XXXXX	XXXXX	XXXXX
c. Customized CyTOF and SC RNAseq Stimulation studies	XXXX	XXXX	XXXXX	XXXXX	XXXXX
DISSEMINATION. Documentation preparation, presentation at national meetings, manuscript submission. Development of Asthma MAP	XXXXX	XXXXX	XXXXX	XXXXX	XXXXX

Protection of Human Subjects

Risks to Human Subjects

a. Human Subjects Involvement and Characteristics. Over the course of this project, we plan to recruit 200 asthmatic and 50 non-asthmatic subjects through the Yale Center for Asthma and Airway Diseases (YCAAD). Each subject will return approximately every 9 months and complete 3 visits over the 5-year grant period. At each of the 3 visits the subject will be administered a questionnaire and perform physiological testing, and blood and sputum samples will be obtained. Asthmatic and non-asthmatic subjects recruited for these studies will be 12 years old or greater. Through a distinct HIC protocol, for these studies we will also recruit asthmatic and non-asthmatic children (age 7-17 years old) undergoing adenotonsillectomy, to donate tonsillar tissue, blood samples, and nasal brushings to the YCAAD biorepository.

Drs. Cohn and Chupp have recruited subjects for the past 15 years through the *Mechanisms and Mediators of Asthma and Chronic Obstructive Pulmonary Disease (COPD) human investigation* protocol (Yale HIC 0102012268, PI Chupp, Co-I Cohn). Over 800 subjects have been recruited to date. Tonsillar tissue will be obtained as part of the *Correlation of Gene Expression Profile with Clinical Response to Adenotonsillectomy* protocol (Yale HIC 1009007345, PI Karas, Co-I Chupp). This is a repository is to investigate the relationship between adenotonsillar hypertrophy and asthma and airway diseases, and identify genes, molecules and pathways that are associated with disease development, progression, and severity.

YCAAD Study Protocol: Exclusions will be based on health considerations. We will exclude any patient with a contraindication to any of the required testing including sputum induction, spirometry, or phlebotomy. These exclusions include severe cardiovascular, renal, or liver disease, bleeding diathesis, or advanced neuromuscular disease. A centerpiece of the YCAAD translational research program and this proposal is the airways disease phenotyping protocol developed to study human asthma. This protocol has been developed to acquire the full complement of clinical information, physiologic measurements and biologic samples to perform genotype-phenotype studies. Following informed consent, subjects are administered a questionnaire by the study coordinator. This includes a detailed asthma and medical history, asthma activity questionnaire (Asthma Control Test), Asthma Quality of Life Questionnaire, and details about medications, histories of smoking, occupational and environmental exposures, history of allergy, sinus disease, gastroesophageal reflux, obesity, and medication compliance. All data is uploaded into a HIPAA compliant web-based database developed by YCAAD. Blood is drawn and processed for the isolation of DNA, RNA, serum and plasma. Samples are aliquoted and banked for future studies. Blood is sent to the clinical laboratory for CBC and differential, serum IgE level, ImmunoCap testing for 22 common aeroallergens and routine chemistries. Following phlebotomy, patients undergo FeNO testing and spirometry (in adherence with ATS guidelines) before and after short acting bronchodilator administration. Sputum induction is done using hypertonic saline and immediately processed. Mucus plugs are removed using a dissecting microscope to remove squamous cell contamination, the cellular and aqueous compartments are separated, cell counts and differentials are quantified, and aliquots of supernatants, cell protein, and cellular RNA are stored for later analysis. The protocol is practical, efficient, and enrolls large numbers of subjects in a timely fashion. We will phenotype up to 5 subjects weekly. Subjects will be scheduled to return for repeat sampling as described, which is important for determination of the stability of their disease and their biological samples.

Asthma Inclusion criteria. Recruitment through YCAAD is designed to enroll a wide-range of asthmatic subjects in terms of age, disease severity and ongoing treatment. Inclusion criteria for asthmatic subjects are: (1) ≥ 12 years of age; (2) < 10 pack years of tobacco, and have not smoked for ≥ 1 year. Asthmatic subjects must have a diagnosis of asthma defined by the following criteria (based on the National Asthma and Education and Prevention Panel Guidelines): (1) a history of chronic symptoms (>2 years) compatible with asthma (determined by a study investigator/pulmonary physician specializing in asthma); and (2) historical evidence of variable airflow obstruction determine by: (a) > 12% improvement in FEV_1 after an inhaled $beta_2$ agonist, inhaled corticosteroids, or an oral prednisone burst; (b) methacholine reactivity with a provocative concentration causing a 20% decrease in FEV_1(PC20) of <8 mg/ml; or (c) 20% diurnal variation of peak expiratory flow rates on 2 days over a 2-3 week period. Patients will be excluded if they cannot safely undergo the studies required for participation or have other chronic lung disease or asthma variant disease (ABPA, Churg-Strauss Syndrome, vocal cord dysfunction, etc.). Normal subjects must have no history of any chronic lung disease. Non-asthmatic control subjects will be excluded if they have a diagnosis of any ongoing acute or chronic lung disease, have smoked ≥10 pack-years or have abnormal spirometry.

Adenotonsillectomy Study Protocol. Parents and patients identified by providers as potential subjects will be

invited to participate in the research study after they have been consented independently for their planned procedure of adenotonsillectomy. The parent and child will complete a YCAAD pediatric questionnaire and an asthma activity score, and spirometry will be done for those children aged six years or older who are capable of completing the test. Intraoperatively, a venous blood sample (5-10ml) and nasal swab sample will be obtained. After a portion of the tissue is sent to surgical pathology, as is standard for adenotonsillectomy, a portion of the removed tonsils and/or adenoid tissue will be taken for study purposes. An additional specimen will be held in the pathology department, flash frozen in OCT in the event more tissue is needed for analysis. Samples will be processed and banked with the assigned de-identified study subject number. A second study visit will take place six months following surgery and includes an interim history using the follow-up questionnaire focused on stability of upper airway and asthma symptoms. Repeat PFT testing will be performed in children aged six years or older, if possible. An additional venous blood sample will be obtained for the repository. Subjects may decline any of the testing at any of these visits.

b. Sources of Materials. In this proposal, the following materials will be collected from human subjects: clinical data (questionnaire, history and physical examination data, lung function data and relevant laboratory test results), blood, sputum, nasal brushings and tonsils/adenoids. Clinical data will be identified with the study subject's name. This information will be maintained in a locked file and these records are kept separate from other clinical records to assure confidentiality. Only Drs. Cohn, Chupp, Niu, and study coordinators will have access to these confidential records. All research specimens, including blood, and sputum specimens are identified by a patient code for correlation purposes. All samples and datasets will be de-identified and only known to study investigators involved in consent and sample acquisition. Investigators and staff will receive only de-identified data and samples for their studies and analyses. All data sets uploaded and samples delivered to Core C and the Yale Center for Genomic Analysis will be de-identified.

The studies in this proposal will adhere to the U.S. Department of Health and Human Services Office for the Protection from Research Risk (OPRR). All information relating to participating subjects will be de-identified. Specimens may only be used in accordance with the conditions stipulated in the Yale Human Investigation Committee (HIC) (see approval letters in Appendix).

c. Potential Risks. Risks associated with the YCAAD study (HIC 0102012268) including pulmonary function tests, blood draw and sputum induction are minimal (adults) and greater than minimal (children 12-17 years old) and are explained to subjects in detail at the time of consent (see consent forms in Appendix). Bronchospasm is a risk of sputum induction, but the safety of sputum induction in asthmatic subjects, even those with severe asthma has been well documented in several studies (ref). Spirometry is repeatedly tested during the procedure to monitor for bronchospasm, and if wheezing or a significant drop in FEV1 occurs, subjects are given bronchodilators. Wheezing, if it occurs, is usually transient and treatable with bronchodilators. A Data and Safety Monitoring (DSM) Plan and DSM Committee are in place to assess adverse events (see Appendix). To date, we have had no serious adverse events in our laboratory, even in severe asthma subjects undergoing sputum induction. Clinical Recruitment and Biostatistics Core Lead (Dr. Cohn) or a research coordinator will monitor all procedures performed. After any procedure performed in YCAAD, subjects are instructed to contact the YCAAD on-call physician if any problem occurs. YCAAD is covered 24 x 7 by pulmonary physicians who are familiar with the management of acute bronchospasm in asthmatic patients. Risks associated with the adenotonsillectomy study (Yale HIC 1009007345) are minimal for the blood draw and there is no added risk of apportioning adenoid and tonsil tissue to the biorepository. Emergency equipment and personnel are immediately available in the event of an adverse reaction at all times during these study visits.

Adequacy of Protection Against Risks

a. Recruitment and Informed Consent. Subjects will be recruited from YCAAD and from the pediatric ENT offices of Dr. Karas. Once a subject is deemed eligible for this study, informed consent will be obtained. The study procedures, risks and benefits will be clearly explained to each subject. All questions will be answered, and time will be allowed for the subject to make an informed decision before signing the consent form. If the subject agrees to enroll, a consent form (see Appendix) will be reviewed and explained, and the subject or consenting adult will be asked to sign it.

b. Protections Against Risk. Risks will be minimized by appropriate subject exclusions, close medical supervision throughout the protocol, and adaptation of testing to identify patients who might be at higher risk. Subjects experiencing adverse events will have access to full inpatient medical facilities of the Yale-New

Haven Hospital if more intensive treatment is necessary to reverse an asthma attack or complication during the study. All patients will be under full-time coordinator supervision throughout every phase of the YCAAD procedures. In the adenotonsillectomy protocol, at least one physician and/or nurse will be present throughout the study visits and procedures. Adverse events will be reported to the Yale IRB (Human Investigation Committee) and the DSMC.

Potential Benefits of the Proposed Research to Human Subjects and Importance of the Knowledge to be Gained

These studies are not designed to provide direct benefit to the participants. However, the knowledge gained from this work will enhance information about the pathogenesis of asthma and may lead to improved diagnosis, treatment and prevention of asthma in the future. All subjects receive asthma education and copies of blood work and lung function tests as part of their visit, and have full access to a YCAAD physician, if desired. If any major test abnormalities are encountered, YCAAD physicians counsel the subjects and provide referrals to appropriate specialists as needed.

Inclusion of Women and Minorities

Subjects will be recruited through the Clinical Recruitment and Biostatistics Core (Core B) and through the office of Dr. Karas for adenotonsillectomy without any bias of race or gender. The YCAAD asthma and control populations and the children undergoing adenotonsillectomy are racially diverse and reflect the population in New Haven and surrounding communities. All minority groups already take part in these research programs and we will continue to actively recruit women and minorities as these studies go forward. Based on demographics from past studies, the asthmatic population in YCAAD is approximately 71% female and 31% Black and Hispanic. The demographics of the adenotonsillectomy population is approximately 37% female, 14% Black and 36% Hispanic.

OMB Number: 0925-0002

Planned Enrollment Report

Study Title: NextGen Biorepository Study

Domestic/Foreign: Domestic

Comments:

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/Alaska Native	0	0	0	0	**0**
Asian	3	2	0	0	**5**
Native Hawaiian or Other Pacific Islander	0	0	0	0	**0**
Black or African American	20	18	8	8	**54**
White	85	84	12	10	**191**
More than One Race	0	0	0	0	**0**
Total	**108**	**104**	**20**	**18**	**250**

Study 1 of 2

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

OMB Number: 0925-0002

Planned Enrollment Report

Study Title: Adenotonsillectomy Biorepository Study

Domestic/Foreign: Domestic

Comments:

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/Alaska Native	0	0	0	0	**0**
Asian	2	2	0	0	**4**
Native Hawaiian or Other Pacific Islander	0	0	0	0	**0**
Black or African American	12	10	6	4	**32**
White	28	22	8	6	**64**
More than One Race	0	0	0	0	**0**
Total	**42**	**34**	**14**	**10**	**100**

Study 2 of 2

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

Inclusion of Children

The proposal will recruit children between the ages of 2 and 21 years (consent and assent form are included in Appendix 1 and 2). The investigative team for Yale #HIC0102012268 will request that the participants (12-21 years old) undergo phlebotomy, sputum induction, lung function testing and complete an asthma questionnaire with the subject and his/her parent/legal guardian. Venipuncture and pulmonary function testing and questioning are part of the routine care of children with asthma. Sputum induction is a low-risk procedure and is outlined in the Appendix. All interviews and procedures will be performed in the Yale Center for Asthma and Airways Disease. Overall, the study poses more than a minimal risk to minor subjects, but its benefits outweigh these risks. The investigative team for Yale # HIC1009007345 will request that the participant (2-18 years old) who was previously scheduled to undergo adenotonsillectomy, consent to have blood drawn intraoperatively, perform spirometry before surgery, if capable, and agree to donate excess tonsil tissue to the biorepository for analyses. These procedures are assessed to be of minimal risk to child subjects. The investigative team has experience caring for children should any adverse events arise during sample acquisition.

Inclusion of children in this study is important since we are interested in the relationship of transcriptomic endophenotypes across the spectrum of asthma. Our studies thus far suggest that there are stable Transcriptional Endophenotypes of Asthma (TEA) in children and adults with severe asthma and, in particular, near fatal asthma. Endotypes seen in adult asthmatics may be evident in young subjects, and this may help us predict the course of disease as they age. Further, we may be able to discern which patients will benefit from more aggressive care and elucidate molecular pathways involved in the pathogenesis of severe asthma. We will follow subjects longitudinally and will define how transcriptomic signatures relate with progression of this disease.

References

1. Centers for Disease C, Prevention. Vital signs: asthma prevalence, disease characteristics, and self-management education: United States, 2001--2009. MMWR Morbidity and mortality weekly report 2011;60:547-52.
2. Fahy JV. Type 2 inflammation in asthma - present in most, absent in many (vol 15, pg 57, 2015). Nat Rev Immunol 2015;15:130-.
3. Huang YJ, Boushey HA. The microbiome in asthma. J Allergy Clin Immun 2015;135:25-30.
4. Thorburn AN, McKenzie CI, Shen S, Stanley D, Macia L, Mason LJ, Roberts LK, Wong CHY, Shim R, Robert R, Chevalier N, Tan JK, Marino E, Moore RJ, Wong L, McConville MJ, Tull DL, Wood LG, Murphy VE, Mattes J, Gibson PG, Mackay CR. Evidence that asthma is a developmental origin disease influenced by maternal diet and bacterial metabolites. Nat Commun 2015;6.
5. Levy BD, Noel PJ, Freemer MM, Cloutier MM, Georas SN, Jarjour NN, Ober C, Woodruff PG, Barnes KC, Bender BG, Camargo CA, Jr., Chupp GL, Denlinger LC, Fahy JV, Fitzpatrick AM, Fuhlbrigge A, Gaston BM, Hartert TV, Kolls JK, Lynch SV, Moore WC, Morgan WJ, Nadeau KC, Ownby DR, Solway J, Szefler SJ, Wenzel SE, Wright RJ, Smith RA, Erzurum SC. Future Research Directions in Asthma: An NHLBI Working Group Report. Am J Respir Crit Care Med 2015.
6. Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. Nat Med 2012;18:716-25.
7. Moore WC, Meyers DA, Wenzel SE, Teague WG, Li H, Li X, D'Agostino R, Jr., Castro M, Curran-Everett D, Fitzpatrick AM, Gaston B, Jarjour NN, Sorkness R, Calhoun WJ, Chung KF, Comhair SA, Dweik RA, Israel E, Peters SP, Busse WW, Erzurum SC, Bleecker ER, National Heart L, Blood Institute's Severe Asthma Research P. Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. Am J Respir Crit Care Med 2010;181:315-23.
8. Yan X, Chu JH, Gomez J, Koenigs M, Holm C, He X, Perez MF, Zhao H, Mane S, Martinez FD, Ober C, Nicolae DL, Barnes KC, London SJ, Gilliland F, Weiss ST, Raby BA, Cohn L, Chupp GL. Noninvasive analysis of the sputum transcriptome discriminates clinical phenotypes of asthma. Am J Respir Crit Care Med 2015;191:1116-25.
9. Gomez JL, Crisafi GM, Holm CT, Meyers DA, Hawkins GA, Bleecker ER, Jarjour N, Severe Asthma Research Program I, Cohn L, Chupp GL. Genetic variation in chitinase 3-like 1 (CHI3L1) contributes to asthma severity and airway expression of YKL-40. The Journal of allergy and clinical immunology 2015;136:51-8 e10.
10. Chupp GL, Lee CG, Jarjour N, Shim YM, Holm CT, He S, Dziura JD, Reed J, Coyle AJ, Kiener P, Cullen M, Grandsaigne M, Dombret MC, Aubier M, Pretolani M, Elias JA. A chitinase-like protein in the lung and circulation of patients with severe asthma. The New England journal of medicine 2007;357:2016-27.
11. Proceedings of the ATS workshop on refractory asthma: current understanding, recommendations, and unanswered questions. American Thoracic Society. Am J Respir Crit Care Med 2000;162:2341-51.
12. Woodruff PG, Modrek B, Choy DF, Jia GQ, Abbas AR, Ellwanger A, Koth LL, Arron JR, Fahy JV. T-helper Type 2-driven Inflammation Defines Major Subphenotypes of Asthma (vol 180, pg 388, 2009). Am J Resp Crit Care 2009;180:796-.
13. Buss G. [Biologics in asthma: what's new?]. Revue medicale suisse 2015;11:20, 2-4.
14. Darveaux J, Busse WW. Biologics in asthma--the next step toward personalized treatment. The journal of allergy and clinical immunology In practice 2015;3:152-60; quiz 61.
15. Hartl D, Lee CG, Da Silva CA, Chupp GL, Elias JA. Novel biomarkers in asthma: chemokines and chitinase-like proteins. Curr Opin Allergy Cl 2009;9:60-6.
16. Zhu Z, Lee CG, Zheng T, Chupp G, Wang JM, Homer RJ, Noble PW, Hamid Q, Elias JA. Airway inflammation and remodeling in asthma - Lessons from interleukin 11 and interleukin 13 transgenic mice. Am J Resp Crit Care 2001;164:S67-S70.
17. Moore WC. The natural history of asthma phenotypes identified by cluster analysis. Looking for chutes and ladders. Am J Respir Crit Care Med 2013;188:521-2.
18. Hastie AT, Moore WC, Meyers DA, Vestal PL, Li H, Peters SP, Bleecker ER, National Heart L, Blood Institute Severe Asthma Research P. Analyses of asthma severity phenotypes and inflammatory proteins in subjects stratified by sputum granulocytes. The Journal of allergy and clinical immunology 2010;125:1028-36 e13.
19. Ober C, Chupp GL. The chitinase and chitinase-like proteins: a review of genetic and functional studies in asthma and immune-mediated diseases. Curr Opin Allergy Clin Immunol 2009;9:401-8.
20. Dickey BF. Exoskeletons and exhalation. The New England journal of medicine 2007;357:2082-4.

21. He CH, Lee CG, Dela Cruz CS, Lee CM, Zhou Y, Ahangari F, Ma B, Herzog EL, Rosenberg SA, Li Y, Nour AM, Parikh CR, Schmidt I, Modis Y, Cantley L, Elias JA. Chitinase 3-like 1 regulates cellular and tissue responses via IL-13 receptor alpha2. Cell reports 2013;4:830-41.
22. Cho WK, Lee CM, Kang MJ, Huang Y, Giordano FJ, Lee PJ, Trow TK, Homer RJ, Sessa WC, Elias JA, Lee CG. IL-13 receptor alpha2-arginase 2 pathway mediates IL-13-induced pulmonary hypertension. American journal of physiology Lung cellular and molecular physiology 2013;304:L112-24.
23. Zhu Z, Zheng T, Homer RJ, Kim YK, Chen NY, Cohn L, Hamid Q, Elias JA. Acidic mammalian chitinase in asthmatic Th2 inflammation and IL-13 pathway activation. Science 2004;304:1678-82.
24. Zhu Z, Homer RJ, Wang Z, Chen Q, Geba GP, Wang J, Zhang Y, Elias JA. Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. J Clin Invest 1999;103:779-88.
25. Lee CG, Hartl D, Lee GR, Koller B, Matsuura H, Da Silva CA, Sohn MH, Cohn L, Homer RJ, Kozhich AA, Humbles A, Kearley J, Coyle A, Chupp G, Reed J, Flavell RA, Elias JA. Role of breast regression protein 39 (BRP-39)/chitinase 3-like-1 in Th2 and IL-13-induced tissue responses and apoptosis. The Journal of experimental medicine 2009;206:1149-66.
26. Elias JA, Zheng T, Lee CG, Homer RJ, Chen Q, Ma B, Blackburn M, Zhu Z. Transgenic modeling of interleukin-13 in the lung. Chest 2003;123:339S-45S.
27. Ahangari F, Sood A, Ma B, Takyar S, Schuyler M, Qualls C, Dela Cruz CS, Chupp GL, Lee CG, Elias JA. Chitinase 3-like-1 Regulates Both Visceral Fat Accumulation and Asthma-like Th2 Inflammation. Am J Resp Crit Care 2015;191:746-57.
28. Gavala ML, Kelly EA, Esnault S, Kukreja S, Evans MD, Bertics PJ, Chupp GL, Jarjour NN. Segmental allergen challenge enhances chitinase activity and levels of CCL18 in mild atopic asthma. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology 2013;43:187-97.
29. Ober C, Tan Z, Sun Y, Possick JD, Pan L, Nicolae R, Radford S, Parry RR, Heinzmann A, Deichmann KA, Lester LA, Gern JE, Lemanske RF, Nicolae DL, Elias JA, Chupp GL. Effect of variation in CHI3L1 on serum YKL-40 level, risk of asthma, and lung function. New Engl J Med 2008;358:1682-91.
30. Rathcke CN, Holmkvist J, Husmoen LL, Hansen T, Pedersen O, Vestergaard H, Linneberg A. Association of polymorphisms of the CHI3L1 gene with asthma and atopy: a populations-based study of 6514 Danish adults. PloS one 2009;4:e6106.
31. Ortega H, Prazma C, Suruki RY, Li H, Anderson WH. Association of CHI3L1 in African-Americans with prior history of asthma exacerbations and stress. The Journal of asthma : official journal of the Association for the Care of Asthma 2013;50:7-13.
32. Cunningham J, Basu K, Tavendale R, Palmer CN, Smith H, Mukhopadhyay S. The CHI3L1 rs4950928 polymorphism is associated with asthma-related hospital admissions in children and young adults. Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology 2011;106:381-6.
33. Yao Y, Liu R, Shin MS, Trentalange M, Allore H, Nassar A, Kang I, Pober JS, Montgomery RR. CyTOF supports efficient detection of immune cell subsets from small samples. Journal of immunological methods 2014;415:1-5.
34. Chupp G, Elias JA. Chitinase-like protein and asthma - Reply. New Engl J Med 2008;358:1075-.
35. Levin JC, Gagnon L, He X, Baum ED, Karas DE, Chupp GL. Improvement in asthma control and inflammation in children undergoing adenotonsillectomy. Pediatric research 2014;75:403-8.
36. Krishnaswamy S, Spitzer MH, Mingueneau M, Bendall SC, Litvin O, Stone E, Pe'er D, Nolan GP. Systems biology. Conditional density-based analysis of T cell signaling in single-cell data. Science 2014;346:1250689.
37. Treutlein B, Brownfield DG, Wu AR, Neff NF, Mantalas GL, Espinoza FH, Desai TJ, Krasnow MA, Quake SR. Reconstructing lineage hierarchies of the distal lung epithelium using single-cell RNA-seq. Nature 2014;509:371-5.
38. Tang H, Sun Y, Shi Z, Huang H, Fang Z, Chen J, Xiu Q, Li B. YKL-40 induces IL-8 expression from bronchial epithelium via MAPK (JNK and ERK) and NF-kappaB pathways, causing bronchial smooth muscle proliferation and migration. Journal of immunology 2013;190:438-46.
39. Gerstein MB, Rozowsky J, Yan KK, Wang D, Cheng C, Brown JB, Davis CA, Hillier L, Sisu C, Li JJ, Pei B, Harmanci AO, Duff MO, Djebali S, Alexander RP, Alver BH, Auerbach R, Bell K, Bickel PJ, Boeck ME, Boley NP, Booth BW, Cherbas L, Cherbas P, Di C, Dobin A, Drenkow J, Ewing B, Fang G, Fastuca M, Feingold EA, Frankish A, Gao G, Good PJ, Guigo R, Hammonds A, Harrow J, Hoskins RA, Howald C, Hu L, Huang H, Hubbard TJ, Huynh C, Jha S, Kasper D, Kato M, Kaufman TC, Kitchen RR, Ladewig E, Lagarde J, Lai E, Leng J, Lu Z, MacCoss M, May G, McWhirter R, Merrihew G, Miller DM, Mortazavi A, Murad R, Oliver B, Olson S, Park PJ,

Pazin MJ, Perrimon N, Pervouchine D, Reinke V, Reymond A, Robinson G, Samsonova A, Saunders GI, Schlesinger F, Sethi A, Slack FJ, Spencer WC, Stoiber MH, Strasbourger P, Tanzer A, Thompson OA, Wan KH, Wang G, Wang H, Watkins KL, Wen J, Wen K, Xue C, Yang L, Yip K, Zaleski C, Zhang Y, Zheng H, Brenner SE, Graveley BR, Celniker SE, Gingeras TR, Waterston R. Comparative analysis of the transcriptome across distant species. Nature 2014;512:445-8.
40. Krishnaswamy S, Kanteti R, Duke-Cohan JS, Loganathan S, Liu W, Ma PC, Sattler M, Singleton PA, Ramnath N, Innocenti F, Nicolae DL, Ouyang Z, Liang J, Minna J, Kozloff MF, Ferguson MK, Natarajan V, Wang YC, Garcia JG, Vokes EE, Salgia R. Ethnic differences and functional analysis of MET mutations in lung cancer. Clinical cancer research : an official journal of the American Association for Cancer Research 2009;15:5714-23.
41. Chupp GL, Lee CG, Jarjour N, Shim YM, Holm CT, He S, Dziura JD, Reed J, Coyle AJ, Kiener P, Cullen M, Grandsaigne M, Dombret M, Aubier M, Pretolani M, Elias JA. A chitinase-like protein in the lung and circulation of patients with severe asthma. New Engl J Med 2007;357:2016-27.
42. Agarwal A, Koppstein D, Rozowsky J, Sboner A, Habegger L, Hillier LW, Sasidharan R, Reinke V, Waterston RH, Gerstein M. Comparison and calibration of transcriptome data from RNA-Seq and tiling arrays. BMC genomics 2010;11:383.
43. Habegger L, Sboner A, Gianoulis TA, Rozowsky J, Agarwal A, Snyder M, Gerstein M. RSEQtools: a modular framework to analyze RNA-Seq data using compact, anonymized data summaries. Bioinformatics 2011;27:281-3.
44. Wang Z, Gerstein M, Snyder M. RNA-Seq: a revolutionary tool for transcriptomics. Nature reviews Genetics 2009;10:57-63.
45. Qian F, Chung L, Zheng W, Bruno V, Alexander RP, Wang Z, Wang X, Kurscheid S, Zhao H, Fikrig E, Gerstein M, Snyder M, Montgomery RR. Identification of genes critical for resistance to infection by West Nile virus using RNA-Seq analysis. Viruses 2013;5:1664-81.
46. Bonneh-Barkay D, Bissel SJ, Kofler J, Starkey A, Wang G, Wiley CA. Astrocyte and macrophage regulation of YKL-40 expression and cellular response in neuroinflammation. Brain pathology 2012;22:530-46.
47. Singh SK, Bhardwaj R, Wilczynska KM, Dumur CI, Kordula T. A complex of nuclear factor I-X3 and STAT3 regulates astrocyte and glioma migration through the secreted glycoprotein YKL-40. The Journal of biological chemistry 2011;286:39893-903.
48. Konradsen JR, James A, Nordlund B, Reinius LE, Soderhall C, Melen E, Wheelock AM, Lodrup Carlsen KC, Lidegran M, Verhoek M, Boot RG, Dahlen B, Dahlen SE, Hedlin G. The chitinase-like protein YKL-40: a possible biomarker of inflammation and airway remodeling in severe pediatric asthma. The Journal of allergy and clinical immunology 2013;132:328-35 e5.
49. Lee CG, Dela Cruz CS, Herzog E, Rosenberg SM, Ahangari F, Elias JA. YKL-40, a chitinase-like protein at the intersection of inflammation and remodeling. Am J Respir Crit Care Med 2012;185:692-4.
50. Steiling K, Christenson SA. Targeting 'types: Precision Medicine in Pulmonary Disease. Am J Respir Crit Care Med 2015;191:1093-4.
51. Baines KJ, Simpson JL, Wood LG, Scott RJ, Gibson PG. Transcriptional phenotypes of asthma defined by gene expression profiling of induced sputum samples. The Journal of allergy and clinical immunology 2011;127:153-60, 60 e1-9.
52. Bosco A, Ehteshami S, Stern DA, Martinez FD. Decreased activation of inflammatory networks during acute asthma exacerbations is associated with chronic airflow obstruction. Mucosal immunology 2010;3:399-409.
53. Benjamini Y, Drai D, Elmer G, Kafkafi N, Golani I. Controlling the false discovery rate in behavior genetics research. Behavioural brain research 2001;125:279-84.
54. Benjamini Y, Hechtlinger Y. Discussion: An estimate of the science-wise false discovery rate and applications to top medical journals by Jager and Leek. Biostatistics 2014;15:13-6; discussion 39-45.
55. Benjamini Y, Yekutieli D. Quantitative trait Loci analysis using the false discovery rate. Genetics 2005;171:783-90.

Resource Sharing Plan

Data generated in these studies will be freely available to members of the research community with a goal to enable dissemination of data, while also protecting the privacy of the participants and the utility of the data, by de-identifying and masking potentially sensitive data elements in compliance with the NIH public data sharing policy. All other resources developed in the course of the proposed studies will be available by request to qualified academic investigators for non-commercial research.

For all studies, we will follow the National Institute of Health's Genomic Data Sharing Policy. The raw datasets corresponding to expression, genomic, and genetic data generated by these studies will be submitted to Gene Expression Omnibus (GEO) or the Sequence Read Archive (SRA) for use by other investigators. As datasets are analyzed, then validated, we will proceed with deposition in ImmPort according to a timeline negotiated with the Program Officer. Sample data in the YCAAD biorepository are, and will continue to be, available on the internet through the YCCI research accelerator, a publicly accessible platform for scientific collaboration (ycci.researchaccelerator.org). In addition, tools, pipelines, derived datasets and analyses will be made available through the website (asthmaMAP.gersteinlab.org) which will serve as an organizational tool for the participants in this cooperative proposal as well as a repository and resource for the greater research community. Details of the contents and construction of the asthma MAP website are in Project 3, Research Proposal Aim 3.

Yale University School of Medicine and all investigators will adhere to the NIH Grants Policy on Sharing of Unique Research Resources including the “Sharing of Biomedical Research Resources: Principles and Guidelines for Recipients of NIH Grants and Contracts” issued in December 1999. Specifically, material transfers would be made with no more restrictive terms than in the Simple Letter Agreement or the UBMTA and without reach through requirements. Should any intellectual property arise which requires a patent, we would ensure that the technology remains widely available to the research community in accordance with the NIH Principles and Guidelines document. In addition, we will provide relevant protocols and published data upon request. Accepted versions of final, peer-reviewed manuscripts emanating from this research will be deposited on-line to PubMed Central in accord with NIH Public Access.

OMB Number: 4040-0001
Expiration Date: 06/30/2016

APPLICATION FOR FEDERAL ASSISTANCE

SF 424 (R&R)

5. APPLICANT INFORMATION **Organizational DUNS*:** 0432075620000

Legal Name*: YALE UNIVERSITY
Department:
Division:
Street1*: OFFICE OF SPONSORED PROJECTS
Street2: 25 Science Park
City*: NEW HAVEN
County:
State*: CT: Connecticut
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 065208237

Person to be contacted on matters involving this application

Prefix:	First Name*:	Middle Name:	Last Name*:	Suffix:
	Marybeth		Brandi	

Position/Title: Proposal Manager
Street1*: 25 Science Park
Street2: 150 Munson Street
City*: New Haven
County:
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Province:
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ZIP / Postal Code*: 06520-8237
Phone Number*: 203-737-3495 Fax Number: Email: marybeth.brandi@yale.edu

7. TYPE OF APPLICANT*

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Novel Immune Pathways Drive Asthma Heterogeneity

12. PROPOSED PROJECT

Start Date*	Ending Date*
07/01/2016	06/30/2021

OMB Number: 4040-0010
Expiration Date: 06/30/2016

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	YALE UNIVERSITY
Duns Number:	0432075620000
Street1*:	300 Cedar St
Street2:	TAC S440
City*:	NEW HAVEN
County:	
State*:	CT: Connecticut
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	065208237
Project/Performance Site Congressional District*:	CT-003

Project/Performance Site Location 1

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	YALE UNIVERSITY
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Street1*:	300 Cedar St
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County:	
State*:	CT: Connecticut
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	065208237
Project/Performance Site Congressional District*:	CT-003

File Name

Additional Location(s)

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ● **Yes** ❍ **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ❍ Yes ● No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6

If NO, is the IRB review Pending? ❍ **Yes** ❍ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ❍ **Yes** ● **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ❍ Yes ❍ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. Is proprietary/privileged information included in the application?* ❍ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ❍ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ❍ Yes ❍ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ❍ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ❍ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Project_2_Project_Summary.pdf
8. Project Narrative*	
9. Bibliography & References Cited	Project_2_References_Cited.pdf
10.Facilities & Other Resources	Facilities_Project_2_Asthma_U19.pdf
11.Equipment	Project_2_Equipment.pdf

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

Project Summary

In Project 2, *Novel Immune Pathways Drive Asthma Heterogeneity,* we bring together senior faculty in the Department of Immunobiology to investigate three novel immune pathways that affect asthma heterogeneity. Each of these pathways is initiated by an environmental stimulus that activates inflammatory pathways that influence asthma. The pathway we will explore will define the role of follicular B helper T (Tfh) cells in promotion of the pathogenic allergen-induced IgE response in endotypes of allergic asthma, based on preliminary findings showing novel populations of Tfh cells in blood of asthmatics and tonsil tissue. The second pathway will determine the role of Dickkopf-1 (Dkk-1) in endotypes of severe asthma based on preliminary studies that show Dkk-1 is important in allergen-induced asthma in animal models and it is highly associated with asthma severity in the YCAAD cohort. The third pathway aims to define which bacteria commensal in the airways influences asthma by defining which of these bacteria are inflammatory and stimulate IgA. These will be the first studies to measure IgA coating bacteria using a novel assay, IgA-Seq, in sputum. Project 2 will probe these pathways in human asthma using the capabilities of YCAAD through Clinical Recruitment Core B that will provide biological samples from adults and children with asthma, including blood, sputum and tonsils. Through the expertise and technologies offered by the Precision Profiling Core C we will conduct novel studies using CyTOF and single cell RNA-Seq to assess the cell and responder populations in these pathways. And with biostatistical support of Core B and pathways analysis defined in Project 3, we will integrate our understanding of the immune pathways, novel cell populations and molecular pathway regulation with asthma phenotypes. These novel pathways to be investigated in asthma through YCAAD will highlight important interactions of environmental stimuli and immune pathways in the lower respiratory tract, help expand definitions of endotypes in asthma, and point to new targets for future therapies.

Facilities and Other Resources - Project 2

Laboratory

The Section of Rheumatology occupies part of the 4th and 5th floors of the newly built The Anlyan Center (TAC) Building at Yale. The building was opened in February 2003. Space devoted to this project is 1,800 square feet of workspace, tissue culture room, general equipment room, and walk-in cold room. The office of Dr. Craft is on the 5th floor of TAC building and directly adjacent to his laboratory.

Dr. Bothwell's laboratory consists of 2100 square feet in the TAC (The Anlyan Center). Other common areas include a cold room, mouse holding and preparatory room, glass washing and media prep room and a darkroom.

The Flavell laboratory comprises of over 5000 square feet of laboratory space as well as office support and computer spaces for the laboratory. This space includes two large laboratories (with 33 bench spaces), two tissue culture rooms, radioactive area, equipment and support rooms. Equipment present in the laboratory used for this project comprise of PCR machines, luminometer, 2 quantitative PCR machines, digital camera, UV and white light imaging system, fluorescent microscope. In particular, we have Nextgen Illumina MiSeq sequencer for the nucleic acid analysis. Moreover, the Department of Immunobiology provides FACS core, which include: three BD LSR II, BD LSR II green, FACS caliber, and Stratedigm. Cell sorters include three BD FACS Arias, BD FACSVantage SE, Beckman Coulter MoFlo, and Sony –Cyt Reflection Bio Rad Bio Plex and two Amis Imagestream imaging stations

Clinical

Yale Center for Asthma and Airways Disease (YCAAD). Yale-New Haven Hospital is the largest referral center in the state, and YCAAD is the only dedicated adult asthma center in the region. This active, rapidly growing center receives over 3000 visits a year and is the hub of the asthma clinical/translational research program at Yale. For the last 15 years, the PI of this proposal, Geoffrey Chupp MD, has built a center and infrastructure to phenotype and endotype human asthma in a longitudinal, high throughput fashion. This protocol (See appendices) has enrolled over 800 subjects over the last 15 years. Six years ago, the protocol was expanded to include a 2 hour study visit that includes an extensive coordinator-administered asthma questionnaire, lung function testing, hypertonic saline sputum induction and blood drawing for genomic level analyses of RNA isolated from the blood and sputum. We now enroll new asthma subjects at a rate of approximately 100 per year and evaluate existing subjects at follow up visits. This infrastructure is central to the studies outlined in this proposal.

Animal

Adequate animal holding facilities are available in the TAC Mouse Unit operated by Yale's Division of Animal Care. Mouse experiments involving lentiviral or retroviral transduced cells or human tumors is carried out in the LSOG mouse facility under BSL2 conditions.

Computer

The Craft laboratory has several up to date Macintosh computers, linked together on the Internet; all postdocs and graduate students in the lab also have up to date Macintosh laptop computers, likewise linked. His office also has an up to date Macintosh computer. The Bothwell lab has several Macintosh computers with access to email, Internet and data bases needed for DNA and protein sequence manipulation and analysis. Two airport transmitters provide internet access to lab members. The Flavell lab has 15 Macintosh and Windows based computers available for use by students and fellows.

Office

Dr. Craft’s office is 200 square feet, and is directly adjacent to his laboratory. Dr. Bothwell's office is 200 square feet and is adjacent to the lab. The lab administrative area consists of an office for the Lab Manager (135 square feet) and the Administrative Assistant (260 square feet). The Flavell lab has over 900 square feet of office space with computer workstations, fax, copiers and shared printers.

General Support Services

Support services readily available at the School of Medicine include: electronic and machine shops; stockroom containing most commonly used chemicals, reagents, glassware, plastic ware, etc.; medical illustration,

computer support services, etc. The Yale Keck Biotechnology Laboratories provides facilities for preparation of synthetic oligonucleotides and peptides, preparation of polyclonal and monoclonal antibodies, microsequencing of proteins and proteomic facilities, and biostatistic and bioinformatic support. The Yale Center for Genome Analysis Laboratory at Yale, one of the largest of its kind in academia, is a world leader in providing genomics services and is well suited to carry out the sequencing work proposed in this study. The YCGA (medicine.yale.edu/keck/ycga/) a full service facility dedicated to providing high throughput DNA and RNA sequencing, RNA expression profiling, DNA genotyping, and microRNA analysis services using Illumina (HiSeq, MiSeq, and GAII), Pacific BioScience, Affymetrix, NimbleGen and Sequenom technologies. Ultrahigh throughput sequencing for ChIP-seq will be performed at the YCGA.

The Immunobiology Dept. is a rich source of colleagues and the faculty, postdocs and students are highly interactive. Yale University provides services including protein, nucleic acid and proteomic core facilities through the Keck Biotechnology Center and image analysis and histologic services through the Yale Comprehensive Cancer Center's Critical Technologies Shared resource in the Department of Pathology.

Other

In the Yale Section of Rheumatology and Department of Immunobiology (where the Craft lab is located) are other investigators with considerable experience in cellular and molecular immunology techniques. These highly interactive faculty include Drs. Richard Flavell, Susan Kaech, Ruth Montgomery, Linda Bockenstedt, Erol Fikrig, Richard Bucala, Al Bothwell, Ann Haberman, João Pereira, Carla Rothlin, Peter Cresswell, Jordan Pober, Bing Su, Kevan Herold, Nancy Ruddle, Ruslan Medzhitov, Akiko Iwasaki, David Schatz, Jordan Pober, Eric Meffre, Kevan Herold, David Hafler, Madav Dhodapkar, Lieping Chen, Jeffrey Bender, Vishwa Dixit, Stephanie Eisenbarth, Daniel Goldstein, and Martin Kriegel. Dr. Craft's office and laboratory are on one of the two floors shared with the Department of Immunobiology in TAC, with his laboratory directly adjacent to those of J. Pereira and A. Haberman (he shares a large open lab with these 2 scientists, and an office suite) and R. Flavell, and down one flight of stairs from that of other colleagues in Immunobiology. In addition to Drs. Pereria, Flavell, and Haberman, other faculty on the same floor as Dr. Craft include Drs. Mamula, Fikrig, Goldstein, and Bucala, with all these labs downstairs one flight from those of Cresswell, Kaech, Iwasaki, Schatz, Medzhitov, Rothlin, and Bothwell. Other faculty in the Department of Immunobiology, including Drs. Pober, Meffre, Herold, Hafler, Dhodapkar, Chen, Bender, Dixit, Eisenbarth, and Kriegel, are in immediately adjacent buildings – Amistad and 300 George Street.

The Section of Pulmonary, Critical Care, and Sleep Medicine is housed on the 4th floor TAC, including the offices/labs of Drs. Geoffrey Chupp and Lauren Cohn, one flight of stairs down from the Section of Rheumatology. The lab and office of Dr. Montgomery in rheumatology is just down the hall from the offices of Drs. Chupp and Cohn.

Equipment - Project 2

Craft Laboratory

Major equipment for this project includes: incubators (including 4 CO_2) and 4 tissue culture hoods, microscopes (including fluorescent), spectrophotometer, a chemical hood, centrifuges (including table tops, micro-, Sorvall and ultra-), freezers (-20 and -80), refrigerators, cold room, a speed vac, gel dryers, automated ELISA reader, orbital shaker, autoclave, film processor, electroporator, automated cell harvester, beta plate reader, scintillation counter, Luminex bioassay and several DNA thermal cyclers.
Proximity to the Craft Laboratory of Other Major Equipment. The Yale Flow Cytometry Core (see following) is housed in Immunobiology space on TAC5 and TAC6; the former is adjacent to the Craft lab, while the latter is one stairway immediately above. The Bio-Rad Bio-Plex System and Amnis Imagestream-X (see below), are in the same space; the CyTOF core is adjacent to the Craft lab in rheumatology space (operated by Dr. Montgomery, appointed in Rheumatology), while the In Vivo Imaging Facility is on TAC6, adjacent to the Flow Core, and just upstairs from the Craft lab.

Other Major Equipment
Flow Cytometry (medicine.yale.edu/labmed/cellsorter/index). There are 8 user-operated analyzers in the TAC Building (LSRII, LSRII Green, LSRII TAC5, two FACSCaliburs, three Stratedigm analyzers), with three in the physically adjacent Amistad facility (LSRII, Stratedigm and FACSCalibur), and a special order LSRII at the equally close George Street Research Building that houses the Human Translational Immunology Program. The Caliburs can analyze up to 4 colors with 488 nm and 633 nm excitation lasers. The Stratedigm STD-8 and STD-13 are capable of analyzing 8 and 13 colors, while the LSRII and LSRII Green have a capability of 12 colors. Machines are checked daily for optimal performance. Technicians are available full time during working hours to troubleshoot. Internet sign-up for analysis time is available from any computer via the Internet for users after completing the training.

Cell Sorting. The following instruments are available in the Flow Cytometry Core: 4 FACS Arias, Beckman Coulter MoFlo, and Sony SY3200. Staff members operate the Beckman Coulter MoFlo, Sony Reflection, and one of the FACSArias; the other FACSArias are operated by staff or can be user-operated after completing the training. We have considerable sorting experience, and typically sort ourselves for convenience and cost. Cells can be sorted into 5 or 15 ml tubes or into various plates as single or multiple cells per well or onto microscopic slides for analysis. In addition, all sorters are capable of simultaneous 4–way sorting. All sorters except MoFlo are also equipped for sorting live human, primate or other potentially biohazardous cells. Users of the Sony Reflection can take advantage of using two sort heads and a lower charge for the second head to be running.

Bio Rad Bio-Plex System (operated by the Flow Cytometry Core). This is a compact, simple, highly flexible platform for a wide range of bead-based assays, directly adjacent to the Craft lab. It combines the concept of flow cytometry with dual red and green lasers for simultaneous bead and reporter detection with up to 100 uniquely identifiable fluorescently labeled microspheres. This combination enables bio-molecule analysis in a highly multiplexed format. Individual microspheres can be complexed with any protein, peptide or nucleic acid.

Amnis Imagestream-X. This imaging flow cytometer (also part of Flow Cytometry Core) provides users with the ability to gain detailed images of a large number of cells in a relatively short period of time and with the opportunity to perform a range of novel applications including co-localization, internalization, stem cell differentiation, and cell-cell interactions.

CyTOF 2. This time-of-flight mass spectrometer is used for high-speed acquisition of highly multi-parametric single cell data (medicine.yale.edu/intmed/rheumat/cytof/index). The CyTOF 2 analyzes cells labeled with stable heavy metal isotopes using time-of-flight atomic mass cytometry technology. It has advantages over traditional flow cytometry: no background, no overlap between channels, and no compensation issues. It handles multi-parametric single cell data, with deep profiling, and can detect up to 34 parameters simultaneously in a single tube (with theoretical limit of 100 parameters) from limited samples. The CyTOF is checked daily for optimal performance. Ala Nassar is available during work hours for consultation. Regarding reagents, labeled antibodies are available from DVS Sciences (DVSSciences.com) including metal-conjugated individual antibodies (>200); ready to use MaxPar Panel Kits comprised of 6-17 well-characterized markers for human or mouse cell populations; and metal labeling reagents for in-house conjugation of antibodies. Note that metal conjugated anti-

fluorochrome antibodies (*e.g.,* anti-FITC) are also available that can be used with existing fluorescent reagents.

In Vivo Imaging Facility (medicine.yale.edu/intmed/rheumat/core/invivo). Two-photon fluorescence microscopy is a powerful research tool that employs ultra-fast pulsed lasers in combination with advanced optical laser scanning techniques to capture high-resolution three-dimensional images of fluorescently tagged specimens within intact tissues samples over time. This research methodology has the advantage of direct visualization of cellular arrangements and events that are often hard to deduce through sole use of in vitro assays and frozen sections, and the added benefit of being able to quantify many aspects of these events. As such, it is particularly well suited to address questions pertaining to the study of dynamic processes in living cells and tissues including, but not limited to, cell interactions, cell differentiation, and cell migration. Such studies can be conducted within virtually any tissue type; given tissue preparations can be developed to overcome the obstacles associated with maintaining native tissue structure and perfusion.

The Yale *In Vivo* Imaging Core Research Facility, supported by a NIAMS Research Core Center Grant (Dr. Craft, PI) provides support for Yale investigators interested in utilizing two-photon fluorescence microscopy to achieve their research goals. The Core is responsible for novel protocol and methods development and is designed to serve as a clearinghouse for expertise and experience, being able to translate newly successful approaches to multiple projects and investigators. It is the Core's goal to assist investigators in experimental design and implementation and to provide training for those whom wish to learn surgical preparations, imaging acquisition, and data analysis at the facilities image workstation utilizing Improvision's Volocity software.

As an additional part of the educational outreach of this Core, we have, in collaboration with the Department of Cell Biology, put on annual hands-on *Microscopy Workshops*. The imaging facility, which includes a dark imaging room and an antechamber for specimen preparations, is located in The Anlyan Center, in room S-614. The imaging workstation is located just down the hall outside of S-600. The In Vivo Imaging Facility's microscope suite is comprised of an antechamber equipped with a sink, lab bench, and dissecting microscope for specimen preparation, and a dark imaging room that houses the microscope. Wall mounted inhalation anesthesia (isoflurane) is available in the dark imaging room to enable longer, safer imaging.

The facility upright, laser scanning, LaVision Biotec TriMScope two-photon microscope is operated with a tunable (680nm – 1080nm) Titanium-Sapphire Laser (Chameleon Vision II, Coherent) purchased in January 2010. The microscope is outfitted with a water immersion, long-working distance Olympus 20X objective lens (NA 0.95) and is capable of imaging both fixed and live specimens. Currently, fluorescent emission from four colors (Far-red, Red, Green, Blue) can be detected simultaneously from a sample utilizing non-descanned photomultiplier tubes (PMTs).

The Mac Pro facility computer workstation is located just down the hall from the microscope suite and is available for data analysis utilizing Imrovision's Volocity software. Volocity utilizes a variety of tools that allow for measurements in both three and four dimensions and the generation of high-resolution images/movies.

Center for Cellular and Molecular Imaging (medicine.yale.edu/ccmi/confocal/index). This facility operates a range of up-to-date imaging tools. For the purposes of this proposal, the Craft laboratory heavily utilized the confocal instruments (medicine.yale.edu/ccmi/confocal/index) with considerable experience in this area (for example, see: Laidlaw B, Zhang N, Marshall H, Staron M, Guan T, Hu Y, Cauley LS, **Craft J***, Kaech S*. $CD4^+$ T Cell Help Guides Formation of $CD103^+$ Lung-Resident Memory $CD8^+$ T Cells during Influenza Viral Infection. *Immunity*. 2014. 41:633-645 (*co-senior and co-corresponding authors), NIHMSID: 632020; Weinstein J, Bertino SA, Hernandez SG, Poholek AC, Teplitzky TB, Nowyhed HN, **Craft J**. B cells in T follicular helper cell development and function: Separable roles in delivery of ICOS ligand and antigen. *J Immunol*. 2014 192:3166-3179. PMCID: PMC3991608; and, Johnson R*, Poholek A*, Yusuf I, DiToro D, Eto D, Barnett B, Dent AL, **Craft J**, Crotty S. Bcl6 and Blimp-1 are reciprocal and antagonistic regulators of follicular helper (T_{FH}) CD4 T cell differentiation. *Science.* 2009. 325:1006-1010 (*co-first authors). PMCID: PMC2766560.)

The confocal facility has two point scanning laser confocal microscopes, a Zeiss LSM 510 and a Zeiss LSM 510 Meta. In addition, the facility also has a combined confocal/two-photon Zeiss LSM 710 Duo NLO. The center also has three image workstations. The microscopes include: 1) Leica SP5. The SP5 has 5 channel detectors, allowing for up to five dyes for simultaneous scanning. The system has been upgraded with a new workstation

and replacement of two of the PMT detectors with Leica's new HyD detectors. These detectors have the advantage of very low noise and high sensitivity. The system is equipped with four lasers, 405nm, multi line Argon, 561nM and 633nm. 2) Zeiss LSM 510 Meta. The Zeiss LSM 510 Meta allows discrimination between fluorophores with closely spaced or overlapping emission spectra due to its "meta detector". The meta feature records a complete spectrum at each pixel of an image allowing sophisticated spectral un-mixing to be performed. This microscope is equipped with 458, 477, 488, 514, 543, and 633nm excitation wavelengths and three detector channels, one of which is the meta detector. This allows the imaging of a wide variety of fluorescent probes among them green fluorescent proteins such as GFP, CFP, YFP, and Ds-Red. 3) Zeiss LSM 710 Duo NLO/Multiphoton Microscope. This microscope's point scanner uses an argon laser and 2 HeNe lasers for excitation (at 458, 488, 514, 543, and 633 nm) and also has three detection channels. The 7-Live scanner is a line scanner capable of rapid acquisition, it is equipped with 405, 488 and 561nm laser lines. This microscope is adapted to examine both fixed and live specimens. The point scanner and line scanner can be used simultaneously for a variety of photo-manipulation experiments such as FRAP, FLIP, FRET, spectral analysis and uncaging. This system is also equipped for multiphoton fluorescence imaging. Multiphoton imaging is particularly useful for imaging of fluorescence deep into tissues (up to 200 micrometers) with bleaching limited to focal planes, and for flash photolysis studies that require imaging in precise subcellular regions. The setup includes a Spectra-Physics MaiTai titanium:sapphire laser for two-photon excitation. The output wavelength of the MaiTai is tunable from ~700-1020 nm. The system has four external non-descanned detectors. Three computer workstations (Dell and Macintosh computers) are available in the facility for off-line analysis and 3D reconstruction of images. They are equipped with the Zeiss LSM510 and Zen programs, Volocity program for 3-4 D reconstruction and volumetric analysis and Photoshop software. The facility has two point scanning laser confocal microscopes, a Zeiss LSM 510 and a Zeiss LSM 510 Meta. In addition, the facility also has a combined confocal/two-photon Zeiss LSM 710 Duo NLO. The center also has three image workstations. The SP5 has 5 channel detectors, allowing for up to five dyes for simultaneous scanning. The system has been upgraded with a new workstation and replacement of two of the PMT detectors with Leica's new HyD detectors. These detectors have the advantage of very low noise and high sensitivity. The system is equipped with four lasers, 405nm, multi line Argon, 561nM and 633nm. The Zeiss LSM 510 Meta allows discrimination between fluorophores with closely spaced or overlapping emission spectra due to its "meta detector". The meta feature records a complete spectrum at each pixel of an image allowing sophisticated spectral un-mixing to be performed. This microscope is equipped with 458, 477, 488, 514, 543, and 633nm excitation wavelengths and three detector channels, one of which is the meta detector. This allows the imaging of a wide variety of fluorescent probes among them green fluorescent proteins such as GFP, CFP, YFP, and Ds-Red. This microscope's point scanner uses an argon laser and 2 HeNe lasers for excitation (at 458, 488, 514, 543, and 633 nm) and also has three detection channels. The 7-Live scanner is a line scanner capable of rapid acquisition, it is equipped with 405, 488 and 561nm laser lines. This microscope is adapted to examine both fixed and live specimens. The point scanner and line scanner can be used simultaneously for a variety of photo-manipulation experiments such as FRAP, FLIP, FRET, spectral analysis and uncaging. This system is also equipped for multiphoton fluorescence imaging. Multiphoton imaging is particularly useful for imaging of fluorescence deep into tissues (up to 200 micrometers) with bleaching limited to focal planes, and for flash photolysis studies that require imaging in precise subcellular regions. The setup includes a Spectra-Physics MaiTai titanium:sapphire laser for two-photon excitation. The output wavelength of the MaiTai is tunable from ~700-1020 nm. The system has four external non-descanned detectors. Three computer workstations (Dell and Macintosh computers) are available in the facility for off-line analysis and 3D reconstruction of images. They are equipped with the Zeiss LSM510 and Zen programs, Volocity program for 3-4 D reconstruction and volumetric analysis and Photoshop software.

Bothwell Laboratory

Dr. Bothwell's laboratory in the TAC building has a common FACS Core Resource Center located on the 6th floor adjacent to the lab that contains 5 FACScan flow cytometers and 3 high speed preparative flow cytometers. Dr. Bothwell's lab is well equipped to perform tissue culture, biochemistry and molecular biology experiments. For tissue culture we have 3 sterilgard hoods, 3 double door CO2 incubators, an inverted microscope and a standard microscope. We also have an ultracentrifuge, a preparative centrifuge, electroporation unit, spectrophotometer, gel scanner, 3 PCR machines and a Biorad real time RT-PCR machine, liquid nitrogen tank, ELISA reader, air shakers and incubators for bacteria, gel electrophoresis equipment and access to a fluorescence microscope, fluorescence plate reader, a scintillation counter, a microtome for cutting tissue sections and a confocal microscope.

We have the ready availability of a common flow cytometry facility that is about 50 feet away that includes 6 user operated analyzers (4 BD FACSCaliburs, a FACScan and an LSR II [12-color analysis]) and 3 high-speed sorters (BD FACSVantage SE, BD Aria, and DakoCytomation MoFlo). Cells can be sorted at the rate of 20K/sec. at 99+% purity, using a variety of commonly used fluorochromes, *e.g.* FITC, PE, PE-Cy5, APC, APC-CY7, PE-CY7, CY5, and PI. The MoFlo has 7-color, Vantage SE 6-color and the Aria has 10-color capability. We also have ready access to transgenic and knockout mouse facilities, and the HHMI Biopolymer/Keck Laboratory and of the NHLBI/Yale Proteomics Center. The latter was funded by a contract from NIH/NHLBI to establish a proteomics center at Yale that supports research by multiple faculties to use ICAT/MS, RNA switch-based, and other approaches for the analysis of protein expression, and to then develop improved, synthetic peptide-based reagents to modulate the interactions of these proteins with their *in vivo* ligands and protein partners.

Available equipment within the department for use includes luminex machines, ELISA plate washers and readers, fluorescence microscope with digital camera attachment, UV box with film processor, and cryostats. Additional facilities within the department include a dark room, cold room, autoclaves and dish washers. The Yale School of Medicine also provides electron and confocal microscopy, DNA sequence analysis, DNA microarray, mass spectrometry, protein sequencing, full animal care facility, irradiator, FACSCaliburs, four LSR IIs (available with a green laser) and several FACS sorters. In addition the facility has a luminex instrument and an Amnis Imagestream for analyzing intracellular proteins using a flow-based method.

Flavell Laboratory

Equipment available includes: 8 laminar flow hoods, 14 CO2 incubators, ultracentrifuges, high and low speed centrifuges, 8 PCR machines, ELISA plate washer and reader, luminometer, 2 quantitative PCR machines, digital camera, UV and white light imaging system, fluorescent microscope, as well as dissecting and viewing microscopes. The laboratory has three microinjection stations for gene targeting. All equipment is available for the support of molecular and cellular immunology. The Department of Immunobiology provides FACS core which includes 8 user-operated analyzers in the TAC Building (LSRII, LSRII Green, LSRII TAC5, two FACSCaliburs, three Stratedigm analyzers), LSRII, Stratedigm and FACSCalibur in Amistad, and at 300 George Street Room 2320H we have a special order LSRII. The Caliburs can analyze up to 4 colors with 488 nm and 633 nm excitation lasers. Stratedigm STD-8 and STD-13 are capable of analyzing 8 and 13 colors. LSRII and LSRII Green have a capability of 12 colors. Stratedigm STD-13, STD-13+L, BD FACSCalibur S, LSRII and LSRII Green are located in room TAC S613; LSRII TAC5, Calibur H, STD-8 TAC5- in room S533. Machines are checked daily for optimal performance. A central darkroom, developer and phosphoimager are available.

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: Joseph Middle Name Edgar Last Name*: Craft Suffix:

Position/Title*: Professor
Organization Name*: YALE UNIVERSITY
Department:
Division:
Street1*: 300 Cedar Street
Street2: Box 208031, Box 208031
City*: New Haven
County:
State*: CT: Connecticut
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 065200000

Phone Number*: 2037371146 Fax Number: E-Mail*: joseph.craft@yale.edu

Credential, e.g., agency login: JOE_CRAFT

Project Role*: Other (Specify) Other Project Role Category: Project Lead

Degree Type: MD,AB Degree Year:

	File Name
Attach Biographical Sketch*:	Craft_Bio_Asthma_U19.pdf
Attach Current & Pending Support:	

Tracking Number: GRANT12004239 Funding Opportunity Number: RFA-AI-15-032 . Received Date: 2015-10-01T14:26:34.000-04:00

PROFILE - Senior/Key Person

Prefix: First Name*: Richard Middle Name A. Last Name*: Flavell Suffix:

Position/Title*: Sterling Professor, Chairman
Organization Name*: YALE UNIVERSITY
Department:
Division:
Street1*: Yale University
Street2: Department of Immunobiology
City*: New Haven
County:
State*: CT: Connecticut
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 065208011

Phone Number*: (203) 737-2216 Fax Number: (203) 737-2958 E-Mail*: richard.flavell@yale.edu

Credential, e.g., agency login: RFLAVELL

Project Role*: Other (Specify) Other Project Role Category: Co-Lead

Degree Type: PHD,FRS Degree Year:

File Name
Attach Biographical Sketch*: Flavell_Bio_Asthma_U19.pdf
Attach Current & Pending Support:

PROFILE - Senior/Key Person

Prefix: First Name*: Alfred Middle Name LM Last Name*: Bothwell Suffix:

Position/Title*: Professor
Organization Name*: YALE UNIVERSITY
Department:
Division:
Street1*: Section of Immunobiology
Street2: PO Box 208011
City*: New Haven
County:
State*: CT: Connecticut
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 065208011

Phone Number*: 203-785-4020 Fax Number: 203-785-4263 E-Mail*: alfred.bothwell@yale.edu

Credential, e.g., agency login: VERTICAL

Project Role*: Other (Specify) Other Project Role Category: Co-Lead

Degree Type: PHD Degree Year:

File Name
Attach Biographical Sketch*: Bothwell_Bio_Asthma_U19.pdf
Attach Current & Pending Support:

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2016 **End Date*:** 06-30-2017 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Joseph	Edgar	Craft		Project Lead		0.6			9,165.00	2,841.00	12,006.00
2.		Richard		Flavell		Co-Lead		0.24			0.00	0.00	0.00
3.		Alfred		Bothwell		Co-Lead		0.6			9,165.00	2,841.00	12,006.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **24,012.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
2	Associate Research Scientist	5.4			23,082.00	7,156.00	30,238.00
1	Research Scientist	3.0			21,583.00	6,691.00	28,274.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**58,512.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**82,524.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2016 **End Date*:** 06-30-2017 **Budget Period: 1**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2016 **End Date*:** 06-30-2017 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	92,476.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**92,476.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**175,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Modified total direct cost	66.5	175,000.00	116,375.00
		Total Indirect Costs	**116,375.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**291,375.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: Project_2_Budget_Justification_Asthma_U19.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2017 **End Date*:** 06-30-2018 **Budget Period: 2**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Joseph	Edgar	Craft		Project Lead		0.6			9,165.00	2,841.00	12,006.00
2.	Richard		Flavell		Co-Lead		0.24			0.00	0.00	0.00
3.	Alfred		Bothwell		Co-Lead		0.6			9,165.00	2,841.00	12,006.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **24,012.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
2	Associate Research Scientist	5.4			23,775.00	7,370.00	31,145.00
1	Research Scientist	3.0			22,231.00	6,892.00	29,123.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**60,268.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**84,280.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2017 **End Date*:** 06-30-2018 **Budget Period: 2**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2017 **End Date*:** 06-30-2018 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	90,720.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**90,720.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**175,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Modified total direct cost	66.5	175,000.00	116,375.00
		Total Indirect Costs	**116,375.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**291,375.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: Project_2_Budget_Justification_Asthma_U19.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2018 **End Date*:** 06-30-2019 **Budget Period: 3**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Joseph	Edgar	Craft		Project Lead		0.6			9,165.00	2,841.00	12,006.00
2.	Richard		Flavell		Co-Lead		0.24			0.00	0.00	0.00
3.	Alfred		Bothwell		Co-Lead		0.6			9,165.00	2,841.00	12,006.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **24,012.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
2	Associate Research Scientist	5.4			24,488.00	7,591.00	32,079.00
1	Research Scientist	3.0			22,898.00	7,099.00	29,997.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**62,076.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**86,088.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2018 **End Date*:** 06-30-2019 **Budget Period: 3**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2018 **End Date*:** 06-30-2019 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	88,912.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**88,912.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**175,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Modified total direct cost	66.5	175,000.00	116,375.00
		Total Indirect Costs	**116,375.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**291,375.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: Project_2_Budget_Justification_Asthma_U19.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2019 **End Date*:** 06-30-2020 **Budget Period: 4**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Joseph	Edgar	Craft		Project Lead		0.6			9,165.00	2,841.00	12,006.00
2.	Richard		Flavell		Co-Lead		0.24			0.00	0.00	0.00
3.	Alfred		Bothwell		Co-Lead		0.6			9,165.00	2,841.00	12,006.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **24,012.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
2	Associate Research Scientist	5.4			25,223.00	7,819.00	33,042.00
1	Research Scientist	3.0			23,585.00	7,311.00	30,896.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**63,938.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**87,950.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2019 **End Date*:** 06-30-2020 **Budget Period: 4**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2019 **End Date*:** 06-30-2020 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	87,050.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**87,050.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**175,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Modified total direct cost	66.5	175,000.00	116,375.00
		Total Indirect Costs	**116,375.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**291,375.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: Project_2_Budget_Justification_Asthma_U19.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2020 **End Date*:** 06-30-2021 **Budget Period: 5**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Joseph	Edgar	Craft		Project Lead		0.6			9,165.00	2,841.00	12,006.00
2.	Richard		Flavell		Co-Lead		0.24			0.00	0.00	0.00
3.	Alfred		Bothwell		Co-Lead		0.6			9,165.00	2,841.00	12,006.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **24,012.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
2	Associate Research Scientist	5.4			25,979.00	8,054.00	34,033.00
1	Research Scientist	3.0			24,292.00	7,531.00	31,823.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**65,856.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**89,868.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2020 **End Date*:** 06-30-2021 **Budget Period: 5**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2020 **End Date*:** 06-30-2021 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	85,132.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**85,132.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**175,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Modified total direct cost	66.5	175,000.00	116,375.00
		Total Indirect Costs	**116,375.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**291,375.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: Project_2_Budget_Justification_Asthma_U19.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

PROJECT 2

BUDGET JUSTIFICATION

Key Personnel

Joseph Craft, MD, (Lead, Project 2). Dr. Craft is the Paul B. Beeson Professor of Medicine (Rheumatology) and Professor of Immunobiology; Section Chief, Rheumatology and Program Director, Investigative Medicine at Yale University School of Medicine. As Project 2 Lead, he will be responsible for overall direction of studies proposed in Aim 2A including the research plan, interpretation of data, and writing all papers for publication. He will insure that the Co-Leads of Aims 2B and 2C along with his research group are working closely with the Cores and other Projects to move these human studies forward. Dr. Craft's effort on this grant will be 0.6 calendar months annually.

Alfred Bothwell, PhD, PhM (Co-Lead, Project 2B). Dr. Bothwell is Professor of Immunobiology at Yale University School of Medicine. As Co-Lead, he will be responsible for the overall direction of studies proposed in Aim 2B including the research plan, interpretation of data, and writing all papers for publication. He has directed all the research in this subproject that has contributed to this application. Dr. Bothwell's effort on this grant will be 0.6 calendar months annually.

Richard Flavell, PhD, FRS (Co-Lead, Project 2C). Dr. Flavell is Sterling Professor of Immunobiology at Yale University School of Medicine and an Investigator of the Howard Hughes Medical Institute (HHMI). As Co-Lead he will be responsible for overall direction of studies proposed in Aim 2C including the research plan, interpretation of data, and writing all papers for publication. Dr. Flavell's effort on this grant will be 0.24 calendar months annually. No funds for salary are being requested for Dr. Flavell, since he is an HHMI investigator.

Non-Key Personnel

Jinyoung Choi, PhD, (Research Scientist). Dr. Choi is a senior member of the Craft lab, with considerable experience in cellular and molecular immunology. She helped produce the preliminary data for this proposal, including characterization of the circulating follicular helper T cells in asthmatics and tonsillar follicular helper T cells, and will be responsible for studies of follicular helper T cell phenotype and function. She meets with Dr. Craft on a weekly (one-to-one) basis, and with other members of the Craft group during their weekly laboratory meeting. She also meets with Dr. Craft on an informal basis several times during the week. Her effort on this grant will be 3.0 calendar months annually.

Piotr Bielecki Ph.D, (Associate Research Scientist). Dr. Bielecki has extensive past experience studying host-pathogen interaction in the lung during his post-doctoral training in Germany and for the past year and one-half in Dr. Flavell's laboratory. He set up and optimized techniques to measure the microbiome in the sputum and is responsible for generating the preliminary studies presented in Aim 3C. He will commit 1.8 calendar months annually.

Wookjin Chae, PhD, (Associate Research Scientist). Dr. Chae has generated all the experimental results related to Dkk-1 contributing to Aim 2B in this application. He will work in the laboratory to measure Dkk-1 in plasma samples, activate sputum and blood cells with Dkk-1 to define its functions and help to define its source in the sputum and blood. His effort will be 3.6 calendar months annually.

Other Expenses

Laboratory Materials and Supplies. The budget for reagents including molecular biology reagents, antibodies, ELISAs, chemicals and general lab supplies is as follows:
Year 1: $92,476
Year 2: $90,720
Year 3: $88,912
Year 4: $87,050
Year 5: $85,132

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		120,060.00
Section B, Other Personnel		310,650.00
Total Number Other Personnel	15	
Total Salary, Wages and Fringe Benefits (A+B)		430,710.00
Section C, Equipment		0.00
Section D, Travel		0.00
1. Domestic	0.00	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		444,290.00
1. Materials and Supplies	444,290.00	
2. Publication Costs	0.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	0.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	0.00	
9. Other 2	0.00	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		875,000.00
Section H, Indirect Costs		581,875.00
Section I, Total Direct and Indirect Costs (G + H)		1,456,875.00
Section J, Fee		0.00

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

1. Project Director / Principal Investigator (PD/PI)

Prefix:
First Name*: GEOFFREY
Middle Name: L
Last Name*: CHUPP
Suffix:

2. Human Subjects

Clinical Trial? ● No ○ Yes
Agency-Defined Phase III Clinical Trial?* ○ No ○ Yes

3. Permission Statement*

If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?

○ Yes ● No

4. Program Income*

Is program income anticipated during the periods for which the grant support is requested? ○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

Budget Period*	Anticipated Amount ($)*	Source(s)*

PHS 398 Cover Page Supplement

5. Human Embryonic Stem Cells

Does the proposed project involve human embryonic stem cells?* ● No ○ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s): ❑ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

6. Inventions and Patents (For renewal applications only)

Inventions and Patents*: ○ Yes ○ No

If the answer is "Yes" then please answer the following:

Previously Reported*: ○ Yes ○ No

7. Change of Investigator / Change of Institution Questions

❑ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:

First Name*:

Middle Name:

Last Name*:

Suffix:

❑ Change of Grantee Institution

Name of former institution*:

PHS 398 Research Plan

Please attach applicable sections of the research plan, below.

OMB Number: 0925-0001

1. Introduction to Application (for RESUBMISSION or REVISION only)	
2. Specific Aims	Project_2_Specific_Aims.pdf
3. Research Strategy*	Project_2_Research_Strategy.pdf
4. Progress Report Publication List	
Human Subjects Sections	
5. Protection of Human Subjects	Protection-HumanSubjects_Asthma_U19.pdf
6. Inclusion of Women and Minorities	Inclusion-Women_Minorities_Asthma_U19.pdf
7. Inclusion of Children	Inclusion-Children_Asthma_U19.pdf
Other Research Plan Sections	
8. Vertebrate Animals	
9. Select Agent Research	
10. Multiple PD/PI Leadership Plan	
11. Consortium/Contractual Arrangements	
12. Letters of Support	
13. Resource Sharing Plan(s)	ResourceSharingPlan_Asthma_U19.pdf
Appendix (if applicable)	
14. Appendix	

Tracking Number: GRANT12004239 Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

Specific Aims

Many factors have been hypothesized to influence the development and activity of asthma. These include genetic susceptibility and environmental exposures, including allergen exposures and the endogenous microbiome. In Project 2, *Novel Immune Pathways Drive Asthma Heterogeneity,* we bring together senior faculty in the Department of Immunobiology to investigate three novel immune pathways that affect asthma heterogeneity. Each of these pathways is initiated by an environmental stimulus that activates inflammatory pathways that influence asthma. In susceptible individuals, allergen exposure leads to the induction of Th2 cells. Our recent studies show that allergens such as house dust mites stimulate release of Dickkopf-1 (Dkk-1) along with TSLP that synergize to stimulate Th2 activation and promote type 2 inflammation. Th2 cells in the lymph nodes and secondary lymphoid organs stimulate follicular B helper T cells (Tfh) to promote B cell allergen-specific IgE production. IgE activates inflammatory pathways in the respiratory tract that lead to asthma, cause disease exacerbations and chronic airway inflammation. How the microbes that live in the lower airways modulate airway inflammation remains unknown, but past studies have shown associations of airway microbes with inflammation in asthma suggesting that alterations in the microbiome will affect disease. With recent advances in bacterial sequencing technologies and our development of techniques to identify inflammation-causing bacteria, we will better identify which of the microbes in the lower respiratory tract of asthmatics modulate disease.

In Project 2 we will probe these pathways in human asthma using the capabilities of YCAAD through Clinical Recruitment Core B that will provide biological samples from adults and children with and without asthma, including blood, sputum and tonsils. Through the expertise and technologies offered by the Precision Profiling Core C we will conduct novel studies using CyTOF and single cell RNA-Seq to assess the cell and responder populations in these pathways. And, finally with biostatistical support of Core B and pathways analysis defined in Project 3, we will integrate our understanding of the immune pathways, novel cell populations and molecular pathway regulation with asthma phenotypes.

These novel pathways to be investigated in asthma through YCAAD will highlight important interactions of environmental stimuli and immune pathways in the lower respiratory tract, help expand definitions of endotypes in asthma, and point to new targets for future therapies.

Aim P2 A. Project Lead: Craft.
Determine the role of follicular B helper T (Tfh) cells in promotion of the pathogenic IgE response in endotypes of asthma.
Our preliminary data show that subpopulations of Tfh cells secrete IL-4 and/or IL-21 stimulate B cells to produce IgE and in asthma Tfh cells will influence pathogenic IgE production. In this proposal we will determine if Tfh cells are elevated in blood and tonsil of asthmatic compared to control subjects and investigate the associations with total and specific IgE. We will define the cytokine expression of circulating and tonsil Tfh cells to assess the impact on IgE levels and allergic asthma subtypes.

Aim P2 B: Co-Lead: Bothwell.
Determine the role of Dkk-1 in endotypes of severe asthma.
Our preliminary studies show that Dkk-1 is important in allergen-induced asthma in animal models and is highly associated with asthma severity in the YCAAD cohort. We will define which subsets of asthmatics express high levels of Dkk-1 to understand how Dkk-1 contributes to disease, define the sources of Dkk-1 in the airways and the immune pathways activated by Dkk-1.

Aim P2 C: Co-Lead: Flavell.
Define inflammatory commensal bacteria in the airway microbiota that are associated with asthma.
We determine differences in total bacterial composition in sputum from asthmatic compared to control subjects using 16S DNA sequencing of sputum. Then, using a novel assay developed in the Flavell laboratory, determine which of these bacteria are inflammatory commensal bacteria through immunoglobin A coating (IgA-Seq) in sputum. These will be the first studies to examine IgA-coating bacteria in the airways with a goal to understand how adaptive immune responses to bacteria in the airways influence asthma.

Significance. Asthma is the most common chronic inflammatory disease of the lung, affects close to 10% of the U.S. and its impact on society is profound (1). 5-10% of asthmatics have severe or refractory disease making up 25-30 million individuals in the U.S. While the manifestations of asthma are universal, including wheezing and airway obstruction that are caused by airway inflammation, many factors have been hypothesized to influence the development and activity of disease. These include genetic susceptibility and environmental exposures such as diet, medications, air quality, geography, and the endogenous microbiome (2, 3). Research investigations over the last 25 years have identified Type 2 inflammation as a driving immunological response in many individuals with asthma (4). Yet, all individuals with a propensity to develop Th2 immune responses do not become asthmatic. Thus, other factors modulate immunity to impact on development and persistence of allergic or Th2-driven airway disease. Indeed, many patients have low or no evidence of Type 2 inflammation. The goals of Project 2 are to define novel immune pathways that affect asthma heterogeneity.

Project 2, *Novel Immune Pathways Drive Asthma Heterogeneity* brings together senior faculty in the Department of Immunobiology. Over the years, investigators in Immunobiology at Yale helped to define the biology of Th2 cells, including the original definition of a Th2 subset that promotes IgE production (5), establishment of GATA-3 as the key transcription factor in Th2 development (6), and an early understanding of the impact of respiratory tract exposures on Th2 development (7, 8). For the past 20 years investigators in Immunobiology and Pulmonary Medicine have interacted closely through collaborative interactions in program grants, seminars, and exchange of trainees. In addition, the ease of scientific interaction is fostered by the juxtaposition of Immunobiology and Pulmonary on the 4th, 5th, and 6th floors of the Anlyan Center, where all of our laboratories and offices are located. In collaboration with PI Chupp in the Yale Center for Asthma and Airway Diseases (YCAAD), members of the Department of Immunobiology have a shared focus to study immune pathways that are active in asthma, specifically three novel pathways relevant to promote disease, and contribute to asthma heterogeneity, and which may identify novel therapeutic targets.

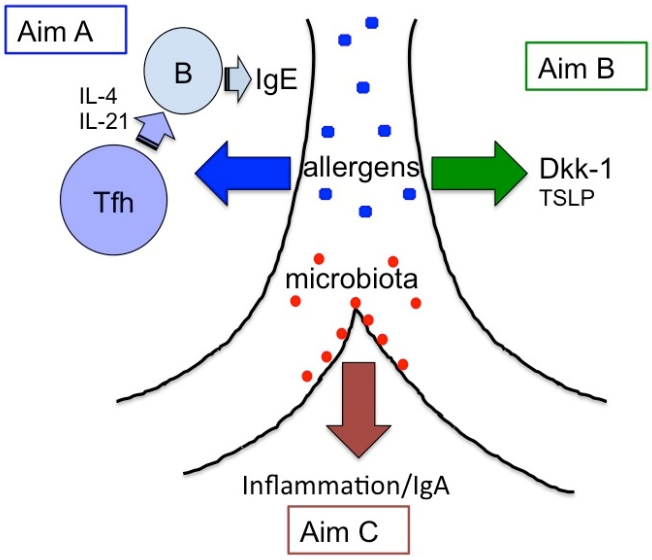

Figure A- Overview of the pathways explored in Project 2 that are initiated with airway exposure to allergen or the microbiota

Each of the Aims, or sub-projects in Project 2 begins with an environmental stimulus that activates an inflammatory pathway that may have an impact in asthma (see Figure A). **Aim 2A** will be led by Project lead Craft to investigate allergen-induced IgE and its generation by follicular B helper T (Tfh) cells. Humoral immune responses at mucosal surfaces are tightly regulated, to balance the need for effective immunity against pathogens and limit development of antibodies against common environmental stimuli or self that results in allergy or autoimmunity. Any class of antibody induced by any stimulus arises as a consequence of lymph node (LN) germinal center (GC) B-cell maturation with development to B-cell memory and long-lived plasma cells (9, 10). Within the GC, B cells undergo repeated rounds of cell division and somatic hypermutation (SHM) under direction of follicular B helper T (Tfh) cells. To understand the pathogenesis of IgE-mediated allergic diseases, it is critical to fully dissect the interactions of T and B cells as they promote the GC response in asthma, in particular analyzing the control of IgE antibody production. We propose to characterize tonsillar Tfh cells and their circulating Tfh counterparts (cTfh cells) in asthma and control subjects to determine their relationship to asthma and endotypes of asthma. Since ongoing inflammation leads to continued development of IgE producing plasma cells, and IgE augments inflammatory responses upon repeated stimulation with allergen, a better understanding of these pathways may lead to identification of new targets for therapy.

Aim 2B will be conducted by Co-Lead Dr. Bothwell, Professor of Immunobiology and Director of the Immunobiology Graduate Program, who has identified an inflammatory pathway mediated by Dickkopf-1 (Dkk-1) that orchestrates chronic type 2 inflammation. After exposure to inhaled house dust mite (HDM) allergen and release of thymic stromal lymphopoietin (TSLP), Dkk-1 synergizes with TSLP to stimulate Th2 development and reactivation. Thus, as a result of exposure to an environmental allergen, release of this platelet-derived factor activates immune pathways that may regulate asthmatic inflammation. Our preliminary studies also show that Dkk1 expression in human sputum has a striking association with asthma severity. Interestingly, Dkk-1 was not elevated in asthmatics of all severities, nor was Dkk-1 associated with atopy or other markers of “Th2 high” asthma. Thus, Dkk-1 elevation in the airway has a specific association with severity. In this project we will measure Dkk-1 protein in the blood to clarify this association with asthma and we will determine the endotypes

of asthma that have elevation of Dkk-1. The U19 program will enable single cell RNA-seq to define the cell populations producing Dkk-1, and CyTOF analysis to define specific effects of Dkk-1 on cells in the airways. Dkk-1 may be a novel marker of severity that helps to define asthma heterogeneity, and this Program will help to define the pathways by which Dkk-1 affects asthma.

Aim 2C will be conducted by Co-Lead Dr. Flavell, Sterling Professor and Chairman of the Department of Immunobiology and Investigator of the Howard Hughes Medical Institute. Dr. Flavell will investigate the impact of the airway microbiome in asthma. While the commensal microbiota in the gastrointestinal tract is appreciated to be a critical regulator of host immune system homeostasis, less is understood about the respiratory tract, since until recently it was considered a sterile organ. A limited number of reports have investigated the changes in the lung microbiota between healthy and diseased individuals, but less is known about the role of the lung microbiome in modulating pulmonary mucosal immune responses. Recent advances in sequencing have revealed the diversity and complexity of the human microbiota (11-13) but failed to identify individual bacterial species that shape disease susceptibility (14). The Flavell Laboratory developed a new technology that uses the host IgA response to the microbiota as a guide to identify specific bacterial species that selectively influence immunity (IgA-Seq). Intestinal bacteria from inflammatory bowel disease subjects selected on the basis of high coating with IgA conferred dramatic susceptibility to colitis in germ-free mice. Importantly, the IgA responses to these bacteria were T cell-dependent, which suggested that IgA-seq largely detects antigen-specific T-dependent IgA responses, and that IgA-seq is likely to identify bacteria that also induce and/or modulate T helper cell responses. Since our preliminary studies show that IgA-seq is feasible using sputum from asthma subjects, we will determine differences in the airway microbiota in the sputum of asthmatic and control subjects. This will be the first use of this technology to define immunogenic bacteria in the respiratory tract. This work would not be possible without robust recruitment and biospecimen acquisition and the analytic capabilities of Cores B and C in the Program.

Overall, these investigations together will identify novel pathways in asthma that connect environmental stimuli to immune pathways in the respiratory tract. Taking findings from animal models and pathways developed in our laboratories, harnessing the unique and powerful YCAAD cohort, the expertise of PI Chupp, the pathway we have outlined in Project 2 will explore new territory to drive an enhanced understanding of heterogeneity in asthma. Data from the Projects and cores will be combined using the extraordinary computational resources of Project Lead Gerstein and studies in Project 3, for analyses to generalize asthma disease observations to a systems-level understanding

Innovation. There are multiple highly innovative features related to this Project:

- ***Identifying novel models of humoral dysregulation in asthma heterogeneity.*** A central theme of this U19 proposal is to develop novel, clinically and pathobiologically meaningful ways to deconstruct the complex disease of asthma and make these tools publicly accessible. By identification of Tfh cell subsets and their control IgE responses in asthma we will identify new targets in asthma pathobiology that may contribute to asthma heterogeneity. Furthermore, in individuals with Type 2 inflammation in the absence of a definable allergen, the regulation of Tfh cells, and how these cells promote B cell responses, are critical to understanding the generation of pathogenic IgE responses in subsets of asthma.
- ***Platelet and vascular dysregulation plays a role in the initiation of type 2 responses.*** The Dkk-1 story outlined in Aim 2 provides a novel mechanism by which platelets release Dkk-1 that drives allergic inflammation. In preliminary studies we identified this novel mediator of inflammation in sputum of severe asthmatics that suggests Dkk-1 is an innovative target for asthma treatment that may block vascular leak and reduce antigen exposure.
- ***Immunogenic microbiota in the airway.*** The impact of airway commensal bacteria on airway diseases is at an early stage of investigation. Using IgA-Seq, it is now feasible to discriminate which commensal organisms may be driving inflammation that modulate the immune response and impact asthma. These will be the first studies that investigate this phenomenon in asthma.
- **Systems-level understanding** of adaptive immune mechanisms that define asthma phenotypes in terms of novel cell populations and molecular pathway regulation and contribute to a publicly accessible, searchable, integrated asthma MAP.

Aim 2A. Project Lead: Craft. Determine the role of follicular B helper T (Tfh) cells in promotion of the pathogenic IgE response in endotypes of asthma.

We hypothesize that Tfh cells drive the IgE response in asthma. We will assess the relationship between circulating and tonsillar Tfh cells and disease heterogeneity, including serum IgE, eosinophilia, and disease severity to define relationships with disease endotypes, followed by defining the phenotypic, functional, and genomic characterization of these cells.

Rationale and Background. Tfh cells regulate the humoral response by providing helper signals to cognate B cells in the LN follicle and GC. The canonical Tfh cytokine IL-21 supports proliferation and differentiation of GC B cells (15, 16). In type 2 infections such as those caused by helminthes, or in allergic illnesses, Tfh cells, like their Th2 counterparts in the tissue, produce IL-4. This cytokine in Tfh cells directs class-switching to isotypes appropriate to the invading pathogen or allergen (*i.e.*, IgE and IgG4), and maintains a robust GC B cell phenotype (17). By contrast to Tfh cells, tissue Th2 cells produce IL-4, IL-5 and IL-13 that support, among other features, eosinophil function and mucous production (18). IgE plays a central role in mediating the pathophysiology of allergic asthma as it is cross-linked on the surface of sensitized mast cells and basophils, activating them to release effector molecules (19, 20). The resultant goblet cell metaplasia and epithelial basement membrane thickening promotes bronchiolar obstruction (21). IgE secretion is strictly regulated, as evidenced by the fact it comprises less than 0.0001% of all serum Ig, with its dysregulation leading to immunopathology, such as anaphylaxis or allergic asthma. It is therefore critical to understand the nuances of IgE generation that may impact on asthma heterogeneity and ultimately point to new targets for therapy. B-cell production of IgE requires Tfh-dependent soluble and contact-dependent signals. IL-4 activates transcription at the IgE isotype-specific switch region via signal transducer and activator of transcription (STAT) 5 and 6 (22), with induction of IgE production by B cells (23, 24), with CD40 signaling activating DNA switch recombination by inducing expression of the enzyme activating induced cytidine deaminase (AID) (25, 26). In human B cells, IL-21 acts synergistically with IL-4 to enhance IgE secretion by naive and memory B cells (27, 28). While past studies have suggested that IL-21 suppresses IgE switching in murine B cells (29-31), more recent data reveals IgE production is dependent on Tfh-cell in GC (9, 32). The independent, and potentially synergistic, roles of IL-4 and IL-21 in IgE production remain unclear. Complicating this analysis are our preliminary data demonstrating that tonsillar Tfh cells, and their circulating counterparts, (a) comprise heterogeneous populations expressing either IL-21 or IL-4, with some expressing both, (b) human studies replicating our preliminary work in mice, in which we have shown populations secreting the individual cytokines are transcriptionally and phenotypically distinct, suggesting their more nuanced role in GC B cell maturation and Ig secretion. Recent studies reported Tfh cell expansion in severe asthma, but there was no in-depth analysis of the phenotype or function of the Tfh cells (33, 34). Therefore, we will investigate how IL-21$^+$ and/or IL-4$^+$ Tfh cells regulate GC responses and the generation of pathogenic IgE in allergic asthma, addressing our **hypothesis that Tfh cells drive the pathogenic IgE response in asthma.** Thus we will characterize Tfh cells from tonsils and blood samples of asthmatics with diverse clinical manifestations, assessing their phenotypes, proteins secreted by the cells, their transcriptional regulation at the single cell level, and their B helper function. Our ultimate goals in this aim are to determine the effects of Tfh cells in asthma and define subsets of asthma associated with aberrant Tfh cell expansion and function.

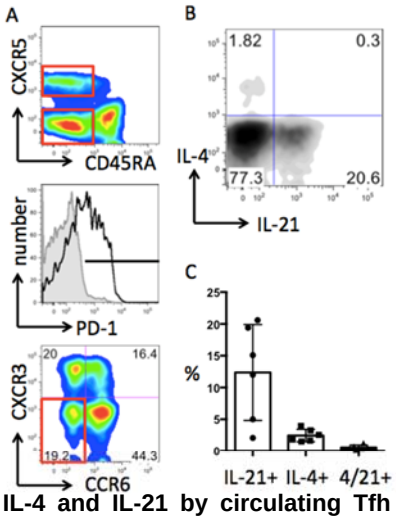

Figure 1. Expression of IL-4 and IL-21 by circulating Tfh (cTfh) cells in asthmatics. (A) CD3$^+$CD4$^+$ T cells were separated into CD45RA$^-$CXCR5^{hi} and CD45RA$^-$CXCR5^{lo} subsets (top panel). cTfh cells were then identified as the CXCR5^{hi}PD-1^{hi} population, with gates set based upon PD-1 expression by naïve CD45RA$^+$CD4$^+$ T cells (middle), followed by gating on the CCR6^{lo}CXCR3^{lo} population to exclude IL-17 and IFN-γ-secreting Th17 and Th1 cells, respectively (bottom). PBMC were stimulated with PMA and ionomycin and stained with antibodies against CD3, CD4, CD45RA, CXCR5, IL-21 and IL-4. (B, C) Density plots of intracellular staining of IL-4 and IL-21 by cTfh cells (B) and the percentage of IL-21$^+$, IL-4$^+$, and IL-4$^+$IL-21$^+$ cTfh cells (n = 6) (C). Horizontal lines represent the mean values with standard deviation of the mean (right). (Student *t*-test).

Preliminary Studies

Characterization of circulating Tfh cells in asthmatics. Tfh cells reside in B cell follicles and in the GCs of secondary lymphoid organs/tissues (SLOs) and they are also blood borne en route to and from these sites, as they re-enter B cell follicles to promote secondary GC responses (35). The blood and secondary lymphoid tissues, such as the tonsils, are optimal sites to analyze Tfh. Circulating Tfh (cTfh) cells are temporarily expanded in the blood from healthy donors after vaccination, with expansion followed by an increase in antibody titers, suggesting a direct relationship (36, 37). This population is also expanded in pathological states,

such as asthma and IgG4-related disease (33, 34), along with other conditions associated with pathogenic antibody formation including SLE and type I diabetes (38, 39), with expansion of cTfh cells positively correlated with disease activity, autoantibodies, and disease severity. These findings suggest cTfh cells in the blood reflect activity of GC responses in SLOs (35). Importantly, it also suggests that pathogenic IgE in certain asthmatics may be driven by heightened Tfh cell responses in SLOs.

Circulating Tfh (cTfh) cells in human asthmatics. We first asked if we could identify cTfh cells in asthmatic patients from the YCAAD cohort. Freshly isolated PBMC were stained with antibodies for CD3, CD4, CD45RA, CXCR5, PD-1, CXCR3, and CCR6 for flow cytometry, with CXCR5 and PD-1 used as Tfh cell markers, as we and others have described in mice and in humans (35, 38, 40-43). Differential expression of the latter chemokine receptors CXCR3 and CCR6 were used to separate cells into effector subsets expressing cytokines characteristic of Th1, Th2, and Th17 cells, with production of IFN-γ, IL-4, and IL-17, respectively (44), with $CXCR3^{lo}CCR6^{lo}$ cells identified as IL-4 producers (data not shown) (44). cTfh cells were identified as activated $CD3^{+}CD4^{+}CD45RA^{-}$ cells bearing the canonical Tfh markers CXCR5 and PD-1 ($CXCR5^{hi}PD1^{hi}$), with minimal expression of the chemokine receptors CCR6 and CXCR3 ($CCR6^{lo}CXCR3^{lo}$), to exclude IL-17 and IFN-γ producing cells, respectively. Such activated $CD4^{+}CXCR5^{hi}PD1^{hi}CCR6^{lo}CXCR3^{lo}$ cTfh subsets support antibody synthesis by B cells differentially based on their cytokine expression (44). Thus, we initially examined cytokine expression by cTfh cells, focusing specifically on IL-4 and IL-21, the latter since cTfh cells produce abundant IL-21 as we and others have shown (38, 44). Freshly isolated PBMC from asthma patients were stimulated with PMA and ionomycin to detect intracellular expression of IL-4 and IL-21 (**Fig. 1**). cTfh cells comprised a mixed population including a relatively small but an exclusively $IL\text{-}4^{+}$ population that was separable from $IL\text{-}21^{+}$ Tfh cells. This result suggests that cTfh cells can be subdivided into those capable of secreting IL-21 or IL-4. We have tentatively labeled these separable populations as cTfh21 and cTfh4 cells. It remains to be determined if these individual subsets function differentially in B cell help and Ig switching. Due to their heterogeneity, we argue that their analysis is necessary to determine the role of Tfh cells in B cell maturation and IgE secretion in asthma.

Identification of Tfh21 and Tfh4 cells in human tonsils. Freshly homogenized tonsillar cells were stimulated and stained as above. Tonsillar Tfh cells revealed a different expression pattern of IL-4 and IL-21, compared to blood cTfh cells (**Fig. 2**). IL-21 was most abundantly expressed (~40% of the tonsillar cells) and in the blood. We also observed $IL\text{-}4^{+}$ Tfh (Tfh4) and $IL\text{-}4^{+}IL\text{-}21^{+}$Tfh cells (Tfh21+4) (**Fig. 2**). By contrast, $PD\text{-}1^{lo}CXCR5^{lo}$ Th2 cells were capable of IL-4 synthesis, a pattern similar to Th2 cells in the blood (not shown). The Tfh21+4 cells are intriguing, given the fact that IL-4 and IL-21 have non-redundant, antagonistic functions in some humoral responses (15, 16, 27, 28, 45). Thus, we will characterize these cells isolated from the tonsils and blood of asthmatics, alongside their single cytokine-producing counterparts, and explore their role in B helper function, studies aided by their transcriptional characterization at a single cell level.

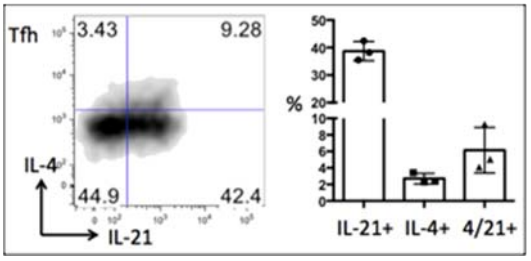

Fig. 2. Expression of IL-4 and IL-21 by tonsillar Tfh cells. Tonsillar cells were stimulated with PMA and ionomycin and stained with anti-CD3, -CD4, -CD45RA, -CXCR5, -IL-21 and -IL-4. Density plots of intracellular staining of IL-4 and IL-21 by tonsillar Tfh cells are shown, along with the percentages of $IL\text{-}21^{+}$, $IL\text{-}4^{+}$, and $IL\text{-}4^{+}IL\text{-}21^{+}$ cells among Tfh cells. Horizontal lines represent the mean values with standard deviation of the mean (right panels).

Murine Tfh cells exist as distinct $IL\text{-}21^{+}$ and $IL\text{-}4^{+}$ secreting populations upon type 2 immune challenge. We recently generated a novel IL-21 reporter mouse using the far red protein Katushka (Kat) (46), and demonstrated its fidelity (46). Crossing this strain with an existing IL-4-eGFP-reporter (eGFP; 4get) strain (47) yielded a dual-reporter system that simultaneously labels *Il21*$^{+}$ and *Il4*$^{+}$ cells *in vivo*. This system utilizes IRES-reporter constructs targeted 3' to the endogenous stop codon but before the endogenous 3' UTR of each cytokine gene. Endogenous cis-regulatory mechanisms act unimpeded, while the reporter proteins are generated whenever mRNA for that gene is transcribed. Using a type 2 immune challenge (with the helminth *Nippostrongylus brasiliensis* or ovalbumin in papain (48), we assessed cytokine (protein) secretion by reporter^{+} cells, asking if those producing IL-21 and IL-4 were developmentally related. Sorted and re-stimulated *Il21*$^{+}$ (Kat^{+}) Tfh cells exclusively secreted IL-21 while sorted *Il4*$^{+}$ ($eGFP^{+}$, $4get^{+}$) Tfh cells contained only IL-4 producers (**Fig. 3A, B**).

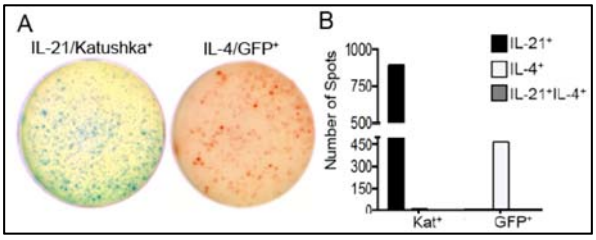

Fig. 3. **A novel reporter mouse reveals discrete subsets of Tfh cells**. (A, B) ELISPOT of Kat^{+} or $eGFP^{+}$ subsets, stimulated for 36hr with PMA/Ionomycin. Blue: IL-21; Red: IL-4. Gated on $CXCR5^{hi}PD\text{-}1^{hi}$ Tfh cells.

T cells producing IL-21 and IL-4 expressed the canonical Tfh cell transcription factor Bcl6 (not shown) (49), supporting their Tfh-cell phenotype. As expected, reporter double-positive cells secreted IL-21 and/or IL-4, and Th2 cells only IL-4 as measured by ELISpot assays (not shown). Given the fidelity of our reporters in marking cytokine secretion, we termed the *Il21* Kat$^+$ Tfh cells as Tfh21 and *Il4* eGFP$^+$ Tfh cells Tfh4. We next asked if Tfh21 and Tfh4 cells were distinct besides expression of IL-21 and IL-4, respectively. Transcriptome analysis of flow cytometrically sorted Tfh21 and Tfh4 populations revealed significant differences in gene expression between these subsets (**Fig. 4A**). Differentially expressed genes included CD40L, necessary for GC B cell maturation to plasma cell formation, as noted above, confirmed by surface staining (**Fig. 4B**). Overall, 1300 genes were differentially expressed (FDR-adjusted *p*-value < 0.05) between Tfh21 and Tfh4 cells. Bioinformatics analysis revealed a pattern of Stat6-bound genes within the top 100 genes enriched in Tfh4 cells, verified by Ingenuity (data not shown), suggesting a role for IL-4 in their development. Together, these data suggest that distinct populations of Tfh cells mature within the murine GC, with IL-21$^+$ (Tfh21) cells distinct from IL-4$^+$ (Tfh4) cells, and that these subsets likely play critical, but distinct, roles in GC B cell maturation.

Experimental Approach. Our first goal is to assess the relationship between Tfh and cTfh cells and their cytokine secretion patterns, and clinical phenotypes. We will ask if these cells are expanded in asthmatics that have high levels of circulating total and specific IgE, with comparison to healthy controls, and if their number and phenotype, particularly cytokine expression of IL-4 and/or IL-21, vary in terms of other parameters of disease activity and severity. If we identify a phenotype (such as, but not limited to, elevated IgE) that displays a strong relationship to Tfh or cTfh cell numbers or phenotype, we will ask in longitudinal analysis if clinical markers such as fluctuation of IgE antibody levels are reflected in the numbers or phenotype of cTfh cells. Second, to explore the functional role of Tfh cells in allergic asthmatics, and their relationship to blood cTfh cells, we will in parallel subject them, and the individual cytokine secreting populations, to phenotypic, functional, and genomic analysis. We will analyze and sort cells using flow cytometry, and perform *in vitro* B helper function assays in the absence or presence of cytokine blockade, asking if Tfh cells aid B cell differentiation to produce IgE antibody, and if Tfh cells transform phenotypically and transcriptionally – is there a pattern cytokine secretion and if so, how is that dictated at the level of gene expression? -- in the progression of T-B collaboration. We will also assess their characteristics using CyTOF, which along with RNA-sequencing, will enable to better determine the relationship among these populations in the tonsil and in the blood

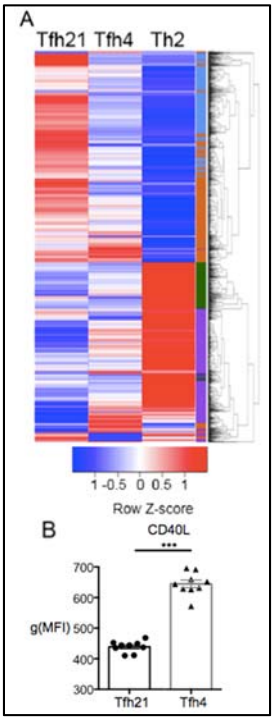

Fig. 4. Tfh21 and Tfh4 subsets are phenotypically distinct. (A) Heatmap of RNA-seq data from Tfh21 and Tfh4 cells, with CXCR5^{lo}PD-1^{lo} Th2 cells shown for comparison. (B) Surface staining for CD40L. Cells were stimulated with PMA/Ionomycin in the presence of anti-CD40L for 30 minutes to capture mobilized surface CD40L. (A-B) Gated on CXCR5^{hi}PD-1^{hi} Tfh cells from day 8 after Nippo.

Patient Cohort. From Core B we will acquire tonsillar tissue and blood from children in the Adenotonsillectomy Biorepository Study.

Sample Size. Objective: compare the Tfh cell proportion between asthmatics and controls. If there are 50 samples per group and we assume that both groups have the same standard deviation (denoted by SD) across samples within each group, there is an 80% power to detect a difference of 0.57xSD between the mean of the two groups at the 0.05 statistical significance level. Preliminary data from a previous study (34) estimated the standard deviation of the CD4+CXCR5+ T cell frequency to be 0.8, 4.1 and 5.0 in the healthy controls and mild asthma patients, respectively. Therefore, using the conservative estimation of SD=5.0, we have 80% power to detect a minimum difference of 2.85 between the two groups. The preliminary data also suggested a difference of 7.8 between healthy controls and mild asthma and 14.5 between healthy controls and severe asthma. The estimated group differences are both much higher than the minimum detectable difference. Therefore, we have enough statistical power to identify the difference of CD4+CXCR5+ T cell frequency between asthmatics and controls. Objective: to correlate the total IgE level with the number of CD4+CXCR5+ T cells: Preliminary data from the previous study suggested a Pearson correlation coefficient of 0.631. Based on this preliminary estimation, if there are 50 asthmatic samples, we will have 99.99% statistical power to identify the suggested correlation at the 0.05 significance level.

Phenotypic and Genomic Analyses. This will first be carried out by flow cytometry, as outlined in our preliminary results. Preliminary studies have shown individual populations of IL-4 and IL-21-producing tonsillar Tfh and circulating Tfh cells, but analysis of a larger cohort is needed to confirm these data and provide clinical correlations. We have considerable experience in analysis of both populations, with comparison to appropriate control cells, including naïve CD4$^+$ T cells, Th2 cells, and central memory CD4$^+$ T (Tcm)

cells. We will perform these assays as we have done in the past in humans (the markers are essentially the same for humans and mice) (27, 29-32). We will in parallel analyze these at the cellular and molecular levels by using CyTOF and single cell RNA-seq, respectively. Core C (Precision Profiling Core) will perform these analyses. Panels for the CyTOF include classical surface proteins of Tfh cells, enabling a distinction among the cytokine producing populations (such as Th1 or Th17 cells), as well as transcription factor analysis, with the goal to group our populations into more discrete subsets, assessing their relationship to one another and to clinical phenotypes. Cells will also be sorted and subjected to RNA-Seq to determine their transcriptional regulation, with the same goal.

Functional Analyses will be assessed in B cell helper assays, using *in vitro* T-B co-culture (35). Flow-cytometry-sorted Tfh cells will be cultured with sorted B cell subsets in the presence or absence of CD3 and CD28 for 5 days. Cells will be stained with plasmablast markers and intracellular Ig as an indicator of plasmablast differentiation, with Ig secreted in culture supernatants assessed by sandwich ELISA assay (27) to detect IgE and IgG isotypes, in particular IgG4 given its IL-4 and IL-21 dependence. We also will assess the Tfh cells in the culture to determine the stability of their phenotypes. To dissect the effect of IL-4 and IL-21 on Ig class switching and plasma cell differentiation, cytokine-blocking antibodies will be used in these co-cultures (27). While cytokine blockade can be effective, this approach does not examine contribution of each cytokine-secreting Tfh cells on B cell helper function. Therefore, we will employ a cytokine capture assay (50), followed by sorting by flow cytometry, to isolate pure populations of IL-4$^+$ Tfh, IL-21$^+$ Tfh, and IL-4$^+$IL-21$^+$ Tfh cells. Tfh subsets will be subject to phenotypic analyses (see above) and assessed for B helper function.

Data Analysis and Integration. Frequencies of cTfh cells will be determined as the percentage of CXC3$^-$CCR6$^-$ population among TCRβ^+CD4$^+$CXCR5$^+$PD-1^{hi}CCR7^{lo} cells, as we have done in the past (27). cTfh cells will be further separated into IL-4$^+$ cTfh and IL-21$^+$ cTfh cells. Data from each patient will be correlated with disease indices, asking if cTfh cells are related to clinical phenotypes, CyTOF results and RNA-Seq data will be analyzed collaboratively via Core C (Precision Profiling Core) and Core B (Clinical Recruitment and Biostatistical Core), respectively. With their expertise in computational biology, the team from Project 3 will analyze results generated independently by CyTOF and RNA-Seq experiments in combination with clinical parameters, with Tfh target molecules and associated pathway analysis, with the ultimate goal to identify targets associated with patient phenotypes. The data generated from CyTOF and single-cell RNA analysis will enable identification of subsets of Tfh and cTfh cells, and their relationships. For example, analysis of IL-4$^+$ cells allows us to dissect the genomic profile of this population, compared to those producing IL-21. The built-in advantage of single-cell analysis should reveal relationships between these populations, and allow for further association with clinical phenotypes. Therefore, precise characterization of these populations, in concert with our knowledge of their secretion profiles, should provide insight into the role of Tfh cell effector function and disease promotion. If it turns out that the frequency or the quality of populations reflects disease activity, this study will provide insights into therapeutic targets as well as a diagnostic tool. We also have the option of testing if cTfh cell cytokine profiles represent a stable phenotype associated with asthma or if they vary upon disease activity. Stability will be investigated on a subset of subjects from the GenEx study for which we will have three blood samples at 9-month intervals. Given our expertise in recruiting patients, and assessing disease activity, and our technical skills in cell sorting and the proposed assays, we do not foresee problems in carrying out these experiments.

Pitfalls and Alternative Approaches. Cytokine expression requires stimulation. This will cause changes in the expression of molecules that differentiate subsets among Tfh cells. To avoid this issue, pre-sorting of Tfh cells may be necessary before stimulation. Activation by PMA and ionomycin together with long incubation times may introduce secretome biases. However, we emphasize that stimulation with PMA and ionomycin drives expression of cytokine genes with open chromatin, with subsequent cytokine secretion, and is thus, a widely accepted method for studies of this kind (51). Treatment with drugs may affect frequency and function of cTfh cells, with these variances to be analyzed statistically using clinical data acquired by Core B.

Expected Outcome and Next Steps. Based on recent papers and our preliminary study on the relationship of cTfh cell subsets and asthma, we anticipate that Tfh cells contribute to the asthmatic phenotype. We expect our study will identify an asthma phenotype that is regulated by Tfh cells and molecules and pathways affected by Tfh cells.

Aim 2B: Co-Lead: Bothwell: Determine the role of Dkk-1 in endotypes of severe asthma

Rationale and Background. Dickkopf-1 (Dkk-1) has been known as an antagonist of the Wnt pathway that has high affinity for its receptor and inhibits transcriptional activation by β-catenin. While Dkk-1 blocks this canonical

Wnt signaling pathway, there are also alternative signaling functions that occur in less well-characterized pathways, described as non-canonical Wnt signaling (52). Our recent studies show that Dkk-1 function is critical for asthma-like pathogenesis in a mouse model of house dust mite (HDM)-induced asthma. Furthermore, our preliminary data show that Dkk-1 expression is associated with asthma severity in humans. Our recent studies show that platelets are an important source of Dkk-1 in both humans and mice, leading to release in the serum (53). Platelet activation in asthma is well known to promote disease through a variety of mechanisms (54). Thus, our new findings provide novel and strong support that Dkk-1 may impact asthma pathogenesis.

Preliminary studies. Using mice expressing only very low levels of Dkk-1 (hypomorphic *doubleridge* mice, Dkk-1 $^{d/d}$) repeatedly exposed to intranasal HDM, we showed that the Type 2 immune response to HDM was almost completely impaired (Fig. 5). To define the mechanism by which Dkk-1 drives this asthma-like response, we tested if recombinant Dkk-1 could induce Gata-3 or other transcription factors essential for T cell development, and found that Dkk-1 strongly stimulates CD4 T cells to become T_H2 cells (not shown). We have shown that

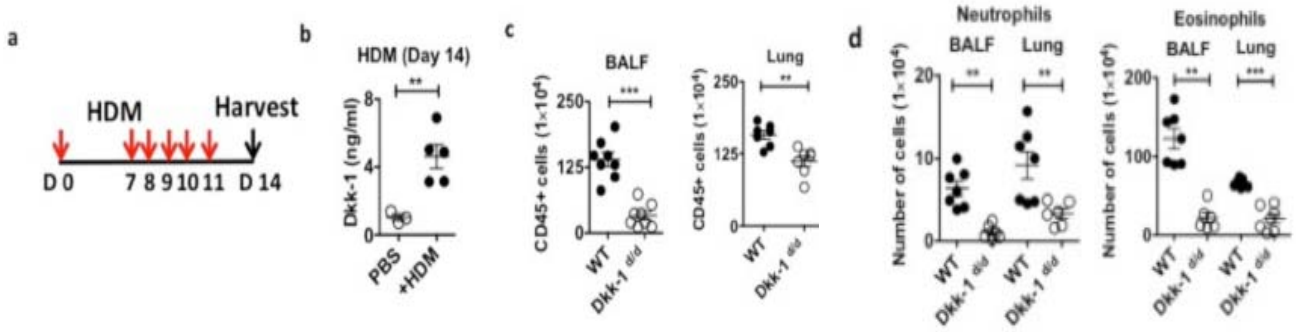

Fig. 5. Intranasal inoculation of HDM induces an asthma response dependent on Dkk-1. On the left is the daily inoculation schedule for C57Bl/6 mice. HDM treatment induces significant increases in Dkk-1 in the blood (b). Mice that are deficient in Dkk-1 or Stat6 are essentially resistant to HDM as judged by induction of neutrophils or eosinophils in the bronchoalveolar lavage (BAL) fluid (C and D).

depletion of platelets by *in vivo* by injection of anti-CD42B also eliminates Dkk-1 from peripheral blood. In addition anti-TSLP also reduces the induction of Dkk-1 in response to HDM. Thus we hypothesize that TSLP is important for the induction of Dkk-1 and may cooperate with Dkk-1 in driving the asthma response perhaps by their receptors on platelets.

Dkk-1 gene expression in the sputum was significantly associated with asthma severity and in the most severe clusters 1 and 2 of the TEA (transcriptomic endotypes of asthma) analysis described by PI Chupp's group (55). Thus, it appears that in asthma, Dkk-1 is highly expressed in the respiratory tract, at the site of inflammation, possibly leading to release of Dkk-1 into the blood. TSLP, shown by us to be required to induce expression of Dkk-1 in platelets, was also increased in sputum expression data, based on asthma severity (p= 0.01, not shown) and in the severe TEA clusters 1 and 2, as shown (Fig. 7). These data show a significant association of the Dkk-1pathway with asthma severity. Dkk-1 expression in sputum was also positively associated with other markers of severe asthma, including number of asthma exacerbations in the past year, dose of inhaled corticosteroid and percentage of bronchial epithelial cells in the sputum, suggesting that epithelial damage and severity may be markers of Dkk-1 elevation in asthma. While a large majority (88%) of the asthmatic subjects in the YCAAD cohort were atopic, Dkk-1 expression in sputum was not elevated in the total asthmatic population, nor was Dkk-1 elevation significantly associated with elevated sputum or blood eosinophils or fraction of exhaled nitric oxide (FENO). These data suggest that Dkk-1 is not merely a marker of atopy or a "Th2 high" asthmatic population. Based on these data, we hypothesize that Dkk-1 is elevated in a subset of asthmatics subjects with severe disease and in these populations Dkk1 induced inflammatory pathways promote persistence of disease. We will define which asthma subpopulations have elevations in Dkk-1 with a focus on epithelial damage and reactivity to specific allergens, and define the biological pathways in the airway that drive Dkk-1.

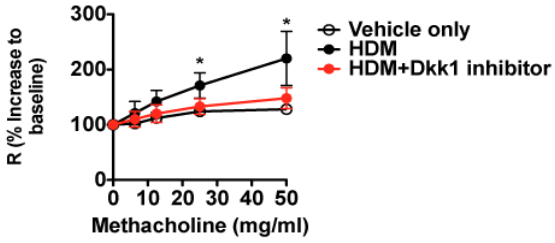

Fig. 6 In wild-type mice treated with HDM, treatment with a small molecule inhibitor of Dkk-1 function blocked airway hyperresponsiveness. Dkk-1 inhibitor was injected i.p. at a dose of 10 mg/kg, N=5 for each group at Day -1, +1, +6. +8. +10, +12 and mice terminated at d14. Newtonian resistance (Rn) is a parameter which represents the resistance of the central or conducting airways. Resistance (R) is dynamic resistance that quantitatively assess the level of constriction in the lungs.

Experimental Approach.

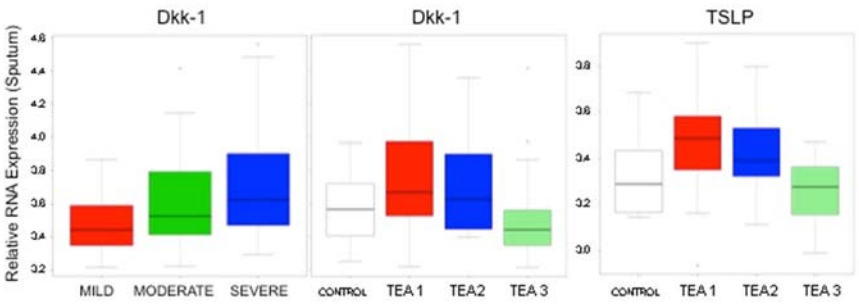

Fig. 7. Increased expression of Dkk-1 and TSLP in sputum microarray analyses. Left panel is from all asthmatics based on clinical criteria of severity ($p=0.004$), middle ($p=5.7 \times 10^{-5}$) and right ($p=9.2 \times 10^{-7}$) panels expression in TEA clusters. X-axis defines the groups.

Our preliminary data show that Dkk-1 expression in sputum correlates with asthma severity. Dkk-1 is produced by activated platelets and was elevated in blood in models of asthma. We hypothesize that Dkk-1 is elevated in a subset of asthmatic subjects and promotes disease. Our goal is to define the subsets of asthmatics with elevations of Dkk-1 and determine the pathways by which Dkk-1 influences disease. We will determine if Dkk-1 in serum is associated with asthma severity. We will measure Dkk-1 in serum and sputum supernatants of 200 asthmatic and 50 non-asthmatic, healthy control subjects by multiplex ELISA (MSD) through Core C. This will assess if Dkk-1 protein in serum parallels its expression in the sputum and if expression in the sputum is reflected in protein in the sputum supernatant.

Samples size calculation: To evaluate the significance of the difference in the DKK1 protein level between asthmatics and controls, when we have 50 non-asthmatic controls and 200 asthmatic patients and assume that both groups have the same standard deviation of DKK1 protein level (denoted by SD), there is an 80% power at the 0.05 significance level to detect a minimum effect size of 0.44. This means that the minimum detectable difference in the group means is 0.44xSD, where SD is the standard deviation of the DKK1 protein level within each group. Preliminary data for the effect size estimation is not available in humans. However, a previous study in intranasal challenged mice showed an effect size of 3.18 --- much higher than 0.44 (53). Therefore, even if the effect size in human decreases by 7 fold, we still have 80% power to identify the difference.

We will define the subset of asthmatics that express high Dkk-1. Using the tools developed by Project 3, we will define which subsets of asthmatics have high expression of Dkk-1. Associations with other pathways will be analyzed. With data from subjects including ImmunCAP, we will investigate if Dkk-1 is associated with subjects with sensitivity to HDM.

We will determine if Dkk-1 is produced in the airway and define its source. Our preliminary studies show that sputum expression of Dkk-1 correlates with asthma severity. We hypothesize that the Dkk-1-producing cells in sputum contribute to inflammation and asthma severity. Our prior analysis shows that the major source is from activated platelets, therefore one possibility is that platelets in the sputum are the source of Dkk-1 (56) might be an important source of Dkk-1 in sputum. Through analysis of RNA-Seq data from sputum and single cell analysis, we will define the *in vivo* source of Dkk-1. We will also assess the cells that express TSLP and TSLP-R to give us insights into the interactions among cells in the sputum. If we find elevation of Dkk-1 in the serum and increased expression of Dkk-1 in platelets, then we will hypothesize that platelets are activated in the respiratory tract where they release Dkk-1.

Define effects of Dkk-1 on sputum and blood cell cytokine production and surface marker expression and cellular differentiation. Blood and sputum cells will be obtained from Core B and stimulated with Dkk-1 *in vitro*. Using CyTOF we will compare cells treated versus untreated for cytokine expression, including type-2 cytokines IL-4, IL-5, IL-13 and IFN-γ and IL-17 and markers of alternative activation (CD206). We will also assess the activation status of platelets from subjects with elevated Dkk-1, as we have described (53), which includes CD62P, TSLP-R and additional adhesion molecules. The cellular responses of platelets to HDM will also be evaluated for induction of CD62P, TSLP-R and additional adhesion molecules.

Expected Outcomes, Pitfalls, and Alternative Approaches. Here we expect to begin to understand hwo Dkk-1 contributes to asthma severity. First, we will define if it elevated in blood, as it was in mouse models of asthma and in sputum where our preliminary data suggest it is elevated. The in-depth measurements proposed through Core C will identify the relevant cell source(s) of Dkk-1 and allow us to study the populations in the sputum that may interact, such as the TSLP-producing and responding cells to further define a role for TSLP as a regulator of Dkk-1 expression.

Aim 2C: Co-Lead: Flavell: Define inflammatory commensal bacteria in the airway microbiota that are associated with asthma.

Rationale and Background

The commensal microbiota is now appreciated to be a critical regulator of host immune system homeostasis and is an emerging area for interdisciplinary research. Disturbances in the composition of commensal bacteria can result in imbalanced immune responses and affect an individual's susceptibility to various diseases, including inflammatory (IBD and colon cancer), autoimmune (e.g., celiac disease, arthritis), allergic (e.g., asthma and atopy) and metabolic (e.g., diabetes, obesity, metabolic syndrome). Investigation of the microbiota in the lower respiratory tract is a relatively new field in comparison to the extensive work on the intestinal tract. In fact, the lung, once considered a sterile organ, was excluded from the original Human Microbiome Project. A limited number of reports have investigated the changes in the lung microbiota between healthy, non-smoking and smoking individuals as well as in patients suffering from Cystic Fibrosis (CF), Chronic Obstructive Pulmonary Disease (COPD) or Asthma (2, 57-60). Despite emerging data on airway microbiota, little is known about the role of the lung microbiome in modulating pulmonary mucosal immune responses and in heterogeneity of asthma. The lung microbiota in humans consists of hundreds of bacterial species per person and exhibits exceptional inter-individual diversity (61). While microbiota likely have dramatic effects on host physiology in health and disease through its interactions with and effects on the immune system, this is the first in-depth investigation of resident airway microbiota from a well-defined cohort of of asthmatic patients.

Recent advances in sequencing have led to an explosion of phylogenetic, taxonomic, and metagenomic studies of the microbiota in health and disease (11, 12). Such studies have revealed the diversity and complexity of the human microbiota (13). However, they have largely failed to identify individual bacterial species in humans that shape the intestinal immune system and disease susceptibility (14). One potential solution to this problem is to further classify members of the microbiota based on functional characteristics (i.e., behavior and effects of that behavior on the host) rather than taxonomy alone. Such a classification would potentially reveal previously hidden patterns and allow for the discovery of specific bacterial species that influence disease in humans. The Flavell Laboratory developed a new technology to address these problems that uses the host Immunoglobulin A (IgA) response to the microbiota as a guide to identify specific intestinal bacterial species that selectively influence immunity in the pathogenesis of inflammatory bowel disease. They tested the hypothesis that IgA-coating specifically marks immunomodulatory members of the microbiota and performed IgA-seq on fecal bacteria from patients with inflammatory bowel disease (IBD). Through extensive anaerobic culturing, it was possible to create personalized, disease-associated gut microbiota culture collections with predefined levels of IgA coating (IgA-Seq). Using these collections, they found that intestinal bacteria selected on the basis of high coating with IgA conferred dramatic susceptibility to colitis in germfree mice. Importantly, the IgA responses to these bacteria were T cell-dependent, which suggested that IgA-Seq largely detects antigen-specific T-dependent IgA responses, and that IgA-Seq is likely to identify bacteria that also induce and/or modulate T helper cell responses in the gut (62). These data thus show that IgA coating selectively marks immunomodulatory intestinal bacteria that confer susceptibility to disease.

Based on these findings and preliminary data showing alterations in the respiratory tract microbiota in chronic inflammatory airway diseases, we hypothesize that the dysbiosis of airway microbiota influences the inflammatory response in asthma. Therefore, by identifying patterns of immune-response-inducing bacteria in the airway, we will better understand asthma heterogeneity and the inflammatory pathways that drive disease, and may identify microbes to target for therapeutic effects in asthma.

Aim 2C.1. To determine differences in total bacterial composition in sputum from asthmatic compared to control subjects.

Preliminary Studies: We previously optimized the protocols for bacterial 16S sequencing from murine lung specimens to improve capture of low abundance bacteria using lung microbiota samples and through 16S sequencing of bronchoalveolar lavage fluid, we discerned differences in bacterial communities in wild-type compared to immunodeficient mice. In preliminary studies for this proposal, we collected sputum samples from asthmatic and control patients, isolated mucus plugs, extracted bacterial DNA and then subjected it to 16S next generation amplicon sequencing of a variable region of the gene encoding 16S rRNA (Fig. 8). In asthmatic

subjects there was a striking increase in abundance of particular bacterial classes compared to non-asthmatic healthy controls. Specifically, we detected elevated proportions of Bacteroidia and a decrease of Actinobacteria in comparison to the healthy controls (sample P2). These data show that the proposed studies are feasible and we are capable of measuring the lower airway microbiota from sputum samples collected in YCAAD. Moreover, we were able to measure the 16S DNA from 100% of samples collected for these studies.

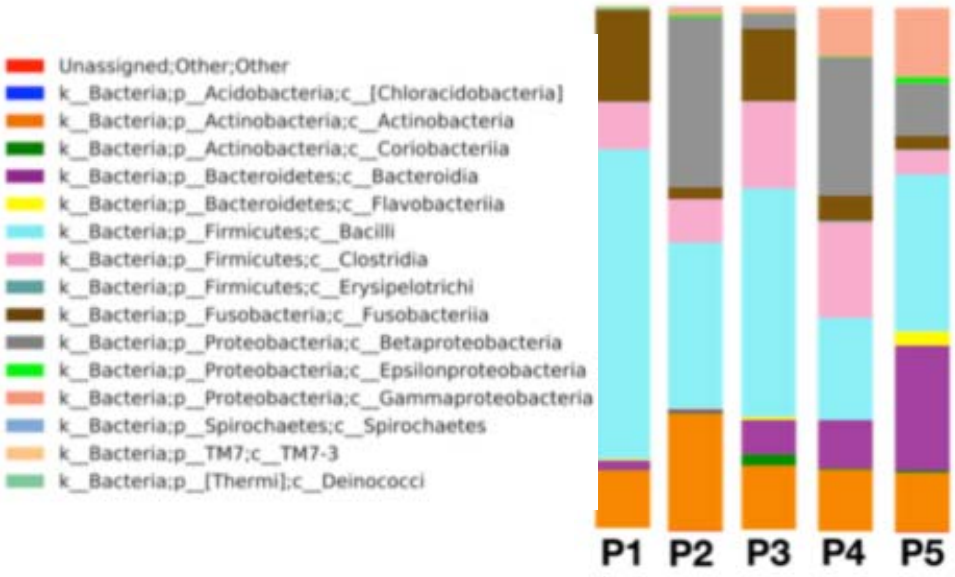

Fig 8. Relative abundance of bacterial classes measured by 16S sequencing. Results represent 5 distinct patients sputum samples. Bacterial DNA was extracted from mucus plugs, which were immediately frozen after sample collection. P2 represent a healthy control, P1, P3, P4 and P5 are diagnosed with asthma (unpublished).

Experimental Approach: To define changes in the airway microbiota in asthma, we will obtain mucus plug samples from 100-120 asthmatic and 50 control subjects from the NextGen Biorepository Study, Core B (sample size calculation below). Bacterial DNA will be extracted according to our established methods. Next-generation sequencing will be used to assess the bacterial composition of the lower respiratory tract. The V4 region of 16S ribosomal RNA will be PCR amplified (28 cycles; primer pair F515/R806) (63). After amplification, PCR products will be quantified with Picogreen (Invitrogen) and pooled at a final concentration of 10 nM before sequencing on a miSeq sequencer (Illumina, 2x250bp paired-end reads, up to 200 samples per sequencing run). Sequencing studies will be conducted with resources of Core C and Dr. Shrikant Mane, Director of Yale's Center for Genomic Analysis. Microbial diversity will be analyzed with the Quantitative Insights Into Microbial Ecology (QIIME) analysis suite (64).

Sample Size calculation: To identify taxa that have a significantly different IgA coating index (ICI) between asthmatics and healthy controls, when there are 50 samples for each group and we assume that both groups have the same standard deviation (denoted by SD), we have 80% statistical power to detect a minimum effect size of 0.5 between the two group means at the 0.05 significance level. Since the effect size represents the ratio of difference in the group means divided by the group standard deviation, this means that the minimum detectable difference between the two group means is 0.5xSD. If the sample size for asthmatics and controls increase to 120 and 80, the minimum detectable effect size will decrease to 0.36 which means we will have 80% statistical power to detect at least a group mean difference of 0.36xSD at statistical significance level of 0.05. Preliminary data on the ICI values in asthmatics is not available. But a previous study (62) investigating IgA-coated gut bacteria in IBD patients suggested an effect size of the differentially IgA-coated species to be from 0.34 to 0.56. Therefore, we expect that with between 100-120 asthmatic and 50 control subjects we will have enough power to identify the taxa that are differentially IgA coated between asthmatics and controls.

Bacterial DNA extraction from sputum: Sputum samples will be subjected to mechanical disruption with glass beads followed by a vortex and slow (50-100g) centrifuge spin, aliquotted and stored at -80°. A sample of bacterial suspension will be subjected to 16S sequencing analysis. Other samples will be used for IgA-Seq (Aim 3 C2). We have already optimized methods to improve bacterial DNA extraction by reducing signal-noise ratio (the ratio of bacterial cells: host cells in the sputum is relatively low), and by improving amplification since the PCR reaction is highly inhibited by excess of host DNA. To do this we first lyse the eukaryotic cells, leaving bacteria intact and then subsequently use both mechanical and chemical lysis of bacteria and ultra-clean microbiome dedicated columns for DNA purification in order to minimize the risk of contamination. We expect to be able to sequence the total bacterial composition (Aim 2C1) and the IgA pull-down bacteria (Aim 2C2) using 100-200 mg mucus provided by Core B, since our initial studies indicate that a fraction of the of mucus was adequate. We will optimize these methods to guarantee sequencing the full-spectrum of bacterial composition while using the least mucus material.

Aim 3C.2. To determine inflammatory commensal bacteria at the level of immunoglobin A coating (IgA-Seq) in sputum from asthmatic compared to control subjects.

Preliminary results: We developed a state-of-the-art technique (IgA-Seq) to identify immunogenic bacteria in the gut. This microbiota analysis profiles IgA-coating of the microbiota in a taxa-specific manner. It is based on the combination of antibody-based bacterial cell sorting and 16S ribosomal RNA (rRNA) gene sequencing to specifically isolate and identify IgA coated bacteria from fecal material (62) and can be easily adopted for microbiota isolated from other mucosal surfaces. Taxa-specific coating is then quantified by calculating an IgA Coating Index (ICI) score (relative abundance (IgA+ fraction)/relative abundance (IgA- fraction)) (see Figure 9 A,B and C for brief explanation of the technique). We performed IgA-Seq on sputum mucus samples from 2 asthmatic subjects (Figure 9D). Antibody-coated bacteria in mucus samples were isolated using a Protein L pull-down technique. The most coated member of microbiota was *Leptotrichia sp. Leptotrichia,* considered an oral commensal bacterium, *can* cause severe pneumonia, were associated with corticosteroid resistant asthma and not corticosteroid sensitive asthma and were increased in abundance in lungs of COPD patients (65-67).

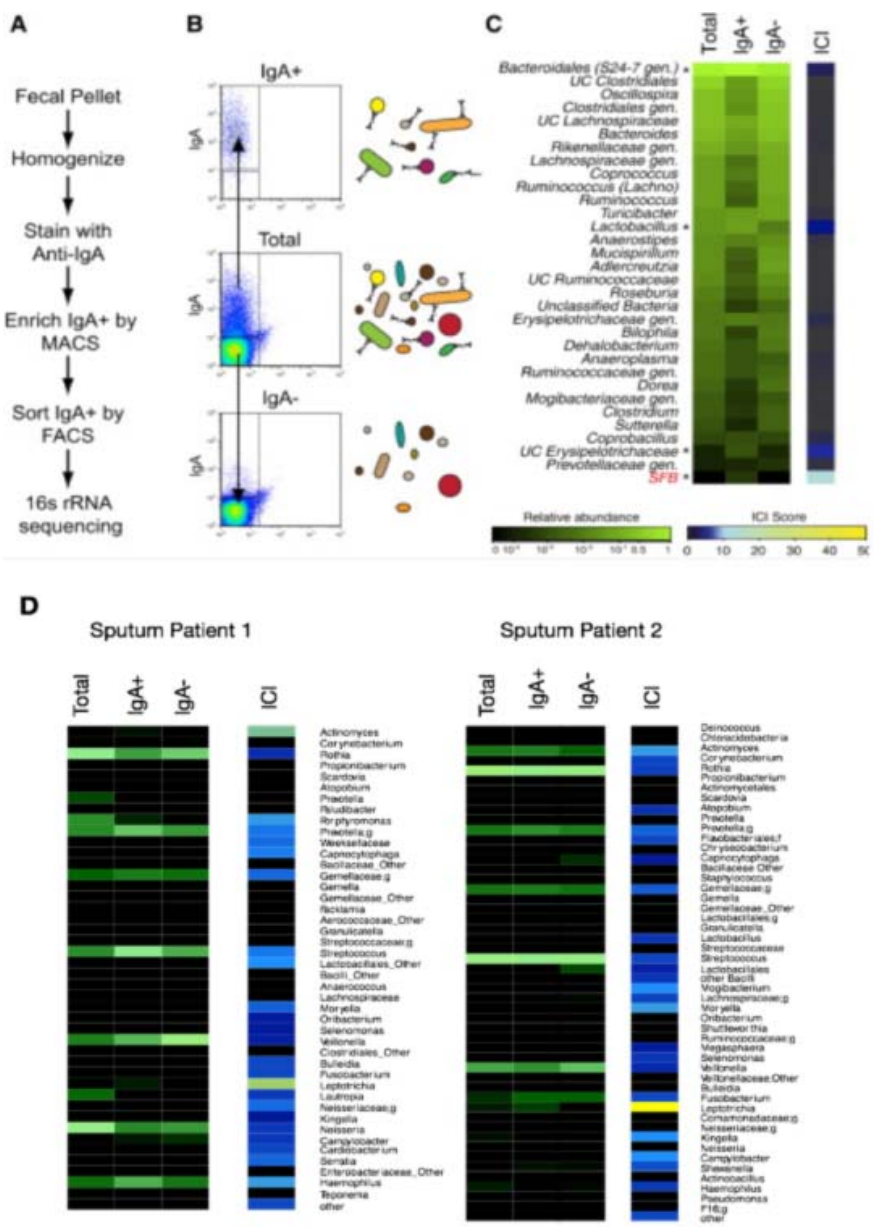

Fig. 9. (A-C) IgA-seq technique explanation. (A) Overview of IgA-based cell sorting of fecal bacteria combined with 16S rRNA gene sequencing (IgA-seq). **(B)** Representative results and a cartoon of cell sorting of IgA+ and IgA− fecal bacteria from mice. **(C)** Heatmap depicting ICI scores and average relative abundance of bacterial genera in Total (Presort), IgA+, and IgA− fractions of fecal bacteria from C57Bl/6 SPF mice (n = 17 samples). Genera that are significantly coated with IgA (significantly higher relative abundance in the IgA+ fraction as compared to the IgA− fraction by LEfSe; $p < 0.05$) are marked with an asterisk. Genera with ICI > 10 are labeled in red. *UC*, unclassified in the Greengenes reference database. *Gen*., classified as a distinct but unnamed genus in the Greengenes reference database. (adopted from Palm NW, de Zoete MR et al., Cell 2014) **(D)** Preliminary data showing two samples from patients suffering form bronchitis. Heatmap depicts ICI scores and average relative abundance of bacterial genera in Total (Presort), IgA+, and IgA− fractions. The ICI color score was set up as more sensitive than above (C), where maximum possible value was 20 (unpublished data).

Experimental Approach

We will use IgA-seq to identify members of the airway microbiota that may play a crucial role in modulation of the immune system and stimulation of chronic inflammation. Bacterial pellets processed in Aim 3 C1 from sputum of asthmatic and control subjects will be resuspended in 100 µl blocking buffer (staining buffer containing 20% Normal Mouse Serum for human samples, Jackson ImmunoResearch), and then stained with PE-conjugated Anti-Human IgA (1:10; Miltenyi Biotec clone IS11-8E10). Anti-IgA stained lung bacteria will be sorted using Magnetic Activated Cell Sorting (MACS) beads (Miltenyi Biotec). After MACS separation, the negative fraction will be used for 16S sequencing analysis (IgA negative fraction). The positive fraction will be then further purified via Fluorescence Activated Cell Sorting (FACSAria; BD Biosciences) as IgA-positive bacteria subject for 16S sequencing analysis. We will use an established bioinformatics analysis pathway to calculate the IgA score (62).

Anticipated Results/Pitfalls/Alternative Approaches: These studies are designed to define the composition of the microbiota in the sputum from asthmatic and control subjects. We will analyze both the differences observed in the total bacterial composition (Aim 2C1) and the IgA coated bacteria (Aim 2C2) using established analytic tools. These studies have never been attempted in sputum, and the preliminary data shown here indicate that identifying IgA-coated bacteria in the sputum is feasible. We expect to find a number of bacterial families that differ in sputum from asthma and control subjects, but we hypothesize that only a fraction of those species will be IgA coated, and thus immunogenic and responsible for modulating immunity in asthma. Our preliminary studies show that few bacteria in the sputum are IgA coated. The species with highest ICI score, *Leptotrichia,* was highly coated in the two asthmatic subjects tested. Once we identify the IgA and non-IgA-coated species, we will work with Project 3 investigators to perform pathways analysis of bulk RNA-Seq data from matched mucus samples from these subjects. Analysis will compare the pathways activated in asthmatic versus control subjects with their patterns of IgA-coated and non-IgA coated bacteria. If we find significant differences in the IgA-coated bacterial families among asthmatic subjects, or between visits of individual asthmatic patients at different stages of disease activity, we will investigate heterogeneity in asthma based on bacterial colonization in the airways. In addition to identifying immune-response inducing bacteria in the airway, these techniques have the potential to define low abundance bacteria that cannot be cultured and are thus, previously unrecognized as pathogens in the airways. We expect to identify specific IgA-coated bacterial taxa, which could be involved in chronic inflammation. Because these studies use tools and techniques that are already established in the Flavell Laboratory, we do not anticipate any technical problems. However, in some subjects the overall number of bacterial cells for FACS sorting of IgA coated bacteria may be too low. If necessary, we will use the pull down technique employing magnetic beads coated with Protein L that was used in preliminary studies. That means we will pull down bacteria coated not only by IgA but as well IgG. In this case, the antibody coating remains consistent with our hypothesis. Another potential concern is that oral contamination of mucus samples will influence sequencing results. Sputum samples with squamous cell contamination of greater than 20% will not be processed further for microbiome analysis (see Core B).

Among asthmatic subjects, many other factors may influence the microbiota in the lung. Microbial diversity was affected in COPD by both age and inhaled corticosteroid use, and in asthma by having corticosteroid resistant disease (65). By association analysis we will use our rich clinical database to determine relationships of microbes with disease features, including age, corticosteroid use, and lung physiology. Data integration analyses will be performed using tools developed in Project 3 to find immune activated pathways that are associated with shifts in microbiota.

Protection of Human Subjects

Risks to Human Subjects

a. Human Subjects Involvement and Characteristics. Over the course of this project, we plan to recruit 200 asthmatic and 50 non-asthmatic subjects through the Yale Center for Asthma and Airway Diseases (YCAAD). Each subject will return approximately every 9 months and complete 3 visits over the 5-year grant period. At each of the 3 visits the subject will be administered a questionnaire and perform physiological testing, and blood and sputum samples will be obtained. Asthmatic and non-asthmatic subjects recruited for these studies will be 12 years old or greater. Through a distinct HIC protocol, for these studies we will also recruit asthmatic and non-asthmatic children (age 7-17 years old) undergoing adenotonsillectomy, to donate tonsillar tissue, blood samples, and nasal brushings to the YCAAD biorepository.

Drs. Cohn and Chupp have recruited subjects for the past 15 years through the *Mechanisms and Mediators of Asthma and Chronic Obstructive Pulmonary Disease (COPD) human investigation* protocol (Yale HIC 0102012268, PI Chupp, Co-I Cohn). Over 800 subjects have been recruited to date. Tonsillar tissue will be obtained as part of the *Correlation of Gene Expression Profile with Clinical Response to Adenotonsillectomy* protocol (Yale HIC 1009007345, PI Karas, Co-I Chupp). This is a repository is to investigate the relationship between adenotonsillar hypertrophy and asthma and airway diseases, and identify genes, molecules and pathways that are associated with disease development, progression, and severity.

YCAAD Study Protocol: Exclusions will be based on health considerations. We will exclude any patient with a contraindication to any of the required testing including sputum induction, spirometry, or phlebotomy. These exclusions include severe cardiovascular, renal, or liver disease, bleeding diathesis, or advanced neuromuscular disease. A centerpiece of the YCAAD translational research program and this proposal is the airways disease phenotyping protocol developed to study human asthma. This protocol has been developed to acquire the full complement of clinical information, physiologic measurements and biologic samples to perform genotype-phenotype studies. Following informed consent, subjects are administered a questionnaire by the study coordinator. This includes a detailed asthma and medical history, asthma activity questionnaire (Asthma Control Test), Asthma Quality of Life Questionnaire, and details about medications, histories of smoking, occupational and environmental exposures, history of allergy, sinus disease, gastroesophageal reflux, obesity, and medication compliance. All data is uploaded into a HIPAA compliant web-based database developed by YCAAD. Blood is drawn and processed for the isolation of DNA, RNA, serum and plasma. Samples are aliquoted and banked for future studies. Blood is sent to the clinical laboratory for CBC and differential, serum IgE level, ImmunoCap testing for 22 common aeroallergens and routine chemistries. Following phlebotomy, patients undergo FeNO testing and spirometry (in adherence with ATS guidelines) before and after short acting bronchodilator administration. Sputum induction is done using hypertonic saline and immediately processed. Mucus plugs are removed using a dissecting microscope to remove squamous cell contamination, the cellular and aqueous compartments are separated, cell counts and differentials are quantified, and aliquots of supernatants, cell protein, and cellular RNA are stored for later analysis. The protocol is practical, efficient, and enrolls large numbers of subjects in a timely fashion. We will phenotype up to 5 subjects weekly. Subjects will be scheduled to return for repeat sampling as described, which is important for determination of the stability of their disease and their biological samples.

Asthma Inclusion criteria. Recruitment through YCAAD is designed to enroll a wide-range of asthmatic subjects in terms of age, disease severity and ongoing treatment. Inclusion criteria for asthmatic subjects are: (1) ≥ 12 years of age; (2) < 10 pack years of tobacco, and have not smoked for ≥ 1 year. Asthmatic subjects must have a diagnosis of asthma defined by the following criteria (based on the National Asthma and Education and Prevention Panel Guidelines): (1) a history of chronic symptoms (>2 years) compatible with asthma (determined by a study investigator/pulmonary physician specializing in asthma); and (2) historical evidence of variable airflow obstruction determine by: (a) > 12% improvement in FEV_1 after an inhaled $beta_2$ agonist, inhaled corticosteroids, or an oral prednisone burst; (b) methacholine reactivity with a provocative concentration causing a 20% decrease in FEV_1(PC20) of <8 mg/ml; or (c) 20% diurnal variation of peak expiratory flow rates on 2 days over a 2-3 week period. Patients will be excluded if they cannot safely undergo the studies required for participation or have other chronic lung disease or asthma variant disease (ABPA, Churg-Strauss Syndrome, vocal cord dysfunction, etc.). Normal subjects must have no history of any chronic lung disease. Non-asthmatic control subjects will be excluded if they have a diagnosis of any ongoing acute or chronic lung disease, have smoked ≥10 pack-years or have abnormal spirometry.

Adenotonsillectomy Study Protocol. Parents and patients identified by providers as potential subjects will be

invited to participate in the research study after they have been consented independently for their planned procedure of adenotonsillectomy. The parent and child will complete a YCAAD pediatric questionnaire and an asthma activity score, and spirometry will be done for those children aged six years or older who are capable of completing the test. Intraoperatively, a venous blood sample (5-10ml) and nasal swab sample will be obtained. After a portion of the tissue is sent to surgical pathology, as is standard for adenotonsillectomy, a portion of the removed tonsils and/or adenoid tissue will be taken for study purposes. An additional specimen will be held in the pathology department, flash frozen in OCT in the event more tissue is needed for analysis. Samples will be processed and banked with the assigned de-identified study subject number. A second study visit will take place six months following surgery and includes an interim history using the follow-up questionnaire focused on stability of upper airway and asthma symptoms. Repeat PFT testing will be performed in children aged six years or older, if possible. An additional venous blood sample will be obtained for the repository. Subjects may decline any of the testing at any of these visits.

b. Sources of Materials. In this proposal, the following materials will be collected from human subjects: clinical data (questionnaire, history and physical examination data, lung function data and relevant laboratory test results), blood, sputum, nasal brushings and tonsils/adenoids. Clinical data will be identified with the study subject's name. This information will be maintained in a locked file and these records are kept separate from other clinical records to assure confidentiality. Only Drs. Cohn, Chupp, Niu, and study coordinators will have access to these confidential records. All research specimens, including blood, and sputum specimens are identified by a patient code for correlation purposes. All samples and datasets will be de-identified and only known to study investigators involved in consent and sample acquisition. Investigators and staff will receive only de-identified data and samples for their studies and analyses. All data sets uploaded and samples delivered to Core C and the Yale Center for Genomic Analysis will be de-identified.

The studies in this proposal will adhere to the U.S. Department of Health and Human Services Office for the Protection from Research Risk (OPRR). All information relating to participating subjects will be de-identified. Specimens may only be used in accordance with the conditions stipulated in the Yale Human Investigation Committee (HIC) (see approval letters in Appendix).

c. Potential Risks. Risks associated with the YCAAD study (HIC 0102012268) including pulmonary function tests, blood draw and sputum induction are minimal (adults) and greater than minimal (children 12-17 years old) and are explained to subjects in detail at the time of consent (see consent forms in Appendix). Bronchospasm is a risk of sputum induction, but the safety of sputum induction in asthmatic subjects, even those with severe asthma has been well documented in several studies (ref). Spirometry is repeatedly tested during the procedure to monitor for bronchospasm, and if wheezing or a significant drop in FEV1 occurs, subjects are given bronchodilators. Wheezing, if it occurs, is usually transient and treatable with bronchodilators. A Data and Safety Monitoring (DSM) Plan and DSM Committee are in place to assess adverse events (see Appendix). To date, we have had no serious adverse events in our laboratory, even in severe asthma subjects undergoing sputum induction. Clinical Recruitment and Biostatistics Core Lead (Dr. Cohn) or a research coordinator will monitor all procedures performed. After any procedure performed in YCAAD, subjects are instructed to contact the YCAAD on-call physician if any problem occurs. YCAAD is covered 24 x 7 by pulmonary physicians who are familiar with the management of acute bronchospasm in asthmatic patients. Risks associated with the adenotonsillectomy study (Yale HIC 1009007345) are minimal for the blood draw and there is no added risk of apportioning adenoid and tonsil tissue to the biorepository. Emergency equipment and personnel are immediately available in the event of an adverse reaction at all times during these study visits.

Adequacy of Protection Against Risks

a. Recruitment and Informed Consent. Subjects will be recruited from YCAAD and from the pediatric ENT offices of Dr. Karas. Once a subject is deemed eligible for this study, informed consent will be obtained. The study procedures, risks and benefits will be clearly explained to each subject. All questions will be answered, and time will be allowed for the subject to make an informed decision before signing the consent form. If the subject agrees to enroll, a consent form (see Appendix) will be reviewed and explained, and the subject or consenting adult will be asked to sign it.

b. Protections Against Risk. Risks will be minimized by appropriate subject exclusions, close medical supervision throughout the protocol, and adaptation of testing to identify patients who might be at higher risk. Subjects experiencing adverse events will have access to full inpatient medical facilities of the Yale-New

Haven Hospital if more intensive treatment is necessary to reverse an asthma attack or complication during the study. All patients will be under full-time coordinator supervision throughout every phase of the YCAAD procedures. In the adenotonsillectomy protocol, at least one physician and/or nurse will be present throughout the study visits and procedures. Adverse events will be reported to the Yale IRB (Human Investigation Committee) and the DSMC.

Potential Benefits of the Proposed Research to Human Subjects and Importance of the Knowledge to be Gained

These studies are not designed to provide direct benefit to the participants. However, the knowledge gained from this work will enhance information about the pathogenesis of asthma and may lead to improved diagnosis, treatment and prevention of asthma in the future. All subjects receive asthma education and copies of blood work and lung function tests as part of their visit, and have full access to a YCAAD physician, if desired. If any major test abnormalities are encountered, YCAAD physicians counsel the subjects and provide referrals to appropriate specialists as needed.

Inclusion of Women and Minorities

Subjects will be recruited through the Clinical Recruitment and Biostatistics Core (Core B) and through the office of Dr. Karas for adenotonsillectomy without any bias of race or gender. The YCAAD asthma and control populations and the children undergoing adenotonsillectomy are racially diverse and reflect the population in New Haven and surrounding communities. All minority groups already take part in these research programs and we will continue to actively recruit women and minorities as these studies go forward. Based on demographics from past studies, the asthmatic population in YCAAD is approximately 71% female and 31% Black and Hispanic. The demographics of the adenotonsillectomy population is approximately 37% female, 14% Black and 36% Hispanic.

OMB Number: 0925-0002

Planned Enrollment Report

Study Title: NextGen Biorepository Study

Domestic/Foreign: Domestic

Comments:

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/Alaska Native	0	0	0	0	**0**
Asian	3	2	0	0	**5**
Native Hawaiian or Other Pacific Islander	0	0	0	0	**0**
Black or African American	20	18	8	8	**54**
White	85	84	12	10	**191**
More than One Race	0	0	0	0	**0**
Total	**108**	**104**	**20**	**18**	**250**

Study 1 of 2

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

OMB Number: 0925-0002

Planned Enrollment Report

Study Title: Adenotonsillectomy Biorepository Study

Domestic/Foreign: Domestic

Comments:

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/Alaska Native	0	0	0	0	**0**
Asian	2	2	0	0	**4**
Native Hawaiian or Other Pacific Islander	0	0	0	0	**0**
Black or African American	12	10	6	4	**32**
White	28	22	8	6	**64**
More than One Race	0	0	0	0	**0**
Total	**42**	**34**	**14**	**10**	**100**

Study 2 of 2

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

Inclusion of Children

The proposal will recruit children between the ages of 2 and 21 years (consent and assent form are included in Appendix 1 and 2). The investigative team for Yale #HIC0102012268 will request that the participants (12-21 years old) undergo phlebotomy, sputum induction, lung function testing and complete an asthma questionnaire with the subject and his/her parent/legal guardian. Venipuncture and pulmonary function testing and questioning are part of the routine care of children with asthma. Sputum induction is a low-risk procedure and is outlined in the Appendix. All interviews and procedures will be performed in the Yale Center for Asthma and Airways Disease. Overall, the study poses more than a minimal risk to minor subjects, but its benefits outweigh these risks. The investigative team for Yale # HIC1009007345 will request that the participant (2-18 years old) who was previously scheduled to undergo adenotonsillectomy, consent to have blood drawn intraoperatively, perform spirometry before surgery, if capable, and agree to donate excess tonsil tissue to the biorepository for analyses. These procedures are assessed to be of minimal risk to child subjects. The investigative team has experience caring for children should any adverse events arise during sample acquisition.

Inclusion of children in this study is important since we are interested in the relationship of transcriptomic endophenotypes across the spectrum of asthma. Our studies thus far suggest that there are stable Transcriptional Endophenotypes of Asthma (TEA) in children and adults with severe asthma and, in particular, near fatal asthma. Endotypes seen in adult asthmatics may be evident in young subjects, and this may help us predict the course of disease as they age. Further, we may be able to discern which patients will benefit from more aggressive care and elucidate molecular pathways involved in the pathogenesis of severe asthma. We will follow subjects longitudinally and will define how transcriptomic signatures relate with progression of this disease.

References Cited

1. Centers for Disease C, Prevention. Vital signs: asthma prevalence, disease characteristics, and self-management education: United States, 2001--2009. MMWR Morbidity and mortality weekly report. 2011;60(17):547-52. Epub 2011/05/06. PubMed PMID: 21544044.
2. Huang YJ, Nariya S, Harris JM, Lynch SV, Choy DF, Arron JR, Boushey H. The airway microbiome in patients with severe asthma: Associations with disease features and severity. The Journal of allergy and clinical immunology. 2015. doi: 10.1016/j.jaci.2015.05.044. PubMed PMID: 26220531.
3. Thorburn AN, McKenzie CI, Shen S, Stanley D, Macia L, Mason LJ, Roberts LK, Wong CH, Shim R, Robert R, Chevalier N, Tan JK, Marino E, Moore RJ, Wong L, McConville MJ, Tull DL, Wood LG, Murphy VE, Mattes J, Gibson PG, Mackay CR. Evidence that asthma is a developmental origin disease influenced by maternal diet and bacterial metabolites. Nat Commun. 2015;6:7320. doi: 10.1038/ncomms8320. PubMed PMID: 26102221.
4. Fahy JV. Type 2 inflammation in asthma--present in most, absent in many. Nat Rev Immunol. 2015;15(1):57-65. doi: 10.1038/nri3786. PubMed PMID: 25534623; PMCID: 4390063.
5. Kim J, Woods A, Becker-Dunn E, Bottomly K. Distinct functional phenotypes of cloned Ia-restricted helper T cells. J Exp Med. 1985;162(1):188-201. PubMed PMID: 3159823; PMCID: PMC2187677.
6. Zheng W, Flavell RA. The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. Cell. 1997;89(4):587-96. PubMed PMID: 9160750.
7. Sokol CL, Barton GM, Farr AG, Medzhitov R. A mechanism for the initiation of allergen-induced T helper type 2 responses. Nat Immunol. 2008;9(3):310-8. doi: 10.1038/ni1558. PubMed PMID: 18300366; PMCID: PMC3888112.
8. Eisenbarth SC, Piggott DA, Huleatt JW, Visintin I, Herrick CA, Bottomly K. Lipopolysaccharide-enhanced, toll-like receptor 4-dependent T helper cell type 2 responses to inhaled antigen. J Exp Med. 2002;196(12):1645-51. PubMed PMID: 12486107; PMCID: PMC2196061.
9. Talay O, Yan D, Brightbill HD, Straney EE, Zhou M, Ladi E, Lee WP, Egen JG, Austin CD, Xu M, Wu LC. IgE(+) memory B cells and plasma cells generated through a germinal-center pathway. Nat Immunol. 2012;13(4):396-404. doi: 10.1038/ni.2256. PubMed PMID: 22366892.
10. Vinuesa CG, Sanz I, Cook MC. Dysregulation of germinal centres in autoimmune disease. Nat Rev Immunol. 2009;9(12):845-57. doi: 10.1038/nri2637. PubMed PMID: 19935804.
11. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, Owens SM, Betley J, Fraser L, Bauer M, Gormley N, Gilbert JA, Smith G, Knight R. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. The ISME journal. 2012;6(8):1621-4. Epub 2012/03/10. doi: 10.1038/ismej.2012.8. PubMed PMID: 22402401; PMCID: 3400413.
12. Goodrich JK, Di Rienzi SC, Poole AC, Koren O, Walters WA, Caporaso JG, Knight R, Ley RE. Conducting a microbiome study. Cell. 2014;158(2):250-62. doi: 10.1016/j.cell.2014.06.037. PubMed PMID: 25036628.
13. Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. Diversity, stability and resilience of the human gut microbiota. Nature. 2012;489(7415):220-30. Epub 2012/09/14. doi: 10.1038/nature11550. PubMed PMID: 22972295; PMCID: 3577372.
14. Blumberg R, Powrie F. Microbiota, disease, and back to health: a metastable journey. Science translational medicine. 2012;4(137):137rv7. Epub 2012/06/08. doi: 10.1126/scitranslmed.3004184. PubMed PMID: 22674557.
15. Linterman MA, Beaton L, Yu D, Ramiscal RR, Srivastava M, Hogan JJ, Verma NK, Smyth MJ, Rigby RJ, Vinuesa CG. IL-21 acts directly on B cells to regulate Bcl-6 expression and germinal center responses. J Exp Med. 2010;207(2):353-63. doi: 10.1084/jem.20091738. PubMed PMID: 20142429; PMCID: 2822609.
16. Zotos D, Coquet JM, Zhang Y, Light A, D'Costa K, Kallies A, Corcoran LM, Godfrey DI, Toellner KM, Smyth MJ, Nutt SL, Tarlinton DM. IL-21 regulates germinal center B cell differentiation and proliferation through a B cell-intrinsic mechanism. J Exp Med. 2010;207(2):365-78. doi: 10.1084/jem.20091777. PubMed PMID: 20142430; PMCID: 2822601.
17. King IL, Mohrs M. IL-4-producing CD4+ T cells in reactive lymph nodes during helminth infection are T follicular helper cells. J Exp Med. 2009;206(5):1001-7. doi: 10.1084/jem.20090313. PubMed PMID: 19380638; PMCID: 2715031.
18. Liang HE, Reinhardt RL, Bando JK, Sullivan BM, Ho IC, Locksley RM. Divergent expression patterns of IL-4 and IL-13 define unique functions in allergic immunity. Nat Immunol. 2012;13(1):58-66. doi: 10.1038/ni.2182. PubMed PMID: 22138715; PMCID: 3242938.
19. Barnes PJ. Immunology of asthma and chronic obstructive pulmonary disease. Nature reviews Immunology. 2008;8(3):183-92. doi: 10.1038/nri2254. PubMed PMID: 18274560.

20. MacGlashan D, Jr. IgE receptor and signal transduction in mast cells and basophils. Current opinion in immunology. 2008;20(6):717-23. doi: 10.1016/j.coi.2008.08.004. PubMed PMID: 18822373.
21. Kraft S, Kinet JP. New developments in FcepsilonRI regulation, function and inhibition. Nature reviews Immunology. 2007;7(5):365-78. doi: 10.1038/nri2072. PubMed PMID: 17438574.
22. Zhu J, Cote-Sierra J, Guo L, Paul WE. Stat5 activation plays a critical role in Th2 differentiation. Immunity. 2003;19(5):739-48. PubMed PMID: 14614860.
23. Coffman RL, Carty J. A T cell activity that enhances polyclonal IgE production and its inhibition by interferon-gamma. Journal of immunology. 1986;136(3):949-54. PubMed PMID: 2934482.
24. Pene J, Rousset F, Briere F, Chretien I, Bonnefoy JY, Spits H, Yokota T, Arai N, Arai K, Banchereau J, et al. IgE production by normal human lymphocytes is induced by interleukin 4 and suppressed by interferons gamma and alpha and prostaglandin E2. Proceedings of the National Academy of Sciences of the United States of America. 1988;85(18):6880-4. PubMed PMID: 2970644; PMCID: 282082.
25. Muramatsu M, Kinoshita K, Fagarasan S, Yamada S, Shinkai Y, Honjo T. Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. Cell. 2000;102(5):553-63. PubMed PMID: 11007474.
26. Xu J, Foy TM, Laman JD, Elliott EA, Dunn JJ, Waldschmidt TJ, Elsemore J, Noelle RJ, Flavell RA. Mice deficient for the CD40 ligand. Immunity. 1994;1(5):423-31. PubMed PMID: 7882172.
27. Avery DT, Ma CS, Bryant VL, Santner-Nanan B, Nanan R, Wong M, Fulcher DA, Cook MC, Tangye SG. STAT3 is required for IL-21-induced secretion of IgE from human naive B cells. Blood. 2008;112(5):1784-93. doi: 10.1182/blood-2008-02-142745. PubMed PMID: 18579794.
28. Kobayashi S, Haruo N, Sugane K, Ochs HD, Agematsu K. Interleukin-21 stimulates B-cell immunoglobulin E synthesis in human beings concomitantly with activation-induced cytidine deaminase expression and differentiation into plasma cells. Human immunology. 2009;70(1):35-40. doi: 10.1016/j.humimm.2008.10.021. PubMed PMID: 19026702.
29. Hiromura Y, Kishida T, Nakano H, Hama T, Imanishi J, Hisa Y, Mazda O. IL-21 administration into the nostril alleviates murine allergic rhinitis. Journal of immunology. 2007;179(10):7157-65. PubMed PMID: 17982108.
30. Ozaki K, Spolski R, Feng CG, Qi CF, Cheng J, Sher A, Morse HC, 3rd, Liu C, Schwartzberg PL, Leonard WJ. A critical role for IL-21 in regulating immunoglobulin production. Science. 2002;298(5598):1630-4. doi: 10.1126/science.1077002. PubMed PMID: 12446913.
31. Suto A, Nakajima H, Hirose K, Suzuki K, Kagami S, Seto Y, Hoshimoto A, Saito Y, Foster DC, Iwamoto I. Interleukin 21 prevents antigen-induced IgE production by inhibiting germ line C(epsilon) transcription of IL-4-stimulated B cells. Blood. 2002;100(13):4565-73. doi: 10.1182/blood-2002-04-1115. PubMed PMID: 12393685.
32. Wu LC, Zarrin AA. The production and regulation of IgE by the immune system. Nature reviews Immunology. 2014;14(4):247-59. doi: 10.1038/nri3632. PubMed PMID: 24625841.
33. Akiyama M, Suzuki K, Yamaoka K, Yasuoka H, Takeshita M, Kaneko Y, Kondo H, Kassai Y, Miyazaki T, Morita R, Yoshimura A, Takeuchi T. Brief Report: Number of Circulating Follicular Helper 2 T Cells Correlates With IgG4 and Interleukin-4 Levels and Plasmablast Numbers in IgG4-Related Disease. Arthritis & rheumatology. 2015;67(9):2476-81. doi: 10.1002/art.39209. PubMed PMID: 25989153.
34. Gong F, Su Q, Jiang D, Chen J, Pan Y, Huang X. High frequency of circulating follicular helper T cells in patients with bronchial asthma. Clinical laboratory. 2014;60(6):963-8. PubMed PMID: 25016701.
35. He J, Tsai LM, Leong YA, Hu X, Ma CS, Chevalier N, Sun X, Vandenberg K, Rockman S, Ding Y, Zhu L, Wei W, Wang C, Karnowski A, Belz GT, Ghali JR, Cook MC, Riminton DS, Veillette A, Schwartzberg PL, Mackay F, Brink R, Tangye SG, Vinuesa CG, Mackay CR, Li Z, Yu D. Circulating Precursor CCR7(lo)PD-1(hi) CXCR5(+) CD4(+) T Cells Indicate Tfh Cell Activity and Promote Antibody Responses upon Antigen Reexposure. Immunity. 2013;39(4):770-81. Epub 2013/10/22. doi: 10.1016/j.immuni.2013.09.007. PubMed PMID: 24138884.
36. Bentebibel SE, Lopez S, Obermoser G, Schmitt N, Mueller C, Harrod C, Flano E, Mejias A, Albrecht RA, Blankenship D, Xu H, Pascual V, Banchereau J, Garcia-Sastre A, Palucka AK, Ramilo O, Ueno H. Induction of ICOS+CXCR3+CXCR5+ TH cells correlates with antibody responses to influenza vaccination. Science translational medicine. 2013;5(176):176ra32. doi: 10.1126/scitranslmed.3005191. PubMed PMID: 23486778; PMCID: 3621097.
37. Locci M, Havenar-Daughton C, Landais E, Wu J, Kroenke MA, Arlehamn CL, Su LF, Cubas R, Davis MM, Sette A, Haddad EK, International AVIPCPI, Poignard P, Crotty S. Human circulating PD-1+CXCR3-CXCR5+ memory Tfh cells are highly functional and correlate with broadly neutralizing HIV antibody responses.

Immunity. 2013;39(4):758-69. doi: 10.1016/j.immuni.2013.08.031. PubMed PMID: 24035365; PMCID: 3996844.
38. Choi JY, Ho JH, Pasoto SG, Bunin V, Kim ST, Carrasco S, Borba EF, Goncalves CR, Costa PR, Kallas EG, Bonfa E, Craft J. Circulating follicular helper-like T cells in systemic lupus erythematosus: association with disease activity. Arthritis & rheumatology. 2015;67(4):988-99. doi: 10.1002/art.39020. PubMed PMID: 25581113; PMCID: 4450082.
39. Kenefeck R, Wang CJ, Kapadi T, Wardzinski L, Attridge K, Clough LE, Heuts F, Kogimtzis A, Patel S, Rosenthal M, Ono M, Sansom DM, Narendran P, Walker LS. Follicular helper T cell signature in type 1 diabetes. The Journal of clinical investigation. 2015;125(1):292-303. doi: 10.1172/JCI76238. PubMed PMID: 25485678; PMCID: 4382272.
40. Poholek AC, Hansen K, Hernandez SG, Eto D, Chandele A, Weinstein JS, Dong X, Odegard JM, Kaech SM, Dent AL, Crotty S, Craft J. In vivo regulation of Bcl6 and T follicular helper cell development. Journal of immunology. 2010;185(1):313-26. doi: 10.4049/jimmunol.0904023. PubMed PMID: 20519643; PMCID: 2891136.
41. Ray JP, Marshall HD, Laidlaw BJ, Staron MM, Kaech SM, Craft J. Transcription factor STAT3 and type I interferons are corepressive insulators for differentiation of follicular helper and T helper 1 cells. Immunity. 2014;40(3):367-77. doi: 10.1016/j.immuni.2014.02.005. PubMed PMID: 24631156; PMCID: 3992517.
42. Weinstein JS, Bertino SA, Hernandez SG, Poholek AC, Teplitzky TB, Nowyhed HN, Craft J. B cells in T follicular helper cell development and function: separable roles in delivery of ICOS ligand and antigen. Journal of immunology. 2014;192(7):3166-79. doi: 10.4049/jimmunol.1302617. PubMed PMID: 24610013; PMCID: 3991608.
43. Weinstein JS, Lezon-Geyda K, Maksimova Y, Craft S, Zhang Y, Su M, Schulz VP, Craft J, Gallagher PG. Global transcriptome analysis and enhancer landscape of human primary T follicular helper and T effector lymphocytes. Blood. 2014;124(25):3719-29. doi: 10.1182/blood-2014-06-582700. PubMed PMID: 25331115; PMCID: 4263981.
44. Morita R, Schmitt N, Bentebibel SE, Ranganathan R, Bourdery L, Zurawski G, Foucat E, Dullaers M, Oh S, Sabzghabaei N, Lavecchio EM, Punaro M, Pascual V, Banchereau J, Ueno H. Human blood CXCR5(+)CD4(+) T cells are counterparts of T follicular cells and contain specific subsets that differentially support antibody secretion. Immunity. 2011;34(1):108-21. doi: 10.1016/j.immuni.2010.12.012. PubMed PMID: 21215658; PMCID: 3046815.
45. Ozaki K, Spolski R, Ettinger R, Kim HP, Wang G, Qi CF, Hwu P, Shaffer DJ, Akilesh S, Roopenian DC, Morse HC, 3rd, Lipsky PE, Leonard WJ. Regulation of B cell differentiation and plasma cell generation by IL-21, a novel inducer of Blimp-1 and Bcl-6. Journal of immunology. 2004;173(9):5361-71. PubMed PMID: 15494482.
46. Shulman Z, Gitlin AD, Weinstein JS, Lainez B, Esplugues E, Flavell RA, Craft JE, Nussenzweig MC. Dynamic signaling by T follicular helper cells during germinal center B cell selection. Science. 2014;345(6200):1058-62. doi: 10.1126/science.1257861. PubMed PMID: 25170154; PMCID: 4519234.
47. Mohrs M, Shinkai K, Mohrs K, Locksley RM. Analysis of type 2 immunity in vivo with a bicistronic IL-4 reporter. Immunity. 2001;15(2):303-11. PubMed PMID: 11520464.
48. Palm NW, Rosenstein RK, Yu S, Schenten DD, Florsheim E, Medzhitov R. Bee venom phospholipase A2 induces a primary type 2 response that is dependent on the receptor ST2 and confers protective immunity. Immunity. 2013;39(5):976-85. doi: 10.1016/j.immuni.2013.10.006. PubMed PMID: 24210353; PMCID: 3852615.
49. Johnston RJ, Poholek AC, DiToro D, Yusuf I, Eto D, Barnett B, Dent AL, Craft J, Crotty S. Bcl6 and Blimp-1 are reciprocal and antagonistic regulators of T follicular helper cell differentiation. Science. 2009;325(5943):1006-10. doi: 10.1126/science.1175870. PubMed PMID: 19608860; PMCID: 2766560.
50. Becker C, Pohla H, Frankenberger B, Schuler T, Assenmacher M, Schendel DJ, Blankenstein T. Adoptive tumor therapy with T lymphocytes enriched through an IFN-gamma capture assay. Nature medicine. 2001;7(10):1159-62. doi: 10.1038/nm1001-1159. PubMed PMID: 11590442.
51. Jacquier V, Estelle J, Schmaltz-Panneau B, Lecardonnel J, Moroldo M, Lemonnier G, Turner-Maier J, Duranthon V, Oswald IP, Gidenne T, Rogel-Gaillard C. Genome-wide immunity studies in the rabbit: transcriptome variations in peripheral blood mononuclear cells after in vitro stimulation by LPS or PMA-Ionomycin. BMC genomics. 2015;16:26. doi: 10.1186/s12864-015-1218-9. PubMed PMID: 25613284; PMCID: 4326531.

52. Park HW, Kim YC, Yu B, Moroishi T, Mo JS, Plouffe SW, Meng Z, Lin KC, Yu FX, Alexander CM, Wang CY, Guan KL. Alternative Wnt Signaling Activates YAP/TAZ. Cell. 2015;162(4):780-94. Epub 2015/08/16. doi: 10.1016/j.cell.2015.07.013. PubMed PMID: 26276632; PMCID: 4538707.
53. Chae W-J, Ehrlich AK, Teixeira AM, Chan PY, Henegariu O, Park J-H, Hao L, Tang WH, Kim S-T, Maher SE, Shin JH, Goldsmith-Pestana K, Hwa J, Krause DS, Rothlin CV, McMahon-Pratt D, Bothwell ALM. The Wnt antagonist Dickkopf-1 promotes pathological type 2 inflammation. Immunity, revision submitted. 2015.
54. Idzko M, Pitchford S, Page C. Role of platelets in allergic airway inflammation. The Journal of allergy and clinical immunology. 2015;135(6):1416-23. doi: 10.1016/j.jaci.2015.04.028. PubMed PMID: 26051948.
55. Yan X, Chu JH, Gomez J, Koenigs M, Holm C, He X, Perez MF, Zhao H, Mane S, Martinez FD, Ober C, Nicolae DL, Barnes KC, London SJ, Gilliland F, Weiss ST, Raby BA, Cohn L, Chupp GL. Noninvasive analysis of the sputum transcriptome discriminates clinical phenotypes of asthma. American journal of respiratory and critical care medicine. 2015;191(10):1116-25. Epub 2015/03/13. doi: 10.1164/rccm.201408-1440OC. PubMed PMID: 25763605; PMCID: 4451618.
56. Porro C, Lepore S, Trotta T, Castellani S, Ratclif L, Battaglino A, Di Gioia S, Martinez MC, Conese M, Maffione AB. Isolation and characterization of microparticles in sputum from cystic fibrosis patients. Respir Res. 2010;11:94. doi: 10.1186/1465-9921-11-94. PubMed PMID: 20618958; PMCID: 2910006.
57. Erb-Downward JR, Thompson DL, Han MK, Freeman CM, McCloskey L, Schmidt LA, Young VB, Toews GB, Curtis JL, Sundaram B, Martinez FJ, Huffnagle GB. Analysis of the lung microbiome in the "healthy" smoker and in COPD. PloS one. 2011;6(2):e16384. doi: 10.1371/journal.pone.0016384. PubMed PMID: 21364979; PMCID: 3043049.
58. Hilty M, Burke C, Pedro H, Cardenas P, Bush A, Bossley C, Davies J, Ervine A, Poulter L, Pachter L, Moffatt MF, Cookson WO. Disordered microbial communities in asthmatic airways. PloS one. 2010;5(1):e8578. doi: 10.1371/journal.pone.0008578. PubMed PMID: 20052417; PMCID: 2798952.
59. Morris A, Beck JM, Schloss PD, Campbell TB, Crothers K, Curtis JL, Flores SC, Fontenot AP, Ghedin E, Huang L, Jablonski K, Kleerup E, Lynch SV, Sodergren E, Twigg H, Young VB, Bassis CM, Venkataraman A, Schmidt TM, Weinstock GM, Lung HIVMP. Comparison of the respiratory microbiome in healthy nonsmokers and smokers. American journal of respiratory and critical care medicine. 2013;187(10):1067-75. doi: 10.1164/rccm.201210-1913OC. PubMed PMID: 23491408; PMCID: 3734620.
60. Zemanick ET, Sagel SD, Harris JK. The airway microbiome in cystic fibrosis and implications for treatment. Current opinion in pediatrics. 2011;23(3):319-24. doi: 10.1097/MOP.0b013e32834604f2. PubMed PMID: 21494150.
61. Dickson RP, Erb-Downward JR, Freeman CM, McCloskey L, Beck JM, Huffnagle GB, Curtis JL. Spatial Variation in the Healthy Human Lung Microbiome and the Adapted Island Model of Lung Biogeography. Ann Am Thorac Soc. 2015;12(6):821-30. doi: 10.1513/AnnalsATS.201501-029OC. PubMed PMID: 25803243.
62. Palm NW, de Zoete MR, Cullen TW, Barry NA, Stefanowski J, Hao L, Degnan PH, Hu J, Peter I, Zhang W, Ruggiero E, Cho JH, Goodman AL, Flavell RA. Immunoglobulin A coating identifies colitogenic bacteria in inflammatory bowel disease. Cell. 2014;158(5):1000-10. doi: 10.1016/j.cell.2014.08.006. PubMed PMID: 25171403; PMCID: 4174347.
63. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, Fierer N, Knight R. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proceedings of the National Academy of Sciences of the United States of America. 2011;108 Suppl 1:4516-22. doi: 10.1073/pnas.1000080107. PubMed PMID: 20534432; PMCID: 3063599.
64. Kuczynski J, Stombaugh J, Walters WA, Gonzalez A, Caporaso JG, Knight R. Using QIIME to analyze 16S rRNA gene sequences from microbial communities. Current protocols in microbiology. 2012;Chapter 1:Unit 1E 5. doi: 10.1002/9780471729259.mc01e05s27. PubMed PMID: 23184592; PMCID: 4477843.
65. Goleva E, Jackson LP, Harris JK, Robertson CE, Sutherland ER, Hall CF, Good JT, Jr., Gelfand EW, Martin RJ, Leung DY. The effects of airway microbiome on corticosteroid responsiveness in asthma. American journal of respiratory and critical care medicine. 2013;188(10):1193-201. doi: 10.1164/rccm.201304-0775OC. PubMed PMID: 24024497; PMCID: 3863730.
66. Kawanami T, Fukuda K, Yatera K, Kido T, Yoshii C, Taniguchi H, Kido M. Severe pneumonia with Leptotrichia sp. detected predominantly in bronchoalveolar lavage fluid by use of 16S rRNA gene sequencing analysis. Journal of clinical microbiology. 2009;47(2):496-8. doi: 10.1128/JCM.01429-08. PubMed PMID: 19052180; PMCID: 2643685.
67. Pragman AA, Kim HB, Reilly CS, Wendt C, Isaacson RE. The lung microbiome in moderate and severe chronic obstructive pulmonary disease. PloS one. 2012;7(10):e47305. doi: 10.1371/journal.pone.0047305. PubMed PMID: 23071781; PMCID: 3469539.

Resource Sharing Plan

Data generated in these studies will be freely available to members of the research community with a goal to enable dissemination of data, while also protecting the privacy of the participants and the utility of the data, by de-identifying and masking potentially sensitive data elements in compliance with the NIH public data sharing policy. All other resources developed in the course of the proposed studies will be available by request to qualified academic investigators for non-commercial research.

For all studies, we will follow the National Institute of Health's Genomic Data Sharing Policy. The raw datasets corresponding to expression, genomic, and genetic data generated by these studies will be submitted to Gene Expression Omnibus (GEO) or the Sequence Read Archive (SRA) for use by other investigators. As datasets are analyzed, then validated, we will proceed with deposition in ImmPort according to a timeline negotiated with the Program Officer. Sample data in the YCAAD biorepository are, and will continue to be, available on the internet through the YCCI research accelerator, a publicly accessible platform for scientific collaboration (ycci.researchaccelerator.org). In addition, tools, pipelines, derived datasets and analyses will be made available through the website (asthmaMAP.gersteinlab.org) which will serve as an organizational tool for the participants in this cooperative proposal as well as a repository and resource for the greater research community. Details of the contents and construction of the asthma MAP website are in Project 3, Research Proposal Aim 3.

Yale University School of Medicine and all investigators will adhere to the NIH Grants Policy on Sharing of Unique Research Resources including the “Sharing of Biomedical Research Resources: Principles and Guidelines for Recipients of NIH Grants and Contracts” issued in December 1999. Specifically, material transfers would be made with no more restrictive terms than in the Simple Letter Agreement or the UBMTA and without reach through requirements. Should any intellectual property arise which requires a patent, we would ensure that the technology remains widely available to the research community in accordance with the NIH Principles and Guidelines document. In addition, we will provide relevant protocols and published data upon request. Accepted versions of final, peer-reviewed manuscripts emanating from this research will be deposited on-line to PubMed Central in accord with NIH Public Access.

OMB Number: 4040-0001
Expiration Date: 06/30/2016

APPLICATION FOR FEDERAL ASSISTANCE

SF 424 (R&R)

5. APPLICANT INFORMATION **Organizational DUNS*:** 0432075620000

Legal Name*: YALE UNIVERSITY
Department:
Division:
Street1*: OFFICE OF SPONSORED PROJECTS
Street2: 25 Science Park
City*: NEW HAVEN
County:
State*: CT: Connecticut
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 065208237

Person to be contacted on matters involving this application

Prefix:	First Name*:	Middle Name:	Last Name*:	Suffix:
	Marybeth		Brandi	

Position/Title: Proposal Manager
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7. TYPE OF APPLICANT*

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Asthma MAP: Computational Tools and Clustering for the Study of Asthma Heterogeneity

12. PROPOSED PROJECT

Start Date*	Ending Date*
07/01/2016	06/30/2021

OMB Number: 4040-0010
Expiration Date: 06/30/2016

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	YALE UNIVERSITY
Duns Number:	0432075620000
Street1*:	300 Cedar St
Street2:	TAC S440
City*:	NEW HAVEN
County:	
State*:	CT: Connecticut
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	065208237
Project/Performance Site Congressional District*:	CT-003

Project/Performance Site Location 1

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	YALE UNIVERSITY
DUNS Number:	0432075620000
Street1*:	266 Whitney Ave
Street2:	Bass 432A
City*:	NEW HAVEN
County:	
State*:	CT: Connecticut
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	065208114
Project/Performance Site Congressional District*:	CT-003

File Name

Additional Location(s)

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ● **Yes** ❍ **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ❍ Yes ● No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6

If NO, is the IRB review Pending? ❍ **Yes** ❍ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ❍ **Yes** ● **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ❍ Yes ❍ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. Is proprietary/privileged information included in the application?* ❍ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ❍ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ❍ Yes ❍ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ❍ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ❍ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Project_3_Project_Summary.pdf
8. Project Narrative*	
9. Bibliography & References Cited	Project_3_References_Cited.pdf
10.Facilities & Other Resources	Project_3_Facilities_and_Resources.pdf
11.Equipment	

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

Project Summary

Asthma can be difficult to treat in part because patients' clinical presentations and responses to different medical interventions vary greatly. Being able to predict an individual's optimal treatment requires a more fundamental understanding both of the extent of asthma heterogeneity and the mechanisms that drive these differences. Next-generation sequencing and high-throughput methods offer promise at revealing much this heterogeneity by reporting on cellular states in the full complexity of the clinical setting. We will apply a novel set of data from a well-defined cohort of patients with different asthma severities to understand the mechanisms that underlie asthma heterogeneity. In our first aim we will provide the pipelines necessary for the asthma-specific and uniform processing of bulk-cell RNA-seq data. We will use these data to cluster patients by transcriptional endotypes and then by genes to speak to the co-expression differences that drive different patient clusters. In our second aim we will then analyze patients at the single-cell level, first by mass cytometry (CyTOF) and then by single-cell RNA-seq. The CyTOF protein information will be clustered to provide high-resolution cell-signatures, yielding accurate numbers of different cell types and their abundances. We will then provide the pipelines for the processing of single-cell RNA-seq data, and then use these data to understand how different cell types vary in their global expression. In our third aim we will integrate these data along with clinical data, external datasets and data from the other Driving Projects in this proposal to generate novel asthma endotypes of patients, cell-signatures and gene regulatory networks. We will provide to the research community all of the data, tools, pipelines and results from project through the website ASTHMA MAP (http://asthmamap.gersteinlab.org).

Facilities and Other Resources - Project 3

The Gerstein laboratory is found in two connected buildings. The laboratory consists of 6 rooms and comprises a total of ~1,900 sq. ft. In addition, three conference rooms that have projectors provide venues for interaction. There are 40 gigabit-ready desks, equipped with one or two 23" and 30" LCD screens. The space is properly air conditioned for supporting a large number of computers.

Gerstein Lab Computer Infrastructure

Laboratory Network and Storage. The lab's computing infrastructure is partitioned into a private and a public network. The entire infrastructure is fully gigabit capable and is connected to the Yale backbone via gigabit optic fibre; the network architecture was designed with computing efficiency and network security in mind. The private network consists of individual laptops, desktops and workstations, as well as communal computational servers, dumb terminals, a central fileserver, a consolidated NAS, and printers. There are also servers that provide essential network services such as NIS, NFS, SMB, DHCP, monitoring and backups. The public network consists of numerous production webservers that are either real or virtual machines. The laboratory maintains its own public subnets of 128 public IP addresses and manages many of its own domains (e.g. gersteinlab.org, molmovdb.org, pseudogenes.org, and partslist.org). The lab has a full-time administrator maintaining the network.

The private and public networks obtain gigabit connectivity through four HP Procurve 5300xl switches that are mutually connected via fibre. The private network is behind a Cisco PIX 525, which is concurrently used as an IPSec VPN gateway into the private network. Within the private network are two NetApp storage appliances with 43Tb of raw space, which is configured with 27.5Tb of working space, thirty custom made 4Tb network disks with a total 120Tb capacity, a Dell NAS with a total of 30TB capacity; the NetApp appliances and Dell NAS are used for live user file space, backups of user files and backups of public production webservers. A seven-day incremental backup and a twelve-month incremental backup are currently being implemented in the lab.

Wireless access is available all throughout the lab. Wireless access connects computers directly to the public network.

Office

Mark Gerstein's office space is 178 square feet.

Computers

There are about forty-seven working laptops in the lab, in which eighteen are recent Macbook Pro models.

In total, the lab has 315u of rack space spread over eight racks. Residing in these racks are a dual CPU twelve core Opteron server with 256GB of memory, a dual CPU six core Opteron server with 128GB of memory, a dual CPU four core Opteron server with 64GB of memory, three Intel blade enclosures with 10 dual CPU Intel blades each, fourteen dual cpu 64 bit Xeons servers and six dual cpu 64 bit Opteron servers; these rack servers are in addition to the NetApp storage appliances and the Dell NAS mentioned above. The rack servers have various uses. The dual CPU Opteron servers are for hosting virtual machines, which function as web hosts. In the private network, five rack servers are for essential network services, four are storage head nodes for the Dell SAN and a few are network support or experimental machines. The rest of the rack servers are in the public network acting as webservers. The private network has seven business class color laserjet printers.

Software

A number of open source software, programs created in-house, and proprietary software is used by the lab researchers for their needs. The lab maintains a set of wiki servers for the documentation of internal information and the public dissemination of information. The lab also manages mailman servers for its mailing

lists. The compute nodes are mainly used to develop and run Java and Perl code and to perform Matlab and Gromacs calculations. The public webservers are used to deploy Java, Perl, PHP and Python applications.

Individual tasks are coordinated by a web group calendar. Web applications and servers are continually being monitored by a Nagios monitoring system.

Yale Life Sciences Supercomputer

The Gerstein laboratory has priority access to two of the Yale supercomputers, namely Louise and BulldogI, and regular access to six other Yale supercomputers. There are two full-time administrators maintaining the supercomputer.

Louise is a cluster with 112 Dell PowerEdge R610 with (2) quad core E5620 nodes, each with 2.4 Ghz cpu cores and 48 GB RAM. They are interconnected with a Force10 network switch. There is therefore a total of 112*8 cores = 896 cores. Louise has 300 TB (raw) of BlueArc parallel file storage.

BulldogI is a cluster consisting of a head node and 170 Dell PowerEdge 1955 nodes, each containing 2 dual core 3.0 Ghz Xeon 64 bit EM64T Intel cpus, for a total of 680 cores. Each node has 16 GB RAM. The network is Gigabit ethernet. BulldogI runs a high performance Lustre filesystem. It is managed via PBS. Three 20Tb Dell Power Vault with storage arrays are attached to BulldogI and are dedicated for Gerstein laboratory use.

The laboratory also has priority access to a SGI F1240 system. This system has 12 Xeon E5345 Quad-Core 2.33GHz CPUs (for a total of 48 processor cores), with 2 x 4M L2 cache per CPU, a 1333MHz front side bus, 96GB of memory, and 6 Raptor 150GB, 10K rpm SATA drives. It runs SUSE Linux Enterprise Server 10 as a system single image. That is, all 48 cores are managed by a single process scheduler, and the 96 GB memory is, in principle, addressable by a single process. In practice, system caches and buffers reduce the maximum amount of memory available to any given process to about 70 GB. In many ways then, the system can be thought of as an SMP, but in terms of hardware architecture it is closer to an infiniband-connected cluster.

Core Lab

The Gerstein Lab is adjacent to the Yale Center for Structural Biology (CSB) Core laboratory. The Core laboratory resources are available to members of the Gerstein lab. The Core laboratory supports the work of all the people associated with the CSB, in total about 200 users and >200 computers. These computers include a number of high-performance graphics workstations for visualizing macromolecular structures and complex data sets. The CSB Core staff of 2 FTE provides support to the associated CSB laboratories as well as the Core computers.

Oracle Server

Yale University has an institutional site license for the Oracle database management system. As a result, many major administrative computing systems at Yale are being developed using Oracle, and Yale's ITS staff has extensive Oracle experience. Yale ITS maintains and operates several Oracle database systems at the School of Medicine, and provides access to these machines to many different projects. There are several advantages to using institutional servers. The ITS staff backs up each database on a regular schedule, typically with full backups weekly and partial backups several times a day. The ITS staff maintains the hardware of the database machine, the system software, and the Oracle software. They perform periodic upgrades when new versions of the software become available. They also handle any systems problems that occur, and are available to help troubleshoot any application problems that arise.

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: Mark Middle Name Bender Last Name*: Gerstein Suffix:

Position/Title*: Albert L. Williams Professor of Biommedical
Organization Name*: YALE UNIVERSITY
Department:
Division:
Street1*: PROGRAM IN COMPUTATIONAL BIOLOGY
Street2: AND BIOINFORMATICS
City*: New Haven
County:
State*: CT: Connecticut
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 065200000

Phone Number*: 203-432-6105 Fax Number: 360-838-7816 E-Mail*: MARK.GERSTEIN@YALE.EDU

Credential, e.g., agency login: MGERSTEIN

Project Role*: Other (Specify) Other Project Role Category: Project Lead

Degree Type: PHD,AB Degree Year:

	File Name
Attach Biographical Sketch*:	Gerstein_Bio_Asthma_U19.pdf
Attach Current & Pending Support:	

Tracking Number: GRANT12004239 Funding Opportunity Number: RFA-AI-15-032 . Received Date: 2015-10-01T14:26:34.000-04:00

PROFILE - Senior/Key Person

Prefix:	First Name*: Smita	Middle Name	Last Name*: Krishnaswamy	Suffix:

Position/Title*:	Assistant Professor
Organization Name*:	YALE UNIVERSITY
Department:	
Division:	
Street1*:	333 Cedar St
Street2:	
City*:	New Haven
County:	
State*:	CT: Connecticut
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	06510-3206

Phone Number*: 203-785-7833	Fax Number:	E-Mail*: smita.krishnaswamy@yale.edu

Credential, e.g., agency login:

Project Role*: Other (Specify)	Other Project Role Category: Co-Lead
Degree Type:	Degree Year:

	File Name
Attach Biographical Sketch*:	Krishnaswamy_Bio_Asthma_U19.pdf
Attach Current & Pending Support:	

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2016 **End Date*:** 06-30-2017 **Budget Period: 1**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Mark	Bender	Gerstein		Project Lead			0.34		6,874.00	2,131.00	9,005.00
2.	Smita		Krishnaswamy		Co-Lead		1.2			13,650.00	4,232.00	17,882.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **26,887.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	4.5			17,648.00	5,470.00	23,118.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
2	Associate Research Scientist	16.0			68,000.00	21,080.00	89,080.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**112,198.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**139,085.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2016 **End Date*:** 06-30-2017 **Budget Period: 1**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
1. server	20,000.00
Total funds requested for all equipment listed in the attached file	
Total Equipment	**20,000.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	5,000.00
2. Foreign Travel Costs	
Total Travel Cost	**5,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2016 **End Date*:** 06-30-2017 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	10,915.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**10,915.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**175,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Modified total direct cost	66.5	155,000.00	103,075.00
		Total Indirect Costs	**103,075.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**278,075.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: Project_3_Budget_Justification_Asthma_U19.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2017 **End Date*:** 06-30-2018 **Budget Period: 2**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Mark	Bender	Gerstein		Project Lead			0.34		6,874.00	2,131.00	9,005.00
2.	Smita		Krishnaswamy		Co-Lead		1.2			14,060.00	4,358.00	18,418.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **27,423.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	4.5			18,178.00	5,635.00	23,813.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
2	Associate Research Scientist	18.0			78,795.00	24,426.00	103,221.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**127,034.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**154,457.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2017 **End Date*:** 06-30-2018 **Budget Period: 2**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	5,000.00
2. Foreign Travel Costs	
Total Travel Cost	**5,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2017 **End Date*:** 06-30-2018 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	25,543.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**25,543.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**185,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Modified total direct cost	66.5	185,000.00	123,025.00
		Total Indirect Costs	**123,025.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**308,025.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: Project_3_Budget_Justification_Asthma_U19.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2018 **End Date*:** 06-30-2019 **Budget Period: 3**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Mark	Bender	Gerstein		Project Lead			0.34		6,874.00	2,131.00	9,005.00
2.	Smita		Krishnaswamy		Co-Lead		1.2			14,481.00	4,489.00	18,970.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **27,975.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	4.5			18,723.00	5,804.00	24,527.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
2	Associate Research Scientist	18.0			81,159.00	25,159.00	106,318.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**130,845.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**158,820.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2018 **End Date*:** 06-30-2019 **Budget Period: 3**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	5,000.00
2. Foreign Travel Costs	
Total Travel Cost	**5,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2018 **End Date*:** 06-30-2019 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	21,180.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**21,180.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**185,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Modified total direct cost	66.5	185,000.00	123,025.00
		Total Indirect Costs	**123,025.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**308,025.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: Project_3_Budget_Justification_Asthma_U19.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12004239 Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2019 **End Date*:** 06-30-2020 **Budget Period: 4**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Mark	Bender	Gerstein		Project Lead			0.34		6,874.00	2,131.00	9,005.00
2.	Smita		Krishnaswamy		Co-Lead		1.2			14,916.00	4,624.00	19,540.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **28,545.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	4.5			19,285.00	5,978.00	25,263.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
2	Associate Research Scientist	18.0			83,594.00	25,913.00	109,507.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**134,770.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**163,315.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2019 **End Date*:** 06-30-2020 **Budget Period: 4**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	5,000.00
2. Foreign Travel Costs	
Total Travel Cost	**5,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2019 **End Date*:** 06-30-2020 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	16,685.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**16,685.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**185,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Modified total direct cost	66.5	185,000.00	123,025.00
		Total Indirect Costs	**123,025.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**308,025.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: Project_3_Budget_Justification_Asthma_U19.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2020 **End Date*:** 06-30-2021 **Budget Period: 5**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Mark	Bender	Gerstein		Project Lead			0.34		6,874.00	2,131.00	9,005.00
2.	Smita		Krishnaswamy		Co-Lead		1.2			15,363.00	4,763.00	20,126.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **29,131.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	4.5			19,863.00	6,158.00	26,021.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
2	Associate Research Scientist	18.0			86,101.00	26,691.00	112,792.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**138,813.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**167,944.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2020 **End Date*:** 06-30-2021 **Budget Period: 5**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	5,000.00
2. Foreign Travel Costs	
Total Travel Cost	**5,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0432075620000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: YALE UNIVERSITY

Start Date*: 07-01-2020 **End Date*:** 06-30-2021 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	12,056.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**12,056.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**185,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Modified total direct cost	66.5	185,000.00	123,025.00
		Total Indirect Costs	**123,025.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**308,025.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: Project_3_Budget_Justification_Asthma_U19.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

PROJECT 3

BUDGET JUSTIFICATION

Key Personnel

Mark Gerstein, Ph.D. (Project Lead). Dr. Gerstein is the Albert Williams Professor of Biomedical Informatics. His lab was one of the first to perform integrated data mining on functional genomics data and to do genome-wide surveys. His tools for analyzing motions and packing are widely used. Most recently, he has designed and developed a wide array of databases and computational tools to mine genome data in humans, as well as in many other organisms. He has worked extensively in the 1000 genomes project in the SV and FIG groups. He also worked in the ENCODE pilot project and currently works extensively in the ENCODE and modENCODE production projects. He is also a PI in DOE KBase and the leader of the Data Analysis Center for the NIH exRNA consortium. In these roles Dr. Gerstein has designed and developed a wide array of databases and computational tools to mine genomic data in humans as well as in many other organisms. He will lead the overall informatics effort in the project. Dr. Gerstein's effort on this grant will be 0.45 summer months, annually.

Smita Krishnaswamy, Ph.D., (Co-Lead). Dr. Krishnaswamy is an Assistant Professor of Genetics and Computer Science. She obtained her Ph.D. in computer Science and engineering in 2008 from the University of Michigan. Prior to joining Yale, she was a postdoctoral fellow at Columbia University in the departments of Systems Biology and Biological Sciences. She also has industrial experience as a research staff member at IBM's TJ Watson Research Center. She has extensive experience in algorithm development in many different contexts and in the analysis and modeling of single-cell data. She will oversee the analysis of single-cell CyTOF and sequencing data generated in the project, including the development and application of novel computational tools, statistical analysis, and interpretation and visualization of results. Dr. Krishnaswamy's effort will be 1.2 calendar months annually.

Non-Key Personnel

Daifeng Wang, Ph.D., is an Associate Research Scientist in the Department of MMBB at Yale University, School of Medicine. He has a strong background in scientific computation and obtained his Ph.D. in The University of Texas at Austin. His background is data modeling, mining and analysis. He used to work on dynamic pattern recognition of large-scale gene expression data. After joining the Gerstein lab, he has been actively working on developing novel algorithms/models to cluster multiplex networks in genomes, identify dynamic patterns in high dimensional genomic datasets, and characterize combinatorial co-operations among regulatory factors in gene regulatory networks. He is also interested in analyzing dynamics of social networks in large scientific projects. Dr. Wang will work on the deconvolution of cell-type signatures and the logical modeling of regulatory pathways (Aim 3). Dr. Wang's effort on this grant will be 12 calendar months annually.

Shaoke Lou, Ph.D., Postdoctoral Assoc. Dr. Lou has multidisciplinary background with a B.S. in Chemistry, an M.S. in Biochemistry and Molecular Biology, and a PhD in Computer Science and Engineering. He earned his PhD from the Chinese University of Hong Kong in 2012. During his PhD study, he mainly works on the development of read-mapping algorithm, next-generation sequencing data analysis, data mining and statistics modeling. He has broad research interests, and is an expert in gene regulatory networks, disease association study and genomic variation prioritization. He was involved in the investigation and data analysis in model organisms: angiogenesis activities of traditional Chinese medicine in zebrafish, regulation of chromatin structure factors (CapH2) in fly, and circadian rhythmic study in mouse. Dr. Lou will work on the annotation of novel asthma-related genetic elements by clustering and network analysis (Aim 1). Dr. Lou's effort ton this grant will be 4.0 calendar months in Year 1, then 6.0 calendar months in Years 2-5).

To Be Named (Postdoctoral Associate). The postdoc in the Krishnaswamy lab will work on development of algorithms, programming, automating statistical analysis and development of graphical software interfaces. His or her annual effort on this grant will be 4.5 calendar months.

Other Expenses

Laboratory Supplies

We are budgeting an incremental amount of supplies for the individuals named above. This supplies budget will be used to cover computer supplies for them, covering such expenses as: diskettes, tapes, and other miscellaneous computer parts (e.g. replacing worn out surge suppressors), software upgrades, web hosting and "cloud computing fees, and reprint charges. These items are needed to complete the proposed research and will solely benefit this project.

The supplies budget for each year is:

Year 1: $10,915
Year 2: $25,273
Year 3: $20,631
Year 4: $15,850
Year 5: $10,926

Travel

We have budgeted $5,000 per year to defray travel costs for the Project Lead and Co-Lead for airfare, lodging and meal expenses to attend scientific meetings annually that benefit the project. In particular, the travel will include at least 1 trip per year to a scientific meeting related to asthma, such as the American Academy of Allergy, Asthma and Immunology annual meeting.

Equipment

In Year 1, $20,000 is budgeted for a Dell Poweredge R815 server with 160GB of memory and four AMD Opteron processors. This will be used for processing our data and digital visualization. This is needed to complete the proposed research and will solely benefit this project.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		139,961.00
Section B, Other Personnel		643,660.00
Total Number Other Personnel	15	
Total Salary, Wages and Fringe Benefits (A+B)		783,621.00
Section C, Equipment		20,000.00
Section D, Travel		25,000.00
1. Domestic	25,000.00	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		86,379.00
1. Materials and Supplies	86,379.00	
2. Publication Costs	0.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	0.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	0.00	
9. Other 2	0.00	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		915,000.00
Section H, Indirect Costs		595,175.00
Section I, Total Direct and Indirect Costs (G + H)		1,510,175.00
Section J, Fee		0.00

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

1. Project Director / Principal Investigator (PD/PI)

Prefix:
First Name*: GEOFFREY
Middle Name: L
Last Name*: CHUPP
Suffix:

2. Human Subjects

Clinical Trial? ● No ○ Yes
Agency-Defined Phase III Clinical Trial?* ○ No ○ Yes

3. Permission Statement*

If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?

○ Yes ● No

4. Program Income*

Is program income anticipated during the periods for which the grant support is requested? ○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

Budget Period*	Anticipated Amount ($)*	Source(s)*

PHS 398 Cover Page Supplement

5. Human Embryonic Stem Cells

Does the proposed project involve human embryonic stem cells?* ● No ❍ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s): ❑ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

6. Inventions and Patents (For renewal applications only)

Inventions and Patents*: ❍ Yes ❍ No

If the answer is "Yes" then please answer the following:

Previously Reported*: ❍ Yes ❍ No

7. Change of Investigator / Change of Institution Questions

❑ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:

First Name*:

Middle Name:

Last Name*:

Suffix:

❑ Change of Grantee Institution

Name of former institution*:

PHS 398 Research Plan

Please attach applicable sections of the research plan, below.

OMB Number: 0925-0001

1. Introduction to Application (for RESUBMISSION or REVISION only)	
2. Specific Aims	Project_3_Specific_Aims.pdf
3. Research Strategy*	Project_3_Research_Strategy.pdf
4. Progress Report Publication List	
Human Subjects Sections	
5. Protection of Human Subjects	Protection-HumanSubjects_Asthma_U19.pdf
6. Inclusion of Women and Minorities	Inclusion-Women_Minorities_Asthma_U19.pdf
7. Inclusion of Children	Inclusion-Children_Asthma_U19.pdf
Other Research Plan Sections	
8. Vertebrate Animals	
9. Select Agent Research	
10. Multiple PD/PI Leadership Plan	
11. Consortium/Contractual Arrangements	
12. Letters of Support	
13. Resource Sharing Plan(s)	ResourceSharingPlan_Asthma_U19.pdf
Appendix (if applicable)	
14. Appendix	

Tracking Number: GRANT12004239 Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

Specific Aims

Asthma is a common disease that has been recognized as highly heterogeneous in both its symptoms and treatment, however, the mechanisms underlying this heterogeneity remain largely unclear. We hypothesize that clusters from a novel combination of bulk-cell and single-cell transcription (RNA-seq) and protein profiling (CyTOF) data from a well-characterized cohort of asthmatic patients can differentiate patients in a way that identifies the mechanisms of asthma heterogeneity. Our goal is to to derive clinically informative clusters of genes, cells, and patients that define asthma phenotypes in terms of novel cell populations and molecular pathway regulation. To this end, we propose the following aims:

Aim 1: Bulk-cell RNA-seq processing pipeline and transcript clustering

We will adapt a comprehensive suite of human RNA-seq tools to generate pipelines for the uniform processing of bulk-cell RNA-seq data isolated from sputum cell populations. This will build on a considerable body of preliminary results that we have from developing human RNA-seq pipelines, both for long and short RNA. We will create a workflow to quantify transcript abundances, determine the degree to which they have been spliced and modified, observe the extent to which the transcripts correspond to annotated portions of the genome, as well as identify non-coding RNAs and transcribed pseudogenes. These pipelines will be passed to Core C for use in generating a uniformly-processed dataset for use by each of the Driving Projects 1 and 2 in this cooperative agreement. We will use these data to generate bulk-cell clusters by patients and by genes. The patient clustering will define asthma endotypes and the gene clustering will define co-expression networks that will speak to the mechanism of asthma disease.

Aim 2: Single-cell processing pipelines and analyses of asthma sputum

We will utilize single-cell measurements of protein and mRNA abundances using mass cytometry (CyTOF) and RNA-seq to deeply characterize populations of cells produced in the airways of individuals with asthma. We will identify cell-signatures from multidimensional CyTOF measurements of signaling and surface proteins based on an unsupervised community detection method. Further, we will match results across samples such that the populations are validated through repeated detection. These cell-signatures will be used to generate protein and cell specific clusters to stratify cell types and signaling pathways. Moreover, we will characterize signaling relationships between proteins and cytokine responses in subpopulations of sputum cells. Further, we will develop a pipeline for single-cell RNA-seq that accounts for the technical variability for each gene and converts from raw reads to gene counts in a biologically meaningful manner. After processing, we will employ our previously developed methods to reduce the data into a few robust dimensions, and cluster the results into metagenes corresponding to pathways. These metagenes will subsequently be analyzed by DREMI and DREVI in order to characterize novel pathways and their interactions in the cell populations present in the sputum. With the understanding of signaling and gene-interaction networks we can characterize the pathways involved in the immune reactions to asthma, and understand their involvement in emerging phenotype.

Aim 3: Integrative clustering and the creation of the AsthmaMAP

We will use the data described in Aims 1 and 2 of this project along with the clinical data generated by Core B and external datasets to make integrative clusters by patients, cells and genes. Cell-type signatures defined in Aim 2 will be used to de-convolve the bulk-cell RNA-seq data to its component cell transcripts, increasing the effective dynamic range of the single-cell data and facilitating integration with the abundance of bulk-RNA-seq datasets (e.g. GTEx for tissue-specific context and ENCODE for transcription factor data). We will use logical modeling within the IP clusters to build regulatory differences between the asthma endotypes. Each of these clusters, networks and models will be evaluated in the context of established clinical measurements (e.g. FEV1 and FeNO) to identify effective measures to stratify patients and how they might give insight to the mechanisms of asthma disease and heterogeneity. To effectively disseminate the data, pipelines and analyses generated by this and the other driving projects in this cooperative proposal, we will create the Asthma MAP website that will include all of the data, tools, pipelines and analyses described here.

This set of aims will enrich our knowledge of asthma, particularly of the gene networks that correlate with patient outcomes and that contribute to the heterogeneity of patients and cells. These analyses will generalize asthma disease observations to a systems-level understanding so that we might speak to the underlying mechanisms that affect patient care.

Research Strategy

1. Significance

Asthma is a chronic inflammatory disease of the airways which afflicts ~7% of the U.S. population [1]. In most individuals, symptoms are easily controlled by treatment with bronchodilators and relatively low doses of inhaled corticosteroids, but as many as 30% of asthmatics do not respond adequately to standard therapies and approximately 5% of asthmatics have a severe, refractory form of the disease. The YCAAD research team (see Project 1) used a novel hierarchical clustering approach to identify three transcriptional endotypes of asthma (sputum TEA clusters) using sputum microarray data that successfully stratified patients with severe disease characteristics, including inflammation, airway remodeling and severe attacks. This represented the first non-invasive transcriptomic stratification of asthma disease severity with the potential to successfully identify high-risk patients and reduce hospitalization. However, the TEA clusters do not have the resolution or dynamic range to elucidate molecular mechanisms by which the individuals responded differently.

We will perform RNA-seq, as well as single-cell measurements with state-of-the-art single-cell technologies including single-cell RNA-seq and CyTOF, on RNA and cells isolated from sputum from a heterogeneous cohort of individuals with asthma. RNA-seq is a new but established technology for genome-wide transcriptomic analysis. It has been widely applied for understanding various diseases and enables discovery of gene clusters associated with common functions, as well as identification of novel transcripts with the same functions. The data will widen our current knowledge on asthma from a few specific pathways to a system-wide level. At the same time, single-cell technologies can greatly expand upon the sensitivity and cell-type specificity of asthma research. On the transcriptomic level, single-cell RNA-seq offers an unbiased measurement of the entire collection of mRNA transcripts produced in each cell. Despite its inherent sparsity, it complements bulk RNA-seq in characterizing the heterogeneity among different cell types. A promising approach for discovering new cell types is to perform unsupervised clustering on single-cell RNA-seq data. Performing both bulk RNA-seq and single-cell RNA-seq will therefore synergize our research and provide the most complete profiling resource to date of a well characterized cohort of asthmatic patients. Single-cell RNA-seq transcriptomics will be complemented by single-cell mass cytometry (CyTOF), which provides in depth measurement of protein abundances that define cell activity and function in a lineage specific manner. Currently, CyTOF detects >42 markers of protein abundance measurements and allows us to examine signaling responses within minutes and hours of exposure to relevant antigens, such as house dust-mites. CyTOF is able to probe the response of various cell types and provides a dynamical element to our study.

2. Innovation

Recent efforts by PI Chupp have shown that the complex and heterogeneous patterns of asthma can be sub-typed into categories using microarray expression data. This proposed work goes several steps further, not only offering transcriptional clustering with unprecedented sensitivity and dynamic range, but does so in a way that will likely offer mechanistic insight and novel therapeutic targets. By using single-cell techniques to interrogate the transcriptional and signaling responses, this work has sufficient resolution to dissect the activities of cells and how they are perturbed in severe disease. The data will be integrated into a model that has the potential to bring personalized medicine to asthma care and provided to the community on a publicly accessible, searchable, integrated asthma MAP.

3. Research Plan

3.A. Plan for Aim 1:Bulk-cell RNA-seq processing pipeline and transcript clustering

3.A.i Rationale

Previous transcriptional analysis of airway cells using microarray technology had some success in clustering endotypes of asthma, but currently RNA-seq has become a standard tool for understanding transcriptional activities in cell populations. Sample collection (Core B) and RNA-seq (Core C) will be carried out as detailed in other parts of the proposal. We will use our extensive analytic experience in this technique as the foundation to generating asthma clusters and the interpretation of our analyses using single-cell techniques described in Aim 2.

3.A.ii Preliminary results

<u>3.A.ii.a Application of RNA-seq processing tools</u>

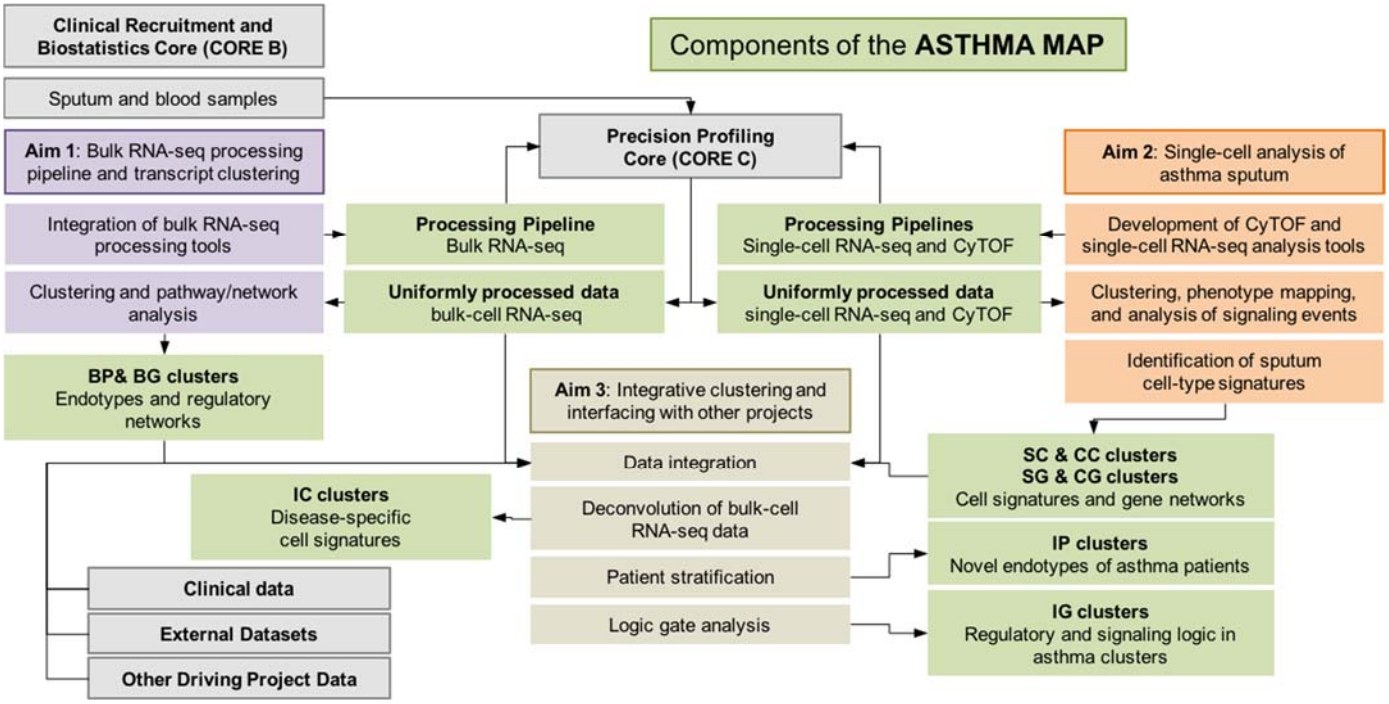

Figure 1. Schematic of the interactions with the Cores and other Driving Projects and the deliverables produced as part of the ASTHMA MAP website.

Of critical importance to the shared use of large datasets is uniform processing. In order to provide a resource to the other projects in this proposal and our own clustering aims, we will build an RNA-seq processing pipeline based on the software suite, RSEQtools, that we have largely developed. These tools consist of a set of modules that perform common tasks such as calculating gene and exon expression values, generating signal tracks of mapped reads and segmenting that signal into actively transcribed regions. Also, implemented within RSEQtools are more specialized analysis pipelines that we have developed (e.g. FusionSeq for fusion transcript detection [2], IQSeq for transcript quantification [3], and DupSeq for analyzing expression patterns of highly homologous genomic regions [4]), as well as thoroughly validated tools such as Bowtie and Tophat [5, 6]. These tools are implemented using Mapped Read Format (MRF), a compact data summary format for short, long and paired-end read alignments that enables the anonymization of confidential sequence information. With this set of tools, we will provide a custom processing pipeline to Core C.

3.A.ii.b: Non-coding RNA (ncRNA) and pseudogene analysis

Other types of transcripts will be important to annotate for analysis of asthma, particularly for the identification of the cell-type signatures that are described in later sections. A fraction of the transcription comes from genomic regions not associated with standard annotations, representing ‘non-canonical transcription’. These transcripts are observable even when experimental protocols use poly-A enrichment [7], as will be performed for the samples in this study. A class of non-canonical transcripts of particular interest is the pseudogene, which recent studies have shown are useful biomarkers to distinguish different cell types. Despite their low abundance, pseudogenes and ncRNAs have been shown to exhibit a greater degree of cell-type specific expression than mRNAs [8] and are therefore useful in several aspects of this study. However, the quantification of pseudogene expression is challenging because of the sequence similarity with its parent genes. To address the issue, we developed DupSeq, which solves this problem by focusing only on those reads and regions that are uniquely mappable [4].

Several other classes of non-coding RNAs have been shown to play regulatory or other roles in the cell. To identify these loci we will apply incRNA, a method that predicts novel ncRNAs using known ncRNAs of various biotypes as a gold standard training set and a minimum-run–maximum-gap algorithm to process reads mapping outside of protein-coding transcripts, pseudogenes and annotated non-coding RNAs [9, 10].

3.A.ii.c: Functional annotation through clustering and network analyses

We have extensive experience in characterizing the functions of genes and non-coding elements via expression data through clustering and network analyses. A group of genes in a co-expression cluster are often responsible for a common function [11]. While there are well known algorithms for expression clustering such as hierarchical clustering, spectral clustering and K-means, we developed several novel methods. We developed a spectral biclustering method for co-clustering genes and conditions. More recently, we developed

a new clustering framework, OrthoClust, for simultaneously clustering network data across different contexts [12]. OrthoClust is able to identify conserved and specific components across different networks. We applied OrthoClust in the comparative transcriptome analysis, and discovered co-expression modules shared in animals and enriched in their developmental genes. Furthermore, expression clusters can be used for annotating functions of unknown transcripts. For example, in modENCODE analysis, by mapping the expression profiles of various ncRNAs to expression clusters, we have used identified functions of various ncRNAs.

The functional relationships between co-expressed genes can further be understood via various molecular networks. Over the past decade, we have identified many relationships between topological properties of genes in networks and their functional genomics features. In terms of local properties, for instance, we found the “essentiality” of a given node is closely related to its tendency to act as a hub or bottleneck [13, 14]. In terms of system-wide organization, we found that gene-regulatory networks are composed of hierarchical structures dominated by downward information flow from master regulators [15]. We further developed methods to determine the hierarchical organization of various regulatory networks, including networks constructed from ENCODE, modENCODE and MCF7 data [16, 17, 18, 19]. Apart from studying individual networks, we also introduced a framework to quantify differences between networks and found a consistent ordering of rewiring rates of different network types. [20].

3.A.ii.d RNA-seq pipeline development for large-scale projects

We have worked on the development and analysis of multiple RNA-seq flows in the context of large consortia, including the implementation of tools we developed and other popular tools such as Bowtie and Tophat. For example, we have been playing a role in such activities for the ENCODE consortium [21], including a recent publication involving the processing and integration of all ENCODE and modENCODE data, which involved 575 experiments and more than 65 billion reads from three organisms. [10]. We are the data integration hub in the Extracellular RNA consortium [22] that generates hundreds of RNA-seq and small RNA-seq samples. Other notable consortia for which we have processed large quantities of data include the BrainSpan project [23] which collected RNA-seq data for 8-16 brain structures in each of 13 developmental stages [24], as well as the PsychENCODE project [25].

3.A.iii Approach

3.A.iii.a Process all the RNA-seq data in a uniform fashion

A critical component to projects that involve a large number of samples sequenced over time is the uniform processing of the data. This is particularly true in cases where clustering will play a role in a generation of conclusions, as it is here that batch effects and sample processing variation can drive artificial organizations of the data. Technical details to minimize experimental variation are in place, see Core C for details. We will process bulk RNA-seq samples in a uniform fashion using the RSEQtools pipeline that we developed, and where appropriate we will combine this with tools like Tophat and Cufflinks (Fig 2). These tools and pipelines have been used extensively by large consortia [10].

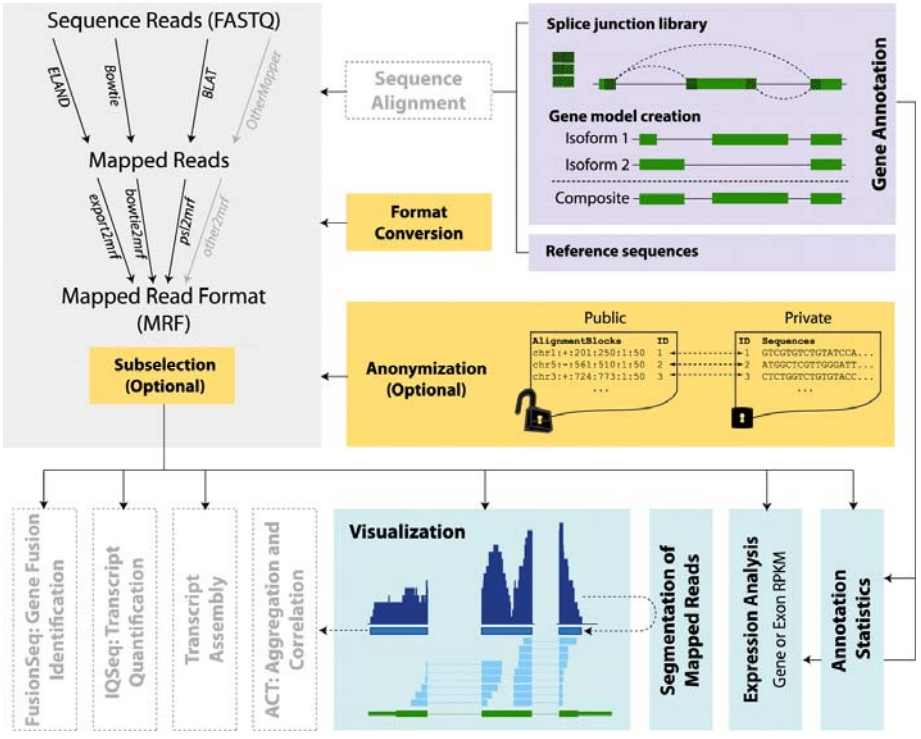

Figure 2. Processing pipeline for RNA-seq tools adapted asthma-specific parameters.

Briefly, sequencing reads with quality scores are mapped to references using several alignment algorithms. The mapped reads are converted to a format that facilitates anonymization and are then processed through a variety of tools including the assembly and quantification of

transcripts, generation of sequence tracks and annotation. In addition to so-called standard gene annotation, as we performed for the GENCODE project [26], other features such as functional RNA structures can be annotated using our tools [21]. Moreover, this process is iterative, in that the exon transcripts are re-aligned to more accurately quantify different gene isoforms. As the components of RSEQtools can be readily assembled and extended to build customizable RNA-seq workflows, additional components like single cell analysis developed in Aim 2, as well as sample deconvolution developed in Aim 3 can be easily incorporated into the pipeline. This pipeline can be easily ported to the core for the universal processing of the data through Yale's dedicated next-generation sequencing supercomputing cluster, or through the RSEQtools container image suitable to cloud computing.

3.A.iii.b Finding ncRNAs and transcribed pseudogenes

We will utilize a statistical approach that compares the levels of expression in the known exon regions to threshold the RNA-seq signal and identify the intergenic and intronic regions that show significant expression. Next, we will utilize the methods we developed (e.g., incRNA [9]) to further classify and characterize these regions. Specifically, we will use the known coding sequences, UTRs, and non-coding RNAs to train a random forest algorithm and apply the trained algorithm to classify the novel transcript regions to one of the classes. Next, we will assign targets to the classified regions by comparing them both with the annotated cis-regulatory elements (e.g. enhancers) and with proximal genes. We will also utilize statistical methods to identify antisense transcripts that have roles in regulating the overlapping transcript.

We will employ our pipeline to identify the transcriptional activity. The essence of the pipeline is to focus on reads and pseudogene regions that are uniquely mappable for the calculation of RPKM. Given previously published results on human pseudogenes with small-scale validation which imply that ~15% of human pseudogenes are transcribed, we can set an RPKM threshold for human analysis such that it gives an approximate agreement with the previous validation [27].

3.A.iii.c Functional annotation through clustering and network analyses

We aim to develop an asthma resource for identifying novel asthma-related genetic elements. Toward this goal, we will employ various clustering algorithms to group transcripts based on purely the RNA-seq data. The clusters will further be validated using biological features such as sequence similarity, genomic distance, and co-regulation. Moreover, we will attempt to predict biological significance of transcripts from biological associations of the modules (e.g. GO terms). As the functions of protein coding genes are more widely known, we will use such clusters to annotate the functions of novel transcripts such as ncRNAs and potentially functional pseudogenes. The clusters will also be used to relate some of the well-known asthma pathways and modules to other less characterized components. The analysis enables us to explore novel asthma-related elements and to examine the relationship between asthma and other pathways in humans. Apart from clustering data, we will perform bi-clustering to obtain samples/patients clusters. Certain clusters provide another dimension of information. They will be used for annotating other clinical information.

Table 1. Clusters produced by Driving Project 3

Aim	Name	Data	Method	Utility
1	BP	Bulk-cell	by Patient	Novel endotypes of asthma by RNA-seq
	BG	RNA-seq	by Gene	Gene networks for understanding novel endotypes
2	SC	Single-cell	by Cell	Cell signatures by transcription
	SG	RNA-seq	by Gene	Consistent expression between cells
	CC	Single-cell	by Cell	Cell signatures by protein levels
	CPr	CyTOF	by Protein	Signaling network analysis
3	IP	All integrated	by Patient	Novel endotypes of asthma by integrated analysis
	IC		by Cell	Cell type signatures of asthma
	IG		by Gene	Logic modeling for disease mechanism

We plan to extend the OrthoClust framework we developed to compare networks constructed by using samples from patients and samples from control, as well as samples in various cell types. For instance, the quantification on the addition and removal of nodes and edges in cross-species analysis can be easily generalized for comparing signaling pathways for asthma study. Furthermore, as a general formalism, OrthoClust can be used to study specific modules contributed to asthma.

3.B Plan for Aim 2: Single-cell analysis of asthma sputum

3.B.i Rationale

Severe asthma is a heterogeneous disease with multiple underlying molecular mechanisms and endotypes. The manifestation of each endotype is the cumulative result of the coordinated and collective behavior of multiple cell types, leading to the phenotypic symptoms. With single-cell technology we can measure with great

precision the cell types involved in asthmatic response and in the particular modes of signaling employed by these cell types that contribute to the heterogeneity of asthma in patients.

Mutations or expression levels can drive differences in signaling and downstream gene expression in different cell types that can contribute to the overall symptoms of severe asthma. As outlined in Project 2, one relevant pathway in a subset of asthmatic patients may be a Th2 inflammatory response to environmental antigen such as dust mites, that stimulate DKK1, then drives naïve CD4+ T cells towards the Th2 lineage. Th2 cells then secrete IL4, IL5, IL-13 and a variety of pro-inflammatory cytokines that mobilize the response of the immune system, including IL-4-producing follicular T helper cells that produce IgE. Therefore, in depth single cell examination of diverse cell types and their functional responses will provide an in-depth picture of relevant triggers that lead to disease heterogeneity.

In this study, we analyze data generated by Precision Profiling Core C, consisting of high-throughput, multi-dimensional single-cell measurements of gene expression and signaling in sputum cells derived from the airways of patients. By analyzing this mixture of inflammatory and epithelial cell types at the single-cell level we will be able to (1) Dissect the phenotypes of immune and other cell types that are present in cohorts of mild and severe asthmatic patients, with particular power to identify rare phenotypes with large effect; (2) understand signaling logic by utilizing cell-to-cell heterogeneity within each phenotype using single cell functional CyTOF data; and (3) understand gene regulatory network and pathways involved downstream of signaling using single-cell RNA-seq.

Bulk-cell RNA-seq as detailed in Aim 1 identifies gene expression from cell samples, and the single-cell technology proposed here has possibility of uncovering the unique transcriptional program of each cell. This will be particularly powerful for analysis of samples from the same patient by both platforms. Additionally, differences between cells can be informative of the underlying relationship or network between proteins and genes. This gives an understanding of both the heterogeneity that exists within cell populations and the cellular logic that generates the heterogeneity in cellular decision-making. Results from the bulk analysis of Aim 1 can be used to validate the populations and relationships found in Aim 2

3.B.ii Preliminary Data

We have previously developed methods for analyzing single-cell data. Our methods are (1) viSNE which is a dimensionality reduction and visualization algorithm for single-cell data analysis [28], (2) DREMI for quantifying signaling interactions in single-cell data [29], and (3) DREVI for characterizing and visualizing relationships between proteins in signaling networks [29].

One of the advantages of multi-dimensional data is the ability to resolve subtle progression of cell populations within a sample. However, it is hard to directly consider all of the dimensions due to visual and computational problems with high dimensions. For the multidimensional data produced by CyTOF, programs developed for flow cytometry (FlowJo) are not adequate and more advanced software infrastructure is required. Therefore, we developed a dimensionality reduction method known as viSNE [28] that derives an optimal low-dimensional embedding that is able to preserve distances between cells in high-dimensions. This enables the efficient resolution of populations of cells and unsupervised clustering.

We have also developed methods for characterizing signaling in populations of cells. A major problem in quantifying signaling relationships is highly biased sampling arising from many cells (especially immune cells) that either do not respond to stimuli or respond stochastically. In such cases the joint density is very peaked and any statistic that is computed from the joint density considers dense regions to be more important than sparse regions, even though dependencies and signal transfer can only be inferred when looking at the system under a whole range of conditions. Conditional-Density Rescaled Visualization (DREVI) is based on conditional density estimation between the independent and dependent variable and reveals the functional shape of the dependency between molecules as well as the stochastic spread in the function along the full dynamic range of molecular operation. Along with DREVI, we developed an information theoretic dependency metric (conditional-Density Resampled Estimate of Mutual Information) for scoring the strength of relationships based on the conditional probability. With DREVI and DREMI, one can quantitatively determine the strength of information transfer and the functions computed by these networks.

The quantitative, behavioral descriptions offered by DREVI and DREMI allow us to tease out subtly altered signaling functionality in closely related cell types (Th1 vs Th2 CD4+ helper cells) or between distinct cohorts of subjects (mild vs severe asthma). Such differences are important because related cell types often contain similarly wired circuits, which reuse the same molecules but behave phenotypically differently. DREMI and DREVI found differences in activation thresholds and shapes of response functions between the signaling networks of naïve and activated T cells. In comparing signaling between naive and antigen-exposed CD4(+) T lymphocytes, we find that although these two cell subtypes had similarly wired networks, naive cells

transmitted more information along a key signaling cascade than did antigen-exposed cells [20]. These methods were also used to track differences in signaling response between T cells from healthy mice and from non-obese diabetic (NOD) mice, which are prone to developing Type 1 diabetes [30].

3.B.iii Approach

We use two key technologies (1) CyTOF or mass cytometry and (2) Fluidigm C1 microfluidic device for single-cell RNA-seq.

3.B.iii.a CyTOF Analysis

The main aims of CyTOF analysis for asthma sputum samples are (1) Determination of heterogeneous cell subpopulations present in patients, (2) Matching of subpopulations and quantification of heterogeneity between patients, and (3) Characterization of signaling responses by higher-dimensional DREVI with a fuzzy logic model for integration with RNA-seq data.

Determination of cell populations: In order to determine cell types within a sample of single-cells, we propose to utilize our previously developed dimensionality reduction methods in conjunction with newly developed unsupervised clustering. Several unsupervised clustering algorithms have been developed in other fields for tackling related problems. Community detection algorithms from social network research seem particularly promising given their speed and utilization of a cell-similarity graph rather than spatial embedding of the data. Recently, the software tool phenograph [31] was developed which heavily utilizes the Louvain Community detection method to discover immune cell types present in leukemia patients. The Louvain method repeatedly and sequentially merges nodes in a cell-similarity graph based on the increase in a measure known as modularity, which quantifies cluster quality. Preliminary results utilizing Phenograph on this data is shown in Fig 3.

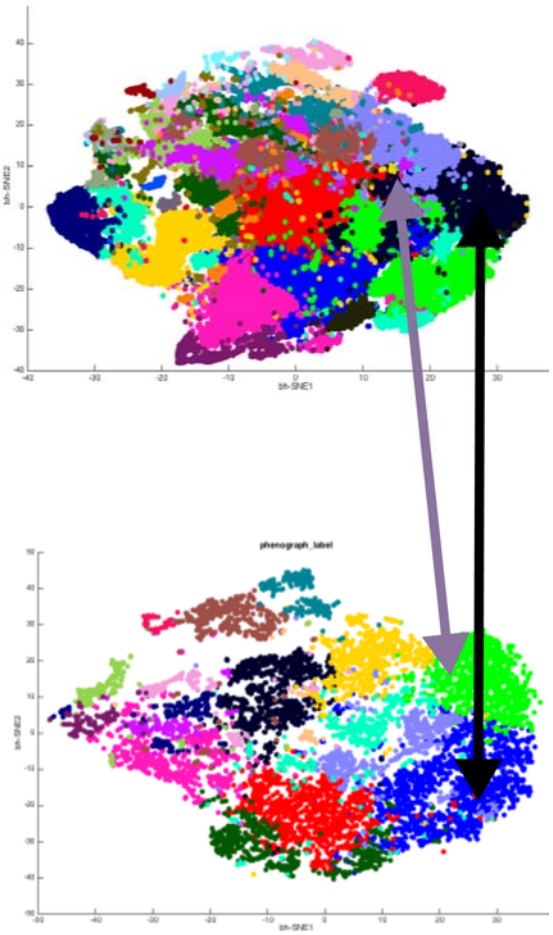

Figure 3. Unsupervised clustering using the Phenograph software on two patient sputum samples. The multidimensional CyTOF measurements are reduced to two dimensions using the tSNE algorithm and each cell is rendered as a point in this space. Additionally, the color given to each point indicates the cluster to which the cell belongs. Two matching clusters are shown using arrows. One of the clusters represents an eosinophil population and another represents a neutrophil population, matched using distribution distances.

Another class of algorithms for unsupervised clustering emerges from literature in VLSI physical placement, where clusters of network elements (logic gates, buffers etcetera) are placed nearby on chips in an attempt to minimize wire length and crowding. Algorithms in this class utilize recursive bisection [32], and spectral methods for clustering [33]} In this project, we will evaluate the robustness of a variety of unsupervised clustering algorithms and utilize the most robust combination of methods to discover novel populations.

3.B.iii.a.1 Subpopulation Characterization and Matching

We propose to find key signaling differences between heterogeneous asthmatic patients and also to identify signaling differences in rare phenotypes to elucidate mechanisms underlying disease and to identify targets for novel therapeutics.

Although Phenograph is able to produce clusters, it does not have the capability of matching clusters between patients in order to find consistently repeating rare cell populations. We propose to develop an approach based on distances between multidimensional distributions in clusters to find matching clusters across individuals with asthma. Each cluster is essentially defined by the multi-dimensional probability density function of its markers. We propose to use kernel density estimation to compute a set of marginal densities and for each cluster in subject X, to find the matching cluster in patient Y by finding the cluster that minimizes the distance between these marginal densities. There are several

methods of computing distances between densities including a simple L1-norm, KL-divergence, as well as Hellinger divergence [34].

3.B.iii.a.2 Analyzing Signaling Relationships in subpopulations

Once the clusters or phenotypes of cells are established, we will gauge signaling response within each cluster with previously developed information theoretic techniques for analyzing signaling interactions, DREMI and DREVI, described in the significance section.

Our goal is to understand how various populations of cells invoke signaling responses to the stimulations detailed in the Precision Profiling Core C. Cells from the sputum of 6 subjects were tested by stimulation with LPS for 6 hours. Future experiments will involve additional types of stimulation such as PMA, and house dust mite antigen. It has been reported previously [35] that triggering the TLR4 receptor on monocytes with its ligand LPS activates several canonical signaling pathways including ERK and NF-KB. Additionally, cells which do not express much TLR also respond, but more slowly with a STAT3 and ITK response. Additional pathways downstream of TLR such as the RIP and TRAF pathways, leading to interferon responses have been reported to be involved. Using an unbiased approach, we will curate a panel of signaling pathways from the results of the bulk-cell RNA-seq data and examine these pathways with a time course. In order to study signal integration along various pathways we will study them using a higher-dimensional extension to DREMI/DREVI. With higher-dimensional DREMI/DREVI we can study how signals from various pathways converge together to form resultant responses in cytokine and transcription factor production. Additionally, higher dimensional DREVI can also be utilized to understand signaling logic.

3.B.i Processing of Single-Cell RNA-Seq Data

Single-cell RNA-seq has the possibility of offering an unbiased view of the pathways that are transcriptionally activated upon immune-system activation at a single-cell resolution, even when cells seem phenotypically similar. However, single-cell sequencing suffers from more technical noise as compared to bulk RNA-seq, arising largely from three sources: (1) sampling inefficiencies which result in only a small fraction of the total number of transcripts being captured; (2) cell-to-cell variations in sequencing efficiency, potentially due to differences in lysis between cells; and (3) amplification bias owing to the small amount of starting material for the RNA-seq. Attempts have been made to address these concerns [36, 37]. However, there is no standard pipeline in place that addresses all of the concerns in going from raw reads from a sequencer (such as the Illumina Hi-Seq) to robust transcript counts. The main steps of such a pipeline, which have been investigated in the literature, include (1) debarcoding and error correction, (2) Aligning reads from each unique molecular identifier (UMI), and (3) quantifying the biological noise in genes.

3.B.i.a Debarcoding and Error Correction

Cell-specific barcodes are the key to identification of the particular collection of transcript sequenced from a single cell. However, these barcodes can be erroneously sequenced, leaving many transcripts unassociated with particular cells. Therefore, an error correction scheme that considers the closest hamming distance barcode from a given barcode could help associate more reads to cells. The design of barcodes with a minimal hamming distance of 3 would allow for the correction of a single error whose probability is estimated by Illumina to be 10^{-6}.

3.B.i.b Aligning Reads from each UMI

After splitting reads into their cell of origin, reads can be further divided into their molecules of origin using the unique molecular identifier or UMI tag, as has been performed for Fluidigm data of this type [38]. Similar to the barcode, the UMI is sequenced along with the read. UMIs are essential to both controlling bias and in identifying the closest element of the transcriptome. Previous works tend to have very specific recommendations for processing the sequencing such as excluding reads mapping to 400 base pairs distance from end of transcript, or identify a minimal set of genes that explain all reads using the hitting set problem [39]. However, this type of highly deterministic procedure with many thresholds is unlikely to yield good results in all situations. Furthermore, the reason for minimizing the set of genes to explain all reads is unclear and could end up missing many valid alignments. Therefore, we propose to develop an alternative, probabilistic procedure where each gene is given a probabilistic alignability score that represents how well the collection of reads align to the particular gene. For each read *r,* the probabilistic score incorporates (1) Pt: How far the read aligns from the end of the transcript, with genes aligning close to the end having a high distribution, modeled as a skewed lognormal distribution; and (2) Pg: How many other genes the read itself aligns to, which is a distribution peaking at 0 with a thin tail such as a Gaussian distribution. A starting probabilistic score could be $\Sigma\, PtPg$ the sum of the product of the values for every read that aligns to the gene. This score would need to be optimized as we generate more data. The UMI would then be assigned to the gene that explains the set with the highest probability.

3.B.i.c Quantifying the biological noise in genes

Quantifying the biological noise of each gene involves separating components for technical variation from biological variation in gene abundances. There are generally thought to be two sources of technical variation.

3.B.i.c.1 Cell-to-cell variability in RNA-seq efficiency

This essentially means that many RNA molecules are captured from some cells whereas few are captured from other cells. Therefore, transcript abundances are sensitive to variations to changes in sequencing efficiency resulting from processing steps such as lysis efficiency. Therefore, normalizing by the library size or total number of transcripts sequenced from a cell can mitigate this type of variation to some extent. However, we wish to explore a generalized linear model to regress the library size variation against each gene individually, in order to normalize scores in a manner robust to outlier genes that are highly expressed.

3.B.i.c.2. RNA sampling from cells

Previous work has quantified the fraction of transcripts that are sequenced using ERCC spike-ins and found the efficiency to be about 3.6%. Grun et al. reported that this sampling probability is distributed such that the variance is equal to the mean of the distribution and therefore can be described as a Poisson distribution [36]. If the complete variation in measured gene expression is due to Poisson sampling then the Fano factor of the gene expression should be equal to 1; higher Fano factors indicate the presence of actual biological variability rather than simply technical variability. Therefore, the amount of information in each gene measurement can be quantified by its fano factor and utilized in selecting genes to analyze.

3.B.ii Single-cell RNA-seq Analysis

After the pipeline steps are completed then we can analyze asthma phenotypes, endotypes, and gene-gene interactions in a similar way as we analyzed CyTOF data. However, one of the keys to successfully extracting information from single-cell RNA-seq data is to be able to use the high-dimensionality of the data to bolster individual (especially low-abundance) gene dimensions that can suffer from dropout. We propose the following steps in order to be able to analyze and cluster single-cell RNA-seq data: (1) use non-linear dimensionality reduction and clustering on genes to form meta-genes, (2) value-impute based on cell clusters and meta-genes, and (3) use the value-imputed data to study gene-gene interactions

3.B.ii.a Non-linear dimensionality-reduction and clustering

Some genes are naturally expressed at low abundances and these can be especially affected by the Poisson sampling process by which RNA is captured from single cells. However, since single-cell RNA-seq data involves measuring thousands of gene dimensions, it is possible to impute values for dropout dimensions using information from a combination of higher-fidelity dimensions. In order to tackle this problem, we propose to reduce the number of dimensions non-linearly by utilizing a method such as bh-SNE [40, 41] or non-linear PCA [42]. After this reduction, we will cluster genes based on the dimensionality-reduced embedding of each cell. We call the resultant cell groupings metagenes. Such metagenes may represent pathways or other functional groupings, which can be examined by enrichment analysis.

3.B.ii.b Cell clustering and value imputation based on meta-genes

Once meta-genes are derived cells can be clustered based on the average expression of meta-genes. Each meta-gene is essentially a cluster of genes that have similar co-occurrences in the population of cells. Therefore, we can use cell clusters derived from meta-genes in order to impute missing values for low-abundance genes. If a cell expresses many members of a metagene, then it can infer a missing value for a gene within the meta-gene by taking a weighted average of cells in its cluster.

3.B.ii.c Use the value imputed data to study gene-gene interactions through DREMI

Once values are imputed into the cell-gene matrix, then it becomes possible to study pairwise gene-gene interaction strengths once again using techniques such as DREMI. We propose to study pairwise DREMI on all pairs of genes exhaustively to derive a gene-gene DREMI matrix. This is essentially an adjacency matrix where the similarity is defined by the mutual information metric DREMI. Next this adjacency matrix can be utilized in graphical or spectral clustering to discover gene modules or pathways through which information is flowing. Note that this is different from the meta-genes because the genes along mutually informative pathways need not have similar expression across cells, they must simply be mutually informative or predictive of one another under probabilistic analysis. In this way we hope to discover new gene-modules or pathways that may be characteristic of cell-subpopulations in asthma patients. These modules can form the basis for additional CyTOF experimentation to discover how signaling is processed along new pathways that have not been studied extensively, and makes for an iterative approach to deepening understanding of the molecular mechanisms underlying asthma heterogeneity.

3.C Plan for Aim 3: Integrative clustering and the creation of the AsthmaMAP

3.C.i Rationale

Each of the methods and data types described in aims 1 and 2 yields unique and valuable information about the biological heterogeneity of asthma. Transforming these analyses into knowledge that can affect patient care requires integrating the data so that each's lessons can be applied to the larger problem. We will integrate the analyses of bulk RNA-seq, single cell RNA-seq and CyTOF measurements with clinical data from Core B to model asthma heterogeneity. This will define the data that best correspond to clinical endotypes in a way that identifies the relevant pathways and identifies potential therapeutic targets.

3.C.ii Preliminary Results

3.C.ii.a Data integration through logical models to characterize clusters

Gene expression is controlled by various gene regulatory factors. Those factors work cooperatively forming a complex regulatory logical circuit on a genome wide scale. Recently, an increasing amount of next generation sequencing data provides great resources to study regulatory activity, so it is possible to go beyond this and systematically study regulatory circuits in terms of logic elements. To this end, we developed Loregic, a computational method integrating gene expression and regulatory network data, to characterize the cooperativity of regulatory factors. Loregic uses all 16 possible two-input-one-output logic gates (e.g. AND or XOR) to describe triplets of two factors regulating a common target [43]. We attempt to find the gate that best matches each triplet's observed gene expression pattern across many conditions. In Loreigc, we also developed a consistency score based on Laplace's rule of succession and permutation test to measure how a triplet is consistent with a logic gate. We made Loregic available as a general-purpose tool [44]. We validated it with known yeast transcription-factor knockout experiments and were able to use human ENCODE ChIP-Seq and TCGA RNA-seq data to demonstrate how Loregic characterizes complex circuits involving both proximally and distally regulating transcription factors (TFs) and also miRNAs in human cancer. In addition, we inter-related Loregic's gate logic with other aspects of regulation, such as indirect binding via protein-protein interactions, feed-forward loop motifs and global regulatory hierarchy. Besides the regulatory logics, we also developed continuous model-based approaches such as DREISS for dynamics of gene expression driven by external and internal regulatory modules based on state space model to help dissect the temporal dynamic effects of different regulatory subsystems on gene expression [45, 46]. This will be the first analysis of this kind to address heterogeneity of asthma endotypes.

3.C.ii.b Further experience developing statistical models of data integration

In addition to integrating RNA-seq and regulatory network data, as described above, we have experience integrating other diverse data types and applying rigorous statistical methods to these novel pairings. For example, we have developed methods to integrate interrelated components that regulate the transcriptional output of a gene, namely transcription factors (TF) and histone modifications [19, 47, 48, 49, 49, 50, 50, 51]. Integrating these models demonstrated a common regulatory mechanism between coding and non-coding genes and determined that the functions of histone modifications are conserved across a broad phylogenetic space [10].

In addition to integrating RNA and DNA sequencing data, we have experience integrating mass spectrometry data for both metabolites and proteins. For example, we integrated RNA-seq and gas-chromatography mass spectrometry data to predict a novel biosynthetic pathway for the biofuel-producing fungus *Ascocoryne sarcoides* [52]. We have have also integrated peptide and protein datasets to predict complex binding dynamics for clinical peptide vaccine search and design using a novel machine learning algorithm that relies on high-order neural networks [53].

3.C.iii Approach

3.C.iii.a Interrelation with external datasets

There are several big-data projects relevant to the analysis and interpretation of the bulk-cell and single-cell RNA-seq data and their interrelation with CyTOF measurements. For example, the NIH Genotype-Tissue Expression Project (GTEx) [54] has tissue-specific transcription data, including lung, which can be used to infer aberrant transcription in the asthma disease states. Data from the ENCODE project (https://genome.ucsc.edu/ENCODE/), particularly the ChIP-Seq data, will give a regulatory framework into which the asthma data can be mapped. We have experience integrating ENCODE data into regulatory networks [17] and studying the impact of transcription factor binding and histone modifications on gene expression [51]. We will leverage this to embed transcripts into cellular regulatory networks and to provide the context needed to understand the role they may play in intercellular signaling. After that, we will identify the key transcripts with high network centralities, and try to predict their functions using "guilt-by-association" with their neighbors.

Besides ENCODE, several other large consortia are generating data systematically across the human genome, resulting in a wealth of functional information of great value to RNA-seq integrative analyses. The Epigenomics Roadmap Project and the International Human Epigenome Consortium have generated rich maps of histone modifications, including deep maps of more than 20 modifications in a small number of cell lines, maps of a few modifications in a large number of cell types, as well as maps of DNA methylation and DNA accessibility. Over 1,200 data samples from primary tissues have been collected and analyzed by GTEx and by integrating the transcripts with the Human Epigenome Atlas we will examine potential effects of a transcript on chromatin modifications in target cells. This is particularly important for those lncRNAs known to regulate histone marks such as H3K27me3 and H3K9me3 through interactions with the members of the Polycomb complex.

Other sources of complementary, large-scale human data include: the NIMH Brainspan Project, the 1000 Genomes Project, and the NCI Cancer Genome Atlas (TCGA) Project. The DOE kbase (of which we are members) [55] provides new genomic toolsets that we will harness. These resources will permit rapid analysis of the airway signaling landscape and provide valuable detailed understanding of factors contributing to asthma heterogeneity.

3.C.iii.b Deconvolution of cell-type signatures from bulk RNA-seq data

In this aim, we want to identify the cell type signatures in terms of gene expression, and find the gene biomarkers from the signatures that can most discriminate asthma patients; e.g., different TEA clusters. We assume that the mixed effects from various related cell types determine the gene expression from each patient's sputum; i.e., mixtures of various cell type signatures. We then try to use both linear and nonlinear approaches to capture the mixed effects as follows.

We first try the linear models that will be computationally efficient. Given the gene expression levels and cell type fractions for each patient, we can use a linear matrix model to identify cell types gene expression signatures. For instance, the patient's *i*th gene expression level can be modeled as a linear superposition of the same gene's expression levels of multiple cell type signatures; i.e., the *i*th gene expression level of *k*th individual person, $x(i,k)$ is the linear combination of this gene's expression levels of different cell type signatures; i.e., $x(i,k)=\sum_{j=1}^{m} w(j,k)s(i,j)$, where $s(i,j)$ is the *i*th gene's expression level in the *j*th cell type, and $w(i,k)$ is the contributing weight of *j*th cell type to *k*th person, which can be the *j*th cell type fraction of *k*th person. If we rewrite this linear model in a matrix form, we have that $X=SW$, where X is the gene expression matrix whose the rows and columns represent genes and persons, W is the cell type fraction matrix whose rows and columns represent cell types and persons, and S is the cell type signature matrix whose the rows and columns represent genes and cell types. The single-cell RNA-seq data described in Aim 2 will yield counts of different cell types, providing the data required for matrix W. The bulk RNA-seq data provided by Precision Profiling Core C after being processed by the pipelines developed in Aim 1 will provide matrix X, so we need to find the optimal S to

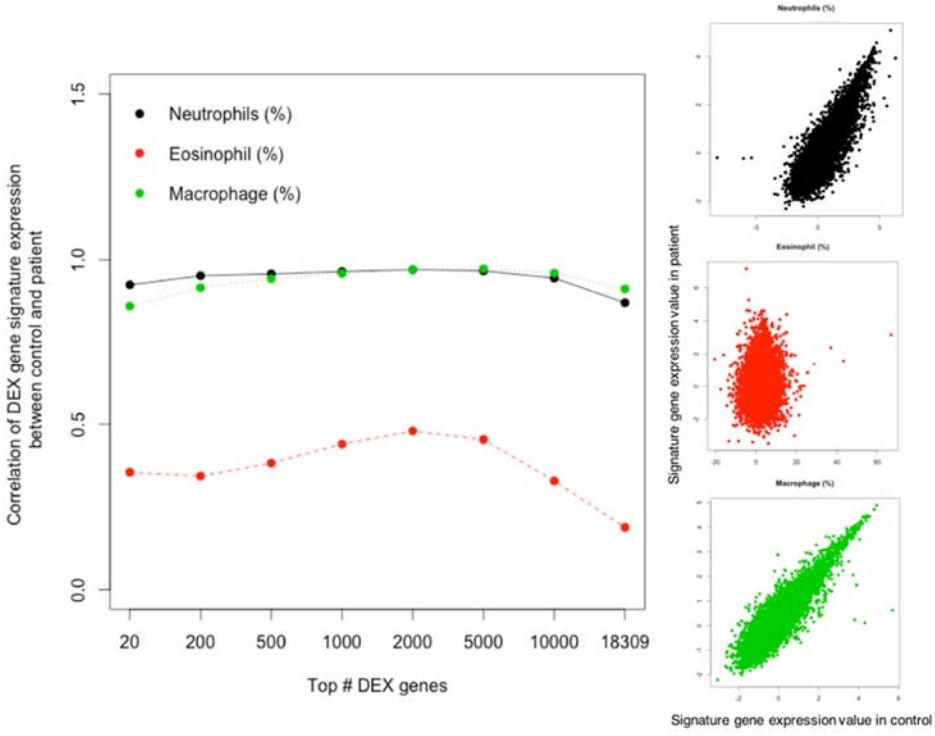

Figure 4. Gene expression signatures of three major cell types in asthma and control. Left, cell type signature correlation between asthma and control for top genes that have highly differential expression levels between asthma and control. Right, all gene expression levels in cell type signatures (x-axis: control, y-axis: asthma). Cell population percentages were obtained by cell staining and light microscopy.

minimize $||X-SW||_F$ given X and W. The optimal solution $S=XW^*$, where W^* is pseudo inverse of W s.t., $WW^*=I$ identity matrix.

We then try to apply advanced models to capture nonlinear effects from different cells to gene expression. For example, we can use machine-learning methods to investigate the gene markers from cell type gene expression signatures for both bulk data and single-cell type. In particular, we would like to use the Denoising Autoencoder (DA), an unsupervised machine-learning framework to extract and characterize cell type signatures. DA is able to discover non-linear expression features from gene expression data using sigmoid transformation. We will apply DA to different patients clusters and compare their non-linear features, and find the genes that have features to most discriminate clusters.

These methods will be compiled into a cell-type signature pipeline that will be distributed to the other Driving Projects for determining the relative fractions of cell-type expression from bulk RNA-seq data. This will be applied to the novel clusters produced in Aim 1 to elucidate the effect of cell-specific transcription in driving the clustering of different samples. Moreover, we will apply this pipeline to established clustering methods, such as TEA clusters, to observe cell type signatures in these contexts. We integrate the analyses of these different clustering methods to identify the cell and gene specific biomarkers that most discriminate clusters.

3.C.iii.c Identification of clinical and CyTOF features of clusters and cell type signatures

Clinical information can be used to classify endotypes of asthma and provide valuable guidelines for diagnosis. Some features like FEV1/FVC have been widely used in endotype clustering. However, the quantitative link between the gene clusters and clinical variables is largely unknown. As some clinical variables represent a certain kind of phenotype of asthma and are reflected by distinct syndromes, distinguishing genes or pathways associated with distinct clinical features may identify targets for novel therapeutic approaches. We will build a regression or classification model using highly scored gene signatures, submodules and pathways in different clusters as the predictor, and clinical information as the target. By means of information gain or gini index, we will characterize the highest associated factors for each clinical phenotype. Finally, we will build a functional representation cluster of clinical variables.

In collaboration with Project 2, three asthma associated pathways will be used to validate and extend the cell responses and gene signatures characterized by CyTOF and RNA-seq. We will begin with the experimentally validated pathways and expand to the whole network, using belief propagation based on experimental results to update and optimize the weight between gene-gene interaction edges. We will apply the Orthoclust framework to identify common and specific regulation or signaling pathways for different cell types and endotypes. Specific modules in signaling response pathways from CyTOF and logic gate analysis will address the dynamic regulation and cascaded signaling transduction in asthma heterogeneity.

3.C.iii.d Logical model-building

In addition to identification of clusters as described above, we will also explore the gene regulatory mechanisms and signaling, which follows certain logics to give rise the phenotypes of these clusters. The gene regulatory factors cooperate to facilitate the correct function. If their cooperation is disrupted, it can cause abnormal gene expression such as those present in asthma. In many cases, the regulatory factors controlling gene expression behave in a discrete fashion and can be modeled using computationally efficient Boolean logical models [56, 57, 58, 59, 60, 61]. Therefore, we will develop computational algorithms based on Boolean models to study the cooperative logics between various regulatory factors.

First, we will obtain TF-gene regulatory and signaling relationships by integrating data on TF binding from the asthma-related cell types such as eosinophils, lymphocytes, and neutrophils from ENCODE and Epigenomics Roadmaps projects and the signaling information from the CyTOF data. Second, given a cluster, we want to identify the regulatory logics among TFs to drive the cluster's expression. We will use regulatory relationships and binarized gene expression datasets across cluster's patients. The binarized gene expression data (on=1 and off=0) is the direct result of the regulatory factors activity on the target genes. Our study will look at regulatory modules; e.g., the simple triplets consisting of two regulatory factors (RFs) and a common target gene T. The main idea is to describe each module using a particular type of logic gate that best matches the binarized expression data for that triplet across all samples. For example, the triplet (RF1, RF2, T) follows an AND logic; i.e., both RF1 and RF2 need to express high to turn on the gene T. Moreover, we will find the logic-circuit behaviors such as cascaded gates for the regulatory pathways. After finding the regulatory logics for different clusters, we will compare them and find the cluster's specific logics. For example, (RF1, RF2, T) may follow AND logic in severe patients, but OR logic in mild patients. We will also compare logical behaviors of pathways across clusters. These studies may predict solutions that guide in vitro studies such as gene knockdowns to modulate the regulatory logics. Finally, we will develop a pipeline for this logical analysis, which will output the regulatory logics to characterize the clusters.

4. Interactions and Deliverables

4.A Interactions with the other members of this U19 Cooperative Proposal

This research will include extensive interaction and collaboration with the other members of this U19 proposal (Fig 1). In our first two aims we will be working closely with the Precision Profiling Core C to generate a processing pipelines for the bulk-RNA-seq, single cell RNA-seq and CyTOF data. These pipelines will be given to the core for implementation, which they will then use to distribute data to all three Driving Projects.

Our final aim will generate a model that will both inform the other driving projects use data from them. For example, Project 1 Aim 3 will use the IP-clusters from Aim 3 to determine cell activities in stimulation assays, and Project 2B will be informed by our logic gate regulation of proteins such as DKK1. We will use the data generated from Project 2C's patients' microbiomes with information about which organisms are coated with IgA. One approach to integrating these data with RNA-seq is to use the microbiome patient clusters as seeds for our IG-clusters, which will reveal how patient groups separated by their microbiomes are responding differently in their transcriptional activities. These findings will be communicated in monthly meetings of the group and more frequent interactions between subgroups and will form the basis for iterative investigation.

4.B Deliverables

The deliverables from this project will be clinically informative clusters of genes, cells and patients that characterize different asthma phenotypes. These clusters, detailed below, are described in Table 1 and speak to a variety of hypotheses from ours and the other projects. The tools and results will be made available to the other members of this project and the research community in a publicly accessible, searchable, integrated ASTHMA MAP website (http://ASTHMAMAP.gersteinlab.org), as we have done for other multi-investigator research efforts [62]. This website will serve as a repository for the pipelines, derived datasets and analyses that are the deliverables from each aim of this research proposal, in addition to the utilization of NIH-sponsored hosts such as Immport.

Aim 1 will produce the pipeline for the processing of bulk RNA-seq data which will be delivered to Core C for execution and made available to the research community. This process will include detailed annotation of transcripts including structural information, ncRNAs and psuedogenes. We will then take these rigorously and uniformly processed data and from Core C and generate BP and BG-clusters using global transcription and co-expression of the genes, respectively. Non-coding RNAs, psuedogenes and other transcripts will be mapped onto BG clusters to suggest possible functions. This unrefined clustering will speak to the global transcriptional activity of the sputum and will be the framework refined by integrating other methods. The BP-clusters, where each patient is clustered by his or her global transcription, will define asthma endotypes similarly to methods used to generate TEA clusters in previous reports and will speak to whether the response from RNA-seq is similar to previous work using microarrays.

Aim 2 will produce software and pipelines for the analysis of both CyTOF and single-cell RNA-seq data as well as results of the analysis of data generated by the Precision Profiling Core C. Each data type will be used to generate clusters by cell signatures and gene networks. The CyTOF analysis will generate CC-clusters from unsupervised clustering of surface markers. These clusters will define the cell population with a new level of precision, including stratifying lymphocytes into component cells types including Th2 and Tfh cells, as is being explored in detail by Project 2. We will produce a method that identifies these subpopulations of cells and tracks changes in their abundances across patients and across the longitudinal sampling of individuals. Moreover, the CyTOF data will be used to generate CPr-clusters, which will define signaling interactions in and between cells using DREVI and fuzzy logic methods.

The single-cell RNA-seq data processing will include debarcoding, quantifying noise, and imputing missing values from low abundance genes. The processed data will deliver SC-clusters that define subpopulations of cells by their transcriptional activities. At the gene level, SG-clusters will identify co-expression networks within specific cell types and generate an output of a DREMI analysis of gene-gene interactions and resultant gene modules.

Aim 3 will integrate the above data with clinical data from Core B, the data generated by other Driving Projects and external datasets to produce integrative clusters by patients, cells and genes (IP, IC and IG-clusters). Bulk-cell RNA-seq data that has been deconvolved with single-cell RNA-seq and CyTOF cell signature data will be merged with other data such as Driving Project 2C's microbiome clusters to yield Patient-level IP-clusters to define novel asthma endotypes. The cell-level IC-clusters will show the populations of cells that are important for disease. Gene-level IG-clusters will define the mechanisms by which those cell populations are different. Specifically, we will produce logic gate models for the different IG-clusters to define the regulatory logic in each asthma subpopulation and identify the optimum targets for intervention.

Protection of Human Subjects

Risks to Human Subjects

a. Human Subjects Involvement and Characteristics. Over the course of this project, we plan to recruit 200 asthmatic and 50 non-asthmatic subjects through the Yale Center for Asthma and Airway Diseases (YCAAD). Each subject will return approximately every 9 months and complete 3 visits over the 5-year grant period. At each of the 3 visits the subject will be administered a questionnaire and perform physiological testing, and blood and sputum samples will be obtained. Asthmatic and non-asthmatic subjects recruited for these studies will be 12 years old or greater. Through a distinct HIC protocol, for these studies we will also recruit asthmatic and non-asthmatic children (age 7-17 years old) undergoing adenotonsillectomy, to donate tonsillar tissue, blood samples, and nasal brushings to the YCAAD biorepository.

Drs. Cohn and Chupp have recruited subjects for the past 15 years through the *Mechanisms and Mediators of Asthma and Chronic Obstructive Pulmonary Disease (COPD) human investigation* protocol (Yale HIC 0102012268, PI Chupp, Co-I Cohn). Over 800 subjects have been recruited to date. Tonsillar tissue will be obtained as part of the *Correlation of Gene Expression Profile with Clinical Response to Adenotonsillectomy* protocol (Yale HIC 1009007345, PI Karas, Co-I Chupp). This is a repository is to investigate the relationship between adenotonsillar hypertrophy and asthma and airway diseases, and identify genes, molecules and pathways that are associated with disease development, progression, and severity.

YCAAD Study Protocol: Exclusions will be based on health considerations. We will exclude any patient with a contraindication to any of the required testing including sputum induction, spirometry, or phlebotomy. These exclusions include severe cardiovascular, renal, or liver disease, bleeding diathesis, or advanced neuromuscular disease. A centerpiece of the YCAAD translational research program and this proposal is the airways disease phenotyping protocol developed to study human asthma. This protocol has been developed to acquire the full complement of clinical information, physiologic measurements and biologic samples to perform genotype-phenotype studies. Following informed consent, subjects are administered a questionnaire by the study coordinator. This includes a detailed asthma and medical history, asthma activity questionnaire (Asthma Control Test), Asthma Quality of Life Questionnaire, and details about medications, histories of smoking, occupational and environmental exposures, history of allergy, sinus disease, gastroesophageal reflux, obesity, and medication compliance. All data is uploaded into a HIPAA compliant web-based database developed by YCAAD. Blood is drawn and processed for the isolation of DNA, RNA, serum and plasma. Samples are aliquoted and banked for future studies. Blood is sent to the clinical laboratory for CBC and differential, serum IgE level, ImmunoCap testing for 22 common aeroallergens and routine chemistries. Following phlebotomy, patients undergo FeNO testing and spirometry (in adherence with ATS guidelines) before and after short acting bronchodilator administration. Sputum induction is done using hypertonic saline and immediately processed. Mucus plugs are removed using a dissecting microscope to remove squamous cell contamination, the cellular and aqueous compartments are separated, cell counts and differentials are quantified, and aliquots of supernatants, cell protein, and cellular RNA are stored for later analysis. The protocol is practical, efficient, and enrolls large numbers of subjects in a timely fashion. We will phenotype up to 5 subjects weekly. Subjects will be scheduled to return for repeat sampling as described, which is important for determination of the stability of their disease and their biological samples.

Asthma Inclusion criteria. Recruitment through YCAAD is designed to enroll a wide-range of asthmatic subjects in terms of age, disease severity and ongoing treatment. Inclusion criteria for asthmatic subjects are: (1) ≥ 12 years of age; (2) < 10 pack years of tobacco, and have not smoked for ≥ 1 year. Asthmatic subjects must have a diagnosis of asthma defined by the following criteria (based on the National Asthma and Education and Prevention Panel Guidelines): (1) a history of chronic symptoms (>2 years) compatible with asthma (determined by a study investigator/pulmonary physician specializing in asthma); and (2) historical evidence of variable airflow obstruction determine by: (a) > 12% improvement in FEV_1 after an inhaled $beta_2$ agonist, inhaled corticosteroids, or an oral prednisone burst; (b) methacholine reactivity with a provocative concentration causing a 20% decrease in FEV_1(PC20) of <8 mg/ml; or (c) 20% diurnal variation of peak expiratory flow rates on 2 days over a 2-3 week period. Patients will be excluded if they cannot safely undergo the studies required for participation or have other chronic lung disease or asthma variant disease (ABPA, Churg-Strauss Syndrome, vocal cord dysfunction, etc.). Normal subjects must have no history of any chronic lung disease. Non-asthmatic control subjects will be excluded if they have a diagnosis of any ongoing acute or chronic lung disease, have smoked ≥10 pack-years or have abnormal spirometry.

Adenotonsillectomy Study Protocol. Parents and patients identified by providers as potential subjects will be

invited to participate in the research study after they have been consented independently for their planned procedure of adenotonsillectomy. The parent and child will complete a YCAAD pediatric questionnaire and an asthma activity score, and spirometry will be done for those children aged six years or older who are capable of completing the test. Intraoperatively, a venous blood sample (5-10ml) and nasal swab sample will be obtained. After a portion of the tissue is sent to surgical pathology, as is standard for adenotonsillectomy, a portion of the removed tonsils and/or adenoid tissue will be taken for study purposes. An additional specimen will be held in the pathology department, flash frozen in OCT in the event more tissue is needed for analysis. Samples will be processed and banked with the assigned de-identified study subject number. A second study visit will take place six months following surgery and includes an interim history using the follow-up questionnaire focused on stability of upper airway and asthma symptoms. Repeat PFT testing will be performed in children aged six years or older, if possible. An additional venous blood sample will be obtained for the repository. Subjects may decline any of the testing at any of these visits.

b. Sources of Materials. In this proposal, the following materials will be collected from human subjects: clinical data (questionnaire, history and physical examination data, lung function data and relevant laboratory test results), blood, sputum, nasal brushings and tonsils/adenoids. Clinical data will be identified with the study subject's name. This information will be maintained in a locked file and these records are kept separate from other clinical records to assure confidentiality. Only Drs. Cohn, Chupp, Niu, and study coordinators will have access to these confidential records. All research specimens, including blood, and sputum specimens are identified by a patient code for correlation purposes. All samples and datasets will be de-identified and only known to study investigators involved in consent and sample acquisition. Investigators and staff will receive only de-identified data and samples for their studies and analyses. All data sets uploaded and samples delivered to Core C and the Yale Center for Genomic Analysis will be de-identified.

The studies in this proposal will adhere to the U.S. Department of Health and Human Services Office for the Protection from Research Risk (OPRR). All information relating to participating subjects will be de-identified. Specimens may only be used in accordance with the conditions stipulated in the Yale Human Investigation Committee (HIC) (see approval letters in Appendix).

c. Potential Risks. Risks associated with the YCAAD study (HIC 0102012268) including pulmonary function tests, blood draw and sputum induction are minimal (adults) and greater than minimal (children 12-17 years old) and are explained to subjects in detail at the time of consent (see consent forms in Appendix). Bronchospasm is a risk of sputum induction, but the safety of sputum induction in asthmatic subjects, even those with severe asthma has been well documented in several studies (ref). Spirometry is repeatedly tested during the procedure to monitor for bronchospasm, and if wheezing or a significant drop in FEV1 occurs, subjects are given bronchodilators. Wheezing, if it occurs, is usually transient and treatable with bronchodilators. A Data and Safety Monitoring (DSM) Plan and DSM Committee are in place to assess adverse events (see Appendix). To date, we have had no serious adverse events in our laboratory, even in severe asthma subjects undergoing sputum induction. Clinical Recruitment and Biostatistics Core Lead (Dr. Cohn) or a research coordinator will monitor all procedures performed. After any procedure performed in YCAAD, subjects are instructed to contact the YCAAD on-call physician if any problem occurs. YCAAD is covered 24 x 7 by pulmonary physicians who are familiar with the management of acute bronchospasm in asthmatic patients. Risks associated with the adenotonsillectomy study (Yale HIC 1009007345) are minimal for the blood draw and there is no added risk of apportioning adenoid and tonsil tissue to the biorepository. Emergency equipment and personnel are immediately available in the event of an adverse reaction at all times during these study visits.

Adequacy of Protection Against Risks

a. Recruitment and Informed Consent. Subjects will be recruited from YCAAD and from the pediatric ENT offices of Dr. Karas. Once a subject is deemed eligible for this study, informed consent will be obtained. The study procedures, risks and benefits will be clearly explained to each subject. All questions will be answered, and time will be allowed for the subject to make an informed decision before signing the consent form. If the subject agrees to enroll, a consent form (see Appendix) will be reviewed and explained, and the subject or consenting adult will be asked to sign it.

b. Protections Against Risk. Risks will be minimized by appropriate subject exclusions, close medical supervision throughout the protocol, and adaptation of testing to identify patients who might be at higher risk. Subjects experiencing adverse events will have access to full inpatient medical facilities of the Yale-New

Haven Hospital if more intensive treatment is necessary to reverse an asthma attack or complication during the study. All patients will be under full-time coordinator supervision throughout every phase of the YCAAD procedures. In the adenotonsillectomy protocol, at least one physician and/or nurse will be present throughout the study visits and procedures. Adverse events will be reported to the Yale IRB (Human Investigation Committee) and the DSMC.

Potential Benefits of the Proposed Research to Human Subjects and Importance of the Knowledge to be Gained

These studies are not designed to provide direct benefit to the participants. However, the knowledge gained from this work will enhance information about the pathogenesis of asthma and may lead to improved diagnosis, treatment and prevention of asthma in the future. All subjects receive asthma education and copies of blood work and lung function tests as part of their visit, and have full access to a YCAAD physician, if desired. If any major test abnormalities are encountered, YCAAD physicians counsel the subjects and provide referrals to appropriate specialists as needed.

Inclusion of Women and Minorities

Subjects will be recruited through the Clinical Recruitment and Biostatistics Core (Core B) and through the office of Dr. Karas for adenotonsillectomy without any bias of race or gender. The YCAAD asthma and control populations and the children undergoing adenotonsillectomy are racially diverse and reflect the population in New Haven and surrounding communities. All minority groups already take part in these research programs and we will continue to actively recruit women and minorities as these studies go forward. Based on demographics from past studies, the asthmatic population in YCAAD is approximately 71% female and 31% Black and Hispanic. The demographics of the adenotonsillectomy population is approximately 37% female, 14% Black and 36% Hispanic.

OMB Number: 0925-0002

Planned Enrollment Report

Study Title: NextGen Biorepository Study

Domestic/Foreign: Domestic

Comments:

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/Alaska Native	0	0	0	0	**0**
Asian	3	2	0	0	**5**
Native Hawaiian or Other Pacific Islander	0	0	0	0	**0**
Black or African American	20	18	8	8	**54**
White	85	84	12	10	**191**
More than One Race	0	0	0	0	**0**
Total	**108**	**104**	**20**	**18**	**250**

Study 1 of 2

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

OMB Number: 0925-0002

Planned Enrollment Report

Study Title: Adenotonsillectomy Biorepository Study

Domestic/Foreign: Domestic

Comments:

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/Alaska Native	0	0	0	0	**0**
Asian	2	2	0	0	**4**
Native Hawaiian or Other Pacific Islander	0	0	0	0	**0**
Black or African American	12	10	6	4	**32**
White	28	22	8	6	**64**
More than One Race	0	0	0	0	**0**
Total	**42**	**34**	**14**	**10**	**100**

Study 2 of 2

Funding Opportunity Number: RFA-AI-15-032. Received Date: 2015-10-01T14:26:34.000-04:00

Inclusion of Children

The proposal will recruit children between the ages of 2 and 21 years (consent and assent form are included in Appendix 1 and 2). The investigative team for Yale #HIC0102012268 will request that the participants (12-21 years old) undergo phlebotomy, sputum induction, lung function testing and complete an asthma questionnaire with the subject and his/her parent/legal guardian. Venipuncture and pulmonary function testing and questioning are part of the routine care of children with asthma. Sputum induction is a low-risk procedure and is outlined in the Appendix. All interviews and procedures will be performed in the Yale Center for Asthma and Airways Disease. Overall, the study poses more than a minimal risk to minor subjects, but its benefits outweigh these risks. The investigative team for Yale # HIC1009007345 will request that the participant (2-18 years old) who was previously scheduled to undergo adenotonsillectomy, consent to have blood drawn intraoperatively, perform spirometry before surgery, if capable, and agree to donate excess tonsil tissue to the biorepository for analyses. These procedures are assessed to be of minimal risk to child subjects. The investigative team has experience caring for children should any adverse events arise during sample acquisition.

Inclusion of children in this study is important since we are interested in the relationship of transcriptomic endophenotypes across the spectrum of asthma. Our studies thus far suggest that there are stable Transcriptional Endophenotypes of Asthma (TEA) in children and adults with severe asthma and, in particular, near fatal asthma. Endotypes seen in adult asthmatics may be evident in young subjects, and this may help us predict the course of disease as they age. Further, we may be able to discern which patients will benefit from more aggressive care and elucidate molecular pathways involved in the pathogenesis of severe asthma. We will follow subjects longitudinally and will define how transcriptomic signatures relate with progression of this disease.

References Cited

1. Moorman, J. E., Zahran, H., Truman, B. I., Molla, M. T., & Centers for Disease Control and Prevention (CDC) (2011) Current asthma prevalence - United States, 2006-2008. *MMWR Surveill Summ* 60 Suppl, 84-6.
2. Sboner, A., Habegger, L., Pflueger, D., Terry, S., Chen, D. Z., Rozowsky, J. S., Tewari, A. K., Kitabayashi, N., Moss, B. J., Chee, M. S., Demichelis, F., Rubin, M. A., & Gerstein, M. B. (2010) FusionSeq: a modular framework for finding gene fusions by analyzing paired-end RNA-sequencing data.. *Genome Biol* 11, R104.
3. Du, J., Leng, J., Habegger, L., Sboner, A., McDermott, D., & Gerstein, M. (2012) IQSeq: integrated isoform quantification analysis based on next-generation sequencing.. *PLoS One* 7, e29175.
4. Habegger, L. (2012) Computational methodologies for transcript analysis in the age of next-generation DNA sequencing. *Thesis (Ph. D.)--Yale University, 2012.* , .
5. Langmead, B., Trapnell, C., Pop, M., & Salzberg, S. L. (2009) Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol* 10, R25.
6. Trapnell, C., Pachter, L., & Salzberg, S. L. (2009) TopHat: discovering splice junctions with RNA-Seq. *Bioinformatics* 25, 1105-11.
7. Guttman, M., Amit, I., Garber, M., French, C., Lin, M. F., Feldser, D., Huarte, M., Zuk, O., Carey, B. W., Cassady, J. P., Cabili, M. N., Jaenisch, R., Mikkelsen, T. S., Jacks, T., Hacohen, N., Bernstein, B. E., Kellis, M., Regev, A., Rinn, J. L., & Lander, E. S. (2009) Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. *Nature* 458, 223-7.
8. Sisu, C., Pei, B., Leng, J., Frankish, A., Zhang, Y., Balasubramanian, S., Harte, R., Wang, D., Rutenberg-Schoenberg, M., Clark, W., Diekhans, M., Rozowsky, J., Hubbard, T., Harrow, J., & Gerstein, M. B. (2014) Comparative analysis of pseudogenes across three phyla. *Proc Natl Acad Sci U S A* 111, 13361-6.
9. Lu, Z. J., Yip, K. Y., Wang, G., Shou, C., Hillier, L. W., Khurana, E., Agarwal, A., Auerbach, R., Rozowsky, J., Cheng, C., Kato, M., Miller, D. M., Slack, F., Snyder, M., Waterston, R. H., Reinke, V., & Gerstein, M. B. (2011) Prediction and characterization of noncoding RNAs in C. elegans by integrating conservation, secondary structure, and high-throughput sequencing and array data. *Genome Res* 21, 276-85.
10. Gerstein, M. B., Rozowsky, J., Yan, K.-K., Wang, D., Cheng, C., Brown, J. B., Davis, C. A., Hillier, L., Sisu, C., Li, J. J., Pei, B., Harmanci, A. O., Duff, M. O., Djebali, S., Alexander, R. P., Alver, B. H., Auerbach, R., Bell, K., Bickel, P. J., Boeck, M. E., Boley, N. P., Booth, B. W., Cherbas, L., Cherbas, P., Di, C., Dobin, A., Drenkow, J., Ewing, B., Fang, G., Fastuca, M., Feingold, E. A., Frankish, A., Gao, G., Good, P. J., Guigó, R., Hammonds, A., Harrow, J., Hoskins, R. A., Howald, C., Hu, L., Huang, H., Hubbard, T. J. P., Huynh, C., Jha, S., Kasper, D., Kato, M., Kaufman, T. C., Kitchen, R. R., Ladewig, E., Lagarde, J., Lai, E., Leng, J., Lu, Z., MacCoss, M., May, G., McWhirter, R., Merrihew, G., Miller, D. M., Mortazavi, A., Murad, R., Oliver, B., Olson, S., Park, P. J., Pazin, M. J., Perrimon, N., Pervouchine, D., Reinke, V., Reymond, A., Robinson, G., Samsonova, A., Saunders, G. I., Schlesinger, F., Sethi, A., Slack, F. J., Spencer, W. C., Stoiber, M. H., Strasbourger, P., Tanzer, A., Thompson, O. A., Wan, K. H., Wang, G., Wang, H., Watkins, K. L., Wen, J., Wen, K., Xue, C., Yang, L., Yip, K., Zaleski, C., Zhang, Y., Zheng, H., Brenner, S. E., Graveley, B. R., Celniker, S. E., Gingeras, T. R., & Waterston, R. (2014) Comparative analysis of the transcriptome across distant species. *Nature* 512, 445-8.
11. D'haeseleer, P., Liang, S., & Somogyi, R. (2000) Genetic network inference: from co-expression clustering to reverse engineering. *Bioinformatics* 16, 707-26.
12. Yan, K.-K., Wang, D., Rozowsky, J., Zheng, H., Cheng, C., & Gerstein, M. (2014) OrthoClust: an orthology-based network framework for clustering data across multiple species. *Genome Biol* 15, R100.
13. Yu, H., Greenbaum, D., Xin Lu, H., Zhu, X., & Gerstein, M. (2004) Genomic analysis of essentiality within protein networks.. *Trends Genet* 20, 227--231.
14. Yu, H., Kim, P. M., Sprecher, E., Trifonov, V., & Gerstein, M. (2007) The importance of bottlenecks in protein networks: correlation with gene essentiality and expression dynamics.. *PLoS Comput Biol* 3, e59.
15. Yu, H. & Gerstein, M. (2006) Genomic analysis of the hierarchical structure of regulatory networks.. *Proc Natl Acad Sci U S A* 103, 14724--14731.
16. Cheng, C., Andrews, E., Yan, K.-K., Ung, M., Wang, D., & Gerstein, M. (2015) An approach for determining and measuring network hierarchy applied to comparing the phosphorylome and the regulome. *Genome Biol* 16, 63.

17. Gerstein, M. B., Kundaje, A., Hariharan, M., Landt, S. G., Yan, K.-K., Cheng, C., Mu, X. J., Khurana, E., Rozowsky, J., Alexander, R., Min, R., Alves, P., Abyzov, A., Addleman, N., Bhardwaj, N., Boyle, A. P., Cayting, P., Charos, A., Chen, D. Z., Cheng, Y., Clarke, D., Eastman, C., Euskirchen, G., Frietze, S., Fu, Y., Gertz, J., Grubert, F., Harmanci, A., Jain, P., Kasowski, M., Lacroute, P., Leng, J., Lian, J., Monahan, H., O'Geen, H., Ouyang, Z., Partridge, E. C., Patacsil, D., Pauli, F., Raha, D., Ramirez, L., Reddy, T. E., Reed, B., Shi, M., Slifer, T., Wang, J., Wu, L., Yang, X., Yip, K. Y., Zilberman-Schapira, G., Batzoglou, S., Sidow, A., Farnham, P. J., Myers, R. M., Weissman, S. M., & Snyder, M. (2012) Architecture of the human regulatory network derived from ENCODE data. *Nature* 489, 91-100.
18. Cheng, C., Yan, K.-K., Hwang, W., Qian, J., Bhardwaj, N., Rozowsky, J., Lu, Z. J., Niu, W., Alves, P., Kato, M., Snyder, M., & Gerstein, M. (2011) Construction and analysis of an integrated regulatory network derived from high-throughput sequencing data. *PLoS Comput Biol* 7, e1002190.
19. Gerstein, M. B., Lu, Z. J., Van Nostrand, E. L., Cheng, C., Arshinoff, B. I., Liu, T., Yip, K. Y., Robilotto, R., Rechtsteiner, A., Ikegami, K., Alves, P., Chateigner, A., Perry, M., Morris, M., Auerbach, R. K., Feng, X., Leng, J., Vielle, A., Niu, W., Rhrissorrakrai, K., Agarwal, A., Alexander, R. P., Barber, G., Brdlik, C. M., Brennan, J., Brouillet, J. J., Carr, A., Cheung, M.-S., Clawson, H., Contrino, S., Dannenberg, L. O., Dernburg, A. F., Desai, A., Dick, L., Dose, A. C., Du, J., Egelhofer, T., Ercan, S., Euskirchen, G., Ewing, B., Feingold, E. A., Gassmann, R., Good, P. J., Green, P., Gullier, F., Gutwein, M., Guyer, M. S., Habegger, L., Han, T., Henikoff, J. G., Henz, S. R., Hinrichs, A., Holster, H., Hyman, T., Iniguez, A. L., Janette, J., Jensen, M., Kato, M., Kent, W. J., Kephart, E., Khivansara, V., Khurana, E., Kim, J. K., Kolasinska-Zwierz, P., Lai, E. C., Latorre, I., Leahey, A., Lewis, S., Lloyd, P., Lochovsky, L., Lowdon, R. F., Lubling, Y., Lyne, R., MacCoss, M., Mackowiak, S. D., Mangone, M., McKay, S., Mecenas, D., Merrihew, G., Miller, D. M. r., Muroyama, A., Murray, J. I., Ooi, S.-L., Pham, H., Phippen, T., Preston, E. A., Rajewsky, N., Ratsch, G., Rosenbaum, H., Rozowsky, J., Rutherford, K., Ruzanov, P., Sarov, M., Sasidharan, R., Sboner, A., Scheid, P., Segal, E., Shin, H., Shou, C., Slack, F. J., Slightam, C., Smith, R., Spencer, W. C., Stinson, E. O., Taing, S., Takasaki, T., Vafeados, D., Voronina, K., Wang, G., Washington, N. L., Whittle, C. M., Wu, B., Yan, K.-K., Zeller, G., Zha, Z., Zhong, M., Zhou, X., Ahringer, J., Strome, S., Gunsalus, K. C., Micklem, G., Liu, X. S., Reinke, V., Kim, S. K., Hillier, L. W., Henikoff, S., Piano, F., Snyder, M., Stein, L., Lieb, J. D., & Waterston, R. H. (2010) Integrative analysis of the Caenorhabditis elegans genome by the modENCODE project.. *Science* 330, 1775--1787.
20. Shou, C., Bhardwaj, N., Lam, H. Y. K., Yan, K.-K., Kim, P. M., Snyder, M., & Gerstein, M. B. (2011) Measuring the evolutionary rewiring of biological networks.. *PLoS Comput Biol* 7, e1001050.
21. Washietl, S., Pedersen, J. S., Korbel, J. O., Stocsits, C., Gruber, A. R., Hackermüller, J., Hertel, J., Lindemeyer, M., Reiche, K., Tanzer, A., Ucla, C., Wyss, C., Antonarakis, S. E., Denoeud, F., Lagarde, J., Drenkow, J., Kapranov, P., Gingeras, T. R., Guigó, R., Snyder, M., Gerstein, M. B., Reymond, A., Hofacker, I. L., & Stadler, P. F. (2007) Structured RNAs in the ENCODE selected regions of the human genome. *Genome Res* 17, 852-64.
22. http://exRNA.org.
23. http://brainspan.org.
24. Miller, J. A., Ding, S.-L., Sunkin, S. M., Smith, K. A., Ng, L., Szafer, A., Ebbert, A., Riley, Z. L., Royall, J. J., Aiona, K., Arnold, J. M., Bennet, C., Bertagnolli, D., Brouner, K., Butler, S., Caldejon, S., Carey, A., Cuhaciyan, C., Dalley, R. A., Dee, N., Dolbeare, T. A., Facer, B. A. C., Feng, D., Fliss, T. P., Gee, G., Goldy, J., Gourley, L., Gregor, B. W., Gu, G., Howard, R. E., Jochim, J. M., Kuan, C. L., Lau, C., Lee, C.-K., Lee, F., Lemon, T. A., Lesnar, P., McMurray, B., Mastan, N., Mosqueda, N., Naluai-Cecchini, T., Ngo, N.-K., Nyhus, J., Oldre, A., Olson, E., Parente, J., Parker, P. D., Parry, S. E., Stevens, A., Pletikos, M., Reding, M., Roll, K., Sandman, D., Sarreal, M., Shapouri, S., Shapovalova, N. V., Shen, E. H., Sjoquist, N., Slaughterbeck, C. R., Smith, M., Sodt, A. J., Williams, D., Zöllei, L., Fischl, B., Gerstein, M. B., Geschwind, D. H., Glass, I. A., Hawrylycz, M. J., Hevner, R. F., Huang, H., Jones, A. R., Knowles, J. A., Levitt, P., Phillips, J. W., Sestan, N., Wohnoutka, P., Dang, C., Bernard, A., Hohmann, J. G., & Lein, E. S. (2014) Transcriptional landscape of the prenatal human brain. *Nature* 508, 199-206.
25. http://psychencode.org.
26. Harrow, J., Frankish, A., Gonzalez, J. M., Tapanari, E., Diekhans, M., Kokocinski, F., Aken, B. L., Barrell, D., Zadissa, A., Searle, S., Barnes, I., Bignell, A., Boychenko, V., Hunt, T., Kay, M., Mukherjee, G., Rajan, J., Despacio-Reyes, G., Saunders, G., Steward, C., Harte, R., Lin, M., Howald, C., Tanzer, A., Derrien, T., Chrast, J., Walters, N., Balasubramanian, S., Pei, B., Tress, M., Rodriguez, J. M., Ezkurdia, I., van Baren, J., Brent, M., Haussler, D., Kellis, M., Valencia, A., Reymond, A., Gerstein, M., Guigo, R., & Hubbard, T. J. (2012) GENCODE: the reference human genome annotation for The ENCODE Project..

Genome Res 22, 1760--1774.
27. Torrents, D., Suyama, M., Zdobnov, E., & Bork, P. (2003) A genome-wide survey of human pseudogenes. *Genome Res* 13, 2559-67.
28. Amir, E.-a. D., Davis, K. L., Tadmor, M. D., Simonds, E. F., Levine, J. H., Bendall, S. C., Shenfeld, D. K., Krishnaswamy, S., Nolan, G. P., & Pe'er, D. (2013) viSNE enables visualization of high dimensional single-cell data and reveals phenotypic heterogeneity of leukemia. *Nat Biotechnol* 31, 545-52.
29. Krishnaswamy, S., Spitzer, M. H., Mingueneau, M., Bendall, S. C., Litvin, O., Stone, E., Pe'er, D., & Nolan, G. P. (2014) Systems biology. Conditional density-based analysis of T cell signaling in single-cell data. *Science* 346, 1250689.
30. Mingueneau, M., Krishnaswamy, S., Spitzer, M. H., Bendall, S. C., Stone, E. L., Hedrick, S. M., Pe'er, D., Mathis, D., Nolan, G. P., & Benoist, C. (2014) Single-cell mass cytometry of TCR signaling: amplification of small initial differences results in low ERK activation in NOD mice. *Proc Natl Acad Sci U S A* 111, 16466-71.
31. Levine, J. H., Simonds, E. F., Bendall, S. C., Davis, K. L., Amir, E.-a. D., Tadmor, M. D., Litvin, O., Fienberg, H. G., Jager, A., Zunder, E. R., Finck, R., Gedman, A. L., Radtke, I., Downing, J. R., Pe'er, D., & Nolan, G. P. (2015) Data-Driven Phenotypic Dissection of AML Reveals Progenitor-like Cells that Correlate with Prognosis. *Cell* 162, 184-97.
32. Caldwell, A.E.; Kahng, A. B. M. I. L. (2000) Can recursive bisection alone produce routable, placements?. *Design Automation Conference, 2000. Proceedings 2000* , 477-482.
33. Alpert, C. J., Kahng, A. B., & Yao, S. Z. (1999) Spectral partitioning with multiple eigenvectors. *Discret Appl. Math.* 90, 3--26.
34. Hellinger, E. (2009) Neue Begründung der Theorie quadratischer Formen von unendlichvielen Veränderlichen.. *Journal für die reine und angewandte Mathematik (Crelle's Journal).* 1909, 210-271.
35. Bodenmiller, B., Zunder, E. R., Finck, R., Chen, T. J., Savig, E. S., Bruggner, R. V., Simonds, E. F., Bendall, S. C., Sachs, K., Krutzik, P. O., & Nolan, G. P. (2012) Multiplexed mass cytometry profiling of cellular states perturbed by small-molecule regulators. *Nat Biotechnol* 30, 858-67.
36. Grün, D., Kester, L., & van Oudenaarden, A. (2014) Validation of noise models for single-cell transcriptomics. *Nat Methods* 11, 637-40.
37. Brennecke, P., Anders, S., Kim, J. K., Kołodziejczyk, A. A., Zhang, X., Proserpio, V., Baying, B., Benes, V., Teichmann, S. A., Marioni, J. C., & Heisler, M. G. (2013) Accounting for technical noise in single-cell RNA-seq experiments. *Nat Methods* 10, 1093-5.
38. Islam, S., Zeisel, A., Joost, S., La Manno, G., Zajac, P., Kasper, M., Lönnerberg, P., & Linnarsson, S. (2014) Quantitative single-cell RNA-seq with unique molecular identifiers. *Nat Methods* 11, 163-6.
39. Klein, A. M., Mazutis, L., Akartuna, I., Tallapragada, N., Veres, A., Li, V., Peshkin, L., Weitz, D. A., & Kirschner, M. W. (2015) Droplet barcoding for single-cell transcriptomics applied to embryonic stem cells. *Cell* 161, 1187-201.
40. Mahfouz, A., van de Giessen, M., van der Maaten, L., Huisman, S., Reinders, M., Hawrylycz, M. J., & Lelieveldt, B. P. F. (2015) Visualizing the spatial gene expression organization in the brain through non-linear similarity embeddings. *Methods* 73, 79-89.
41. Van Der Maaten, L. (2014) Accelerating t-SNE Using Tree-based Algorithms. *J. Mach. Learn. Res.* 15, 3221--3245.
42. Scholz, M., Kaplan, F., Guy, C. L., Kopka, J., & Selbig, J. (2005) Non-linear PCA: a missing data approach. *Bioinformatics* 21, 3887-95.
43. Wang, D., Yan, K.-K., Sisu, C., Cheng, C., Rozowsky, J., Meyerson, W., & Gerstein, M. B. (2015) Loregic: a method to characterize the cooperative logic of regulatory factors. *PLoS Comput Biol* 11, e1004132.
44. http://github.com/gersteinlab/loregic.
45. Daifeng Wang, Fei He, S. M. M. G. DREISS: Using state-space models to infer the dynamics of gene expression driven by external and internal regulatory networks. *Minor revision, PLoS Computational Biology*.
46. https://github.com/gersteinlab/Dreiss.
47. Cheng, C., Alexander, R., Min, R., Leng, J., Yip, K. Y., Rozowsky, J., Yan, K.-K., Dong, X., Djebali, S., Ruan, Y., Davis, C. A., Carninci, P., Lassman, T., Gingeras, T. R., Guigó, R., Birney, E., Weng, Z., Snyder, M., & Gerstein, M. (2012) Understanding transcriptional regulation by integrative analysis of transcription factor binding data. *Genome Res* 22, 1658-67.
48. ENCODE Project Consortium, Dunham, I., Kundaje, A., Aldred, S. F., Collins, P. J., Davis, C. A., Doyle,

F., Epstein, C. B., Frietze, S., Harrow, J., Kaul, R., Khatun, J., Lajoie, B. R., Landt, S. G., Lee, B.-K., Pauli, F., Rosenbloom, K. R., Sabo, P., Safi, A., Sanyal, A., Shoresh, N., Simon, J. M., Song, L., Trinklein, N. D., Altshuler, R. C., Birney, E., Brown, J. B., Cheng, C., Djebali, S., Dong, X., Dunham, I., Ernst, J., Furey, T. S., Gerstein, M., Giardine, B., Greven, M., Hardison, R. C., Harris, R. S., Herrero, J., Hoffman, M. M., Iyer, S., Kelllis, M., Khatun, J., Kheradpour, P., Kundaje, A., Lassman, T., Li, Q., Lin, X., Marinov, G. K., Merkel, A., Mortazavi, A., Parker, S. C. J., Reddy, T. E., Rozowsky, J., Schlesinger, F., Thurman, R. E., Wang, J., Ward, L. D., Whitfield, T. W., Wilder, S. P., Wu, W., Xi, H. S., Yip, K. Y., Zhuang, J., Bernstein, B. E., Birney, E., Dunham, I., Green, E. D., Gunter, C., Snyder, M., Pazin, M. J., Lowdon, R. F., Dillon, L. A. L., Adams, L. B., Kelly, C. J., Zhang, J., Wexler, J. R., Green, E. D., Good, P. J., Feingold, E. A., Bernstein, B. E., Birney, E., Crawford, G. E., Dekker, J., Elinitski, L., Farnham, P. J., Gerstein, M., Giddings, M. C., Gingeras, T. R., Green, E. D., Guigó, R., Hardison, R. C., Hubbard, T. J., Kellis, M., Kent, W. J., Lieb, J. D., Margulies, E. H., Myers, R. M., Snyder, M., Starnatoyannopoulos, J. A., Tennebaum, S. A., Weng, Z., White, K. P., Wold, B., Khatun, J., Yu, Y., Wrobel, J., Risk, B. A., Gunawardena, H. P., Kuiper, H. C., Maier, C. W., Xie, L., Chen, X., Giddings, M. C., Bernstein, B. E., Epstein, C. B., Shoresh, N., Ernst, J., Kheradpour, P., Mikkelsen, T. S., Gillespie, S., Goren, A., Ram, O., Zhang, X., Wang, L., Issner, R., Coyne, M. J., Durham, T., Ku, M., Truong, T., Ward, L. D., Altshuler, R. C., Eaton, M. L., Kellis, M., Djebali, S., Davis, C. A., Merkel, A., Dobin, A., Lassmann, T., Mortazavi, A., Tanzer, A., Lagarde, J., Lin, W., Schlesinger, F., Xue, C., Marinov, G. K., Khatun, J., Williams, B. A., Zaleski, C., Rozowsky, J., Röder, M., Kokocinski, F., Abdelhamid, R. F., Alioto, T., Antoshechkin, I., Baer, M. T., Batut, P., Bell, I., Bell, K., Chakrabortty, S., Chen, X., Chrast, J., Curado, J., Derrien, T., Drenkow, J., Dumais, E., Dumais, J., Duttagupta, R., Fastuca, M., Fejes-Toth, K., Ferreira, P., Foissac, S., Fullwood, M. J., Gao, H., Gonzalez, D., Gordon, A., Gunawardena, H. P., Howald, C., Jha, S., Johnson, R., Kapranov, P., King, B., Kingswood, C., Li, G., Luo, O. J., Park, E., Preall, J. B., Presaud, K., Ribeca, P., Risk, B. A., Robyr, D., Ruan, X., Sammeth, M., Sandu, K. S., Schaeffer, L., See, L.-H., Shahab, A., Skancke, J., Suzuki, A. M., Takahashi, H., Tilgner, H., Trout, D., Walters, N., Wang, H., Wrobel, J., Yu, Y., Hayashizaki, Y., Harrow, J., Gerstein, M., Hubbard, T. J., Reymond, A., Antonarakis, S. E., Hannon, G. J., Giddings, M. C., Ruan, Y., Wold, B., Carninci, P., Guigó, R., Gingeras, T. R., Rosenbloom, K. R., Sloan, C. A., Learned, K., Malladi, V. S., Wong, M. C., Barber, G. P., Cline, M. S., Dreszer, T. R., Heitner, S. G., Karolchik, D., Kent, W. J., Kirkup, V. M., Meyer, L. R., Long, J. C., Maddren, M., Raney, B. J., Furey, T. S., Song, L., Grasfeder, L. L., Giresi, P. G., Lee, B.-K., Battenhouse, A., Sheffield, N. C., Simon, J. M., Showers, K. A., Safi, A., London, D., Bhinge, A. A., Shestak, C., Schaner, M. R., Kim, S. K., Zhang, Z. Z., Mieczkowski, P. A., Mieczkowska, J. O., Liu, Z., McDaniell, R. M., Ni, Y., Rashid, N. U., Kim, M. J., Adar, S., Zhang, Z., Wang, T., Winter, D., Keefe, D., Birney, E., Iyer, V. R., Lieb, J. D., Crawford, G. E., Li, G., Sandhu, K. S., Zheng, M., Wang, P., Luo, O. J., Shahab, A., Fullwood, M. J., Ruan, X., Ruan, Y., Myers, R. M., Pauli, F., Williams, B. A., Gertz, J., Marinov, G. K., Reddy, T. E., Vielmetter, J., Partridge, E. C., Trout, D., Varley, K. E., Gasper, C., Bansal, A., Pepke, S., Jain, P., Amrhein, H., Bowling, K. M., Anaya, M., Cross, M. K., King, B., Muratet, M. A., Antoshechkin, I., Newberry, K. M., McCue, K., Nesmith, A. S., Fisher-Aylor, K. I., Pusey, B., DeSalvo, G., Parker, S. L., Balasubramanian, S., Davis, N. S., Meadows, S. K., Eggleston, T., Gunter, C., Newberry, J. S., Levy, S. E., Absher, D. M., Mortazavi, A., Wong, W. H., Wold, B., Blow, M. J., Visel, A., Pennachio, L. A., Elnitski, L., Margulies, E. H., Parker, S. C. J., Petrykowska, H. M., Abyzov, A., Aken, B., Barrell, D., Barson, G., Berry, A., Bignell, A., Boychenko, V., Bussotti, G., Chrast, J., Davidson, C., Derrien, T., Despacio-Reyes, G., Diekhans, M., Ezkurdia, I., Frankish, A., Gilbert, J., Gonzalez, J. M., Griffiths, E., Harte, R., Hendrix, D. A., Howald, C., Hunt, T., Jungreis, I., Kay, M., Khurana, E., Kokocinski, F., Leng, J., Lin, M. F., Loveland, J., Lu, Z., Manthravadi, D., Mariotti, M., Mudge, J., Mukherjee, G., Notredame, C., Pei, B., Rodriguez, J. M., Saunders, G., Sboner, A., Searle, S., Sisu, C., Snow, C., Steward, C., Tanzer, A., Tapanan, E., Tress, M. L., van Baren, M. J., Walters, N., Washieti, S., Wilming, L., Zadissa, A., Zhengdong, Z., Brent, M., Haussler, D., Kellis, M., Valencia, A., Gerstein, M., Raymond, A., Guigó, R., Harrow, J., Hubbard, T. J., Landt, S. G., Frietze, S., Abyzov, A., Addleman, N., Alexander, R. P., Auerbach, R. K., Balasubramanian, S., Bettinger, K., Bhardwaj, N., Boyle, A. P., Cao, A. R., Cayting, P., Charos, A., Cheng, Y., Cheng, C., Eastman, C., Euskirchen, G., Fleming, J. D., Grubert, F., Habegger, L., Hariharan, M., Harmanci, A., Iyenger, S., Jin, V. X., Karczewski, K. J., Kasowski, M., Lacroute, P., Lam, H., Larnarre-Vincent, N., Leng, J., Lian, J., Lindahl-Allen, M., Min, R., Miotto, B., Monahan, H., Moqtaderi, Z., Mu, X. J., O'Geen, H., Ouyang, Z., Patacsil, D., Pei, B., Raha, D., Ramirez, L., Reed, B., Rozowsky, J., Sboner, A., Shi, M., Sisu, C., Slifer, T., Witt, H., Wu, L., Xu, X., Yan, K.-K., Yang, X., Yip, K. Y., Zhang, Z., Struhl, K., Weissman, S. M., Gerstein, M., Farnham, P. J., Snyder, M., Tenebaum, S. A., Penalva, L. O., Doyle, F., Karmakar, S., Landt, S. G., Bhanvadia, R. R., Choudhury, A., Domanus, M., Ma, L., Moran, J., Patacsil, D.,

Slifer, T., Victorsen, A., Yang, X., Snyder, M., White, K. P., Auer, T., Centarin, L., Eichenlaub, M., Gruhl, F., Heerman, S., Hoeckendorf, B., Inoue, D., Kellner, T., Kirchmaier, S., Mueller, C., Reinhardt, R., Schertel, L., Schneider, S., Sinn, R., Wittbrodt, B., Wittbrodt, J., Weng, Z., Whitfield, T. W., Wang, J., Collins, P. J., Aldred, S. F., Trinklein, N. D., Partridge, E. C., Myers, R. M., Dekker, J., Jain, G., Lajoie, B. R., Sanyal, A., Balasundaram, G., Bates, D. L., Byron, R., Canfield, T. K., Diegel, M. J., Dunn, D., Ebersol, A. K., Ebersol, A. K., Frum, T., Garg, K., Gist, E., Hansen, R. S., Boatman, L., Haugen, E., Humbert, R., Jain, G., Johnson, A. K., Johnson, E. M., Kutyavin, T. M., Lajoie, B. R., Lee, K., Lotakis, D., Maurano, M. T., Neph, S. J., Neri, F. V., Nguyen, E. D., Qu, H., Reynolds, A. P., Roach, V., Rynes, E., Sabo, P., Sanchez, M. E., Sandstrom, R. S., Sanyal, A., Shafer, A. O., Stergachis, A. B., Thomas, S., Thurman, R. E., Vernot, B., Vierstra, J., Vong, S., Wang, H., Weaver, M. A., Yan, Y., Zhang, M., Akey, J. A., Bender, M., Dorschner, M. O., Groudine, M., MacCoss, M. J., Navas, P., Stamatoyannopoulos, G., Kaul, R., Dekker, J., Stamatoyannopoulos, J. A., Dunham, I., Beal, K., Brazma, A., Flicek, P., Herrero, J., Johnson, N., Keefe, D., Lukk, M., Luscombe, N. M., Sobral, D., Vaquerizas, J. M., Wilder, S. P., Batzoglou, S., Sidow, A., Hussami, N., Kyriazopoulou-Panagiotopoulou, S., Libbrecht, M. W., Schaub, M. A., Kundaje, A., Hardison, R. C., Miller, W., Giardine, B., Harris, R. S., Wu, W., Bickel, P. J., Banfai, B., Boley, N. P., Brown, J. B., Huang, H., Li, Q., Li, J. J., Noble, W. S., Bilmes, J. A., Buske, O. J., Hoffman, M. M., Sahu, A. O., Kharchenko, P. V., Park, P. J., Baker, D., Taylor, J., Weng, Z., Iyer, S., Dong, X., Greven, M., Lin, X., Wang, J., Xi, H. S., Zhuang, J., Gerstein, M., Alexander, R. P., Balasubramanian, S., Cheng, C., Harmanci, A., Lochovsky, L., Min, R., Mu, X. J., Rozowsky, J., Yan, K.-K., Yip, K. Y., & Birney, E. (2012) An integrated encyclopedia of DNA elements in the human genome. *Nature* 489, 57-74.

49. Cheng, C. & Gerstein, M. (2012) Modeling the relative relationship of transcription factor binding and histone modifications to gene expression levels in mouse embryonic stem cells.. *Nucleic Acids Res* 40, 553--568.

50. Dong, X., Greven, M. C., Kundaje, A., Djebali, S., Brown, J. B., Cheng, C., Gingeras, T. R., Gerstein, M., Guigó, R., Birney, E., & Weng, Z. (2012) Modeling gene expression using chromatin features in various cellular contexts. *Genome Biol* 13, R53.

51. Cheng, C., Yan, K.-K., Yip, K. Y., Rozowsky, J., Alexander, R., Shou, C., & Gerstein, M. (2011) A statistical framework for modeling gene expression using chromatin features and application to modENCODE datasets. *Genome Biol* 12, R15.

52. Gianoulis, T. A., Griffin, M. A., Spakowicz, D. J., Dunican, B. F., Alpha, C. J., Sboner, A., Sismour, A. M., Kodira, C., Egholm, M., Church, G. M., Gerstein, M. B., & Strobel, S. A. (2012) Genomic analysis of the hydrocarbon-producing, cellulolytic, endophytic fungus Ascocoryne sarcoides.. *PLoS Genet* 8, e1002558.

53. Kuksa, P. P., Min, M. R., Dugar, R., & Gerstein, M. (2015) High-order neural networks and kernel methods for peptide-MHC binding prediction. *Bioinformatics*.

54. http://www.gtexportal.org/ () NIH Genotype-Tissue Expression Project (GTEx).

55. http://kbase.us/.

56. Peter, I. S. & Davidson, E. H. (2011) Evolution of gene regulatory networks controlling body plan development. *Cell* 144, 970-85.

57. Mangan, S. & Alon, U. (2003) Structure and function of the feed-forward loop network motif. *Proc Natl Acad Sci U S A* 100, 11980-5.

58. Tu, S., Pederson, T., & Weng, Z. (2013) Networking development by Boolean logic. *Nucleus* 4, 89-91.

59. Siuti, P., Yazbek, J., & Lu, T. K. (2013) Synthetic circuits integrating logic and memory in living cells. *Nat Biotechnol* 31, 448-52.

60. Fenno, L. E., Mattis, J., Ramakrishnan, C., Hyun, M., Lee, S. Y., He, M., Tucciarone, J., Selimbeyoglu, A., Berndt, A., Grosenick, L., Zalocusky, K. A., Bernstein, H., Swanson, H., Perry, C., Diester, I., Boyce, F. M., Bass, C. E., Neve, R., Huang, Z. J., & Deisseroth, K. (2014) Targeting cells with single vectors using multiple-feature Boolean logic. *Nat Methods* 11, 763-72.

61. Silva-Rocha, R. & de Lorenzo, V. (2008) Mining logic gates in prokaryotic transcriptional regulation networks. *FEBS Lett* 582, 1237-44.

62. https://www.encodeproject.org/comparative/.

Resource Sharing Plan

Data generated in these studies will be freely available to members of the research community with a goal to enable dissemination of data, while also protecting the privacy of the participants and the utility of the data, by de-identifying and masking potentially sensitive data elements in compliance with the NIH public data sharing policy. All other resources developed in the course of the proposed studies will be available by request to qualified academic investigators for non-commercial research.

For all studies, we will follow the National Institute of Health's Genomic Data Sharing Policy. The raw datasets corresponding to expression, genomic, and genetic data generated by these studies will be submitted to Gene Expression Omnibus (GEO) or the Sequence Read Archive (SRA) for use by other investigators. As datasets are analyzed, then validated, we will proceed with deposition in ImmPort according to a timeline negotiated with the Program Officer. Sample data in the YCAAD biorepository are, and will continue to be, available on the internet through the YCCI research accelerator, a publicly accessible platform for scientific collaboration (ycci.researchaccelerator.org). In addition, tools, pipelines, derived datasets and analyses will be made available through the website (asthmaMAP.gersteinlab.org) which will serve as an organizational tool for the participants in this cooperative proposal as well as a repository and resource for the greater research community. Details of the contents and construction of the asthma MAP website are in Project 3, Research Proposal Aim 3.

Yale University School of Medicine and all investigators will adhere to the NIH Grants Policy on Sharing of Unique Research Resources including the “Sharing of Biomedical Research Resources: Principles and Guidelines for Recipients of NIH Grants and Contracts” issued in December 1999. Specifically, material transfers would be made with no more restrictive terms than in the Simple Letter Agreement or the UBMTA and without reach through requirements. Should any intellectual property arise which requires a patent, we would ensure that the technology remains widely available to the research community in accordance with the NIH Principles and Guidelines document. In addition, we will provide relevant protocols and published data upon request. Accepted versions of final, peer-reviewed manuscripts emanating from this research will be deposited on-line to PubMed Central in accord with NIH Public Access.