PROGRAM CONTACT:      ( Privileged Communication )      *Release Date:*    **05/06/2016**
Jerry Li
240-276-6210                                                     *Revised Date:*
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*Application Number:*    **1 R01 CA209781-01**

**Principal Investigator**

**GERSTEIN, MARK BENDER**

**Applicant Organization:**   **YALE UNIVERSITY**

| | |
|---|---|
| *Review Group:* | **ZRG1 OBT-D (55)** |
| | **Center for Scientific Review Special Emphasis Panel** |
| | **Cancer Health Disparities/Diversity in Basic Cancer Research** |

| | | | |
|---|---|---|---|
| *Meeting Date:* | **04/11/2016** | *RFA/PA:* | **PAR15-093** |
| *Council:* | **MAY 2016** | *PCC:* | **J3SB** |
| *Requested Start:* | **07/01/2016** | | |

| | |
|---|---|
| *Project Title:* | **The Genomic Basis of Racial Disparities in Kidney Cancer** |

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|---|---|
| *SRG Action:* | **++** |
| *Next Steps:* | **Visit http://grants.nih.gov/grants/next_steps.htm** |
| **Human Subjects:** | **10-No human subjects involved** |
| **Animal Subjects:** | **10-No live vertebrate animals involved for competing appl.** |

| Project Year | Direct Costs Requested |
|---|---|
| 1 | 250,000 |
| 2 | 250,000 |
| 3 | 250,000 |
| 4 | 250,000 |
| 5 | 250,000 |
| **TOTAL** | **1,250,000** |

**++NOTE TO APPLICANT: Members of the Scientific Review Group (SRG) were asked to identify those applications with the highest scientific merit, generally the top half. Written comments, criterion scores, and preliminary impact scores were submitted by the assigned reviewers prior to the SRG meeting. At the meeting, the more meritorious applications were discussed and given final impact scores; by concurrence of the full SRG, the remaining applications, including this application, were not discussed or scored. The reviewers' comments (largely unedited by NIH staff) and criterion scores for this application are provided below. Because applications deemed by the SRG to have the highest scientific merit generally are considered for funding first, it is highly unlikely that an application with an ND recommendation will be funded. Each applicant should read the written critiques carefully and, if there are questions about the review or future options for the project, discuss them with the Program Contact listed above.**

**1R01CA209781-01 Gerstein, Mark**

**DESCRIPTION (provided by applicant):** Recent studies have shown significant racial disparities in kidney cancer including early disease onset for African-Americans, different histologic distribution, and worst disease outcome, even when controlling for treatment. No study has evaluated a biologic or cause of racial disparities in kidney cancer. In our study, we intend to investigate whether these disparities are due to recurrent coding or non-coding germline and somatic alterations and whether these alterations are more common in African-American or Caucasian individuals with clear cell and papillary kidney cancer. For this purpose, we have developed four specific aims. In our first aim, we intend to perform whole genome sequencing of 15 African-Americans in order to increase the number of African- Americans currently existing in TCGA with kidney cancer. For these individuals, we intend to perform high-quality mutation calls for structural and genomic variation including SNPs, indels, inversions and copy number variations for both coding and non- coding regions. Our second aim is to assemble a comprehensive list of somatic & germline mutations associated with cancer and prioritize regions with greatest impact. To complete our aim, we will use our novel and already developed tools including i) FunSeq, a sophisticated algorithm that prioritizes high impact variants, ii) LARVA, a burden test algorithm that identifies significant mutation enrichment in non-coding elements, iii) an extension of FunSeq to construct connected genetic modules based on molecular and protein interactions and iv) an extension of LARVA to include additional covariates such as mutation rate, replication time etc. In our third aim, we will test a list of prioritized regions and modules obtained from aim 2 to identify racial genomic differences. More specifically, we intend to test for disparities across i) germline mutations in coding regions using WES data, ii) genomic regions with higher mutational burdens, iii) germline mutations in non-coding prioritized regions using WGS data and iv) somatic mutations in prioritized regions. Finally, in our fourth objective, we intend to validate racial disparities from a list of 550 prioritized regions using a Yale Validation Cohort, a cohort consisting of total 384 individuals; an equal number of 96 Caucasian and African-American clear cell and papillary tumors. To validate for racial disparity, we will use the MassArray system, a highly accurate technology for rapid detection of known or suspected somatic or germline alterations.

**PUBLIC HEALTH RELEVANCE:** We investigate the genomic cause of racial disparities in renal cell carcinoma. We will evaluate clear cell and papillary renal tumors from African American and Caucasians to identify and prioritize both common and rare, germline and somatic variants, in coding and non-coding regions. Utilizing a range of cancer genome data and novel bioinformatics tools, our goal is to prioritize variants contributing to racial disparity.

**CRITIQUE 1:**

Significance: 2
Investigator(s): 1
Innovation: 2
Approach: 4
Environment: 1

**Overall Impact:** The proposed study is built on the hypotheses that 1) recurrent non-coding as well as coding germline and somatic alterations are associated with kidney cancer and 2) specific germline and somatic alterations are more common in African-Americans with kidney cancer. Using a cohort of African-American and Caucasians patients treated at Yale with clear cell RCC and papillary RCC, these hypotheses will be tested by: 1) Perform whole genome sequencing (WGS) of African-Americans with ccRCC to complete a missing aspect of the cancer genome atlas (TCGA). 2) Assemble a set of coding and non-coding regions associated with kidney cancer, both in terms of somatic and germline alterations. 3) Identify genomic regions differing most between African-Americans and Caucasians with kidney cancer. 4) Validate specific regions with either germline or somatic mutations suspected of

contributing to kidney cancer racial disparity. The proposed study is well thought as regards the recruitment of the AA patients, collection of the samples, sequencing and analyzing the bioinformatics data with in-house developed programs and expertise of the investigator at Yale. The feasibility of the study is also not an issue as the proposed LARVA/FunSeq analysis has already been performed by the investigator on 32 WGS on KIRP group. With the lack of WGS of AA patients, this study will be useful in filling that gap of resources and sequencing data. Availability of such data in the public domain may also benefit other studies, which are not feasible at present due to the lack of representation of AA WGS. However, there are several weak points in the study as follows. As described by the investigator, the age-adjusted incidence of kidney cancer in Caucasian is 15.5 cases per 100,000 as compared to 18.8 cases per 100,000 in AA. Thus, the risk of kidney cancer in AA is only marginally higher (0.003%) than Caucasians. This marginally higher risk may also include disparity in AA due to causes such as obesity, metabolic syndrome, hypertension other than genetic links. It may also be important to decipher any genetic differences between pRCC and ccRCC before searching for the genetic links distinguishing racial health disparity of this cancer. Another issue is how the variants (mutations or SNPs) will be segregated or ruled out of analysis, which are not due to race but influenced by other factors such as age, sex, metabolic syndrome or other clinical problems in the individuals. No information has been presented if similar parameters have already been used on the available WGS from the TCGA database (not aiming disparity) to link the coding/non-coding regions to pRCC or ccRCC.These concerns diminishes the enthusiasm for this otherwise excellent study and reduces the impact of the study to a medium to moderate level.

## 1. Significance:

**Strengths**

- The area of investigation is significant since it involves the need of a better understanding of kidney cancer disparity between Caucasians and AA.

- With the lack of WGS of AA patients, this study will be useful in filling that gap of resources and sequencing data. Availability of such data in the public domain may also benefit other studies, which are not feasible at present due to the lack of representation of AA WGS.

**Weaknesses**

- As described by the investigator, the age-adjusted incidence of kidney cancer in Caucasian is 15.5 cases per 100,000 as compared to 18.8 cases per 100,000 in AA according to the NIH SEER data. It is emphasized in this proposal that incidence in AA is 30% greater than in Caucasians. The 30% higher incidence in AA is huge and very alarming; however, if we calculate the risk of having kidney cancer in AA from the given values, it is marginally higher (0.003%) than Caucasians. This slightly higher level of risk also includes disparity in AA due to causes such as obesity, metabolic syndrome, hypertension other than genetic links. There is no discussion to better understand this difference.

- From previous studies, genetic basis of kidney cancer is not very well linked except few known published genetic mutations. It is also not clear what are the genetic differences between pRCC and ccRCC. It may be important to further investigate these missing gaps in addition to or before searching for the genetic links distinguishing racial health disparity of this cancer.

## 2. Investigator(s):

**Strengths**

- The investigator is well established in the field and has an extensive experience in the proposed study.

- Dr. Gerstein has several cutting-edge publications related to this research field.

- The proposed LARVA/FunSeq analysis has already been performed by the investigator on 32 WGS on KIRP group.

**Weaknesses**

- None are noted.

### 3. Innovation:

**Strengths**

- While multiple studies have characterized epidemiologic differences, to date, there has been no comprehensive investigation into a biologic or genetic cause of kidney cancer health disparity.

- Proposed research plan will improve our understanding of the genomic cause of racial disparity in RCC and may have implications beyond the scope of this project.

**Weaknesses**

- No significant weaknesses are noted.

### 4. Approach:

**Strengths**

- The proposed study is well thought out as regards the recruitment of the AA patients, collection of the samples, sequencing and analyzing the bioinformatics data with in-house developed programs and expertise of the investigator at Yale.

- The feasibility of the study is good as the proposed LARVA/FunSeq analysis has already been performed by the investigator on 32 WGS on KIRP group.

**Weaknesses**

- There is no question on the collection of samples, sequencing and bioinformatics analytical tools and the availability of necessary expertise in this study. However, one issue is how the variants (mutations or SNPs) will be segregated or ruled out of analysis, which are not due to race but influenced by other factors such as age, sex, metabolic syndrome or other clinical problems in the individuals.

- There is a gap between finding the coding or non-coding mutations and clinical features of the patients, which decreases the chances of benefits from the endpoints of the study.

- It will be interesting to know if similar parameters have already been used on the available WGS from the TCGA database (not aiming disparity) to link the coding/non-coding regions to pRCC or ccRCC.

### 5. Environment:

**Strengths**

- The research environment is excellent and seems to have all the necessary facilities for the development and execution of this proposal.

**Weaknesses**

- None are noted.

**Protections for Human Subjects:**

Not Applicable (No Human Subjects)


**Vertebrate Animals:**

Not Applicable (No Vertebrate Animals)


**Biohazards:**

Not Applicable (No Biohazards)


**Resource Sharing Plans:**

Acceptable


**Budget and Period of Support:**

Recommend as Requested


**CRITIQUE 2:**

Significance: 7
Investigator(s): 1
Innovation: 7
Approach: 7
Environment: 1


**Overall Impact:** The goal of this application is focused on genome sequencing of clear cell renal cell carcinoma (CCRCC) so that improved understanding of genomic cause of racial disparity in renal cell carcinoma. While the proposed comparative genomic analysis of clear cell and non-clear cell renal cell carcinoma from Caucasians and African Americans is clearly of potential significance, the proposed approach is essentially adding new information to that available in TCGA. Neither the concept nor the methodology is very innovative. Overall, based on the concerns with the significance and innovation the impact is rated as fair.


**1. Significance:**

**Strengths**

- Comparative genomic analysis clear cell and non-clear cell renal cell carcinoma from Caucasians and African Americans is clearly of potential significance.

**Weaknesses**

- A large body of published data on genomic analysis of clear cell and non-clear cell renal cell carcinoma is available including data at TCGA.

- Difficult to ascertain what new information of high impact will be obtained from the proposed studies based on currently available genomic information.

**2. Investigator(s):**

**Strengths**

- The investigator Dr. Gerstein obtained a Ph.D. in Bioinformatics/Chemistry from Cambridge University in 1993 and subsequent post-doctoral training in Bioinformatics at Stanford University, Stanford, CA. Dr. Gerstein currently a Professor of Computer Science and Molecular Biophysics & Biochemistry at Yale University, New Haven, CT has an excellent publication record on bioinformatics related to genomic analyses.

- The co-investigator, Dr. Shuch, obtained his MD from New York University School of Medicine in 2004 and subsequent training in translational investigation and urology at UCLA School of Medicine and the NCI. Dr. Shuch has a good publication record relevant to kidney cancer and his leadership role with the biorepository and translational research is essential for the proposed studies.

**Weaknesses**

- None are noted.

**3. Innovation:**

**Strengths**

- None are noted.

**Weaknesses**

- There is limited innovation in concept and methodology.

**4. Approach:**

**Strengths**

- None are noted.

**Weaknesses**

- Methodology will be that already used by TCGA.

- No strategy or preliminary data to support that the proposed genomic analysis of clear cell and non-clear cell renal cell carcinoma will reveal novel data not already available from published information or the TCGA.

- No table of planned enrollment report making it difficult to ascertain the actual ethnic distribution of clear cell and non-clear cell renal cell carcinoma specimens.

**5. Environment:**

**Strengths**

- The scientific environment and resources at Yale University are excellent for the proposed studies.

**Weaknesses**

- None are noted.

**Protections for Human Subjects:**

Not Applicable (No Human Subjects)


**Vertebrate Animals:**

Not Applicable (No Vertebrate Animals)


**Biohazards:**

Not Applicable (No Biohazards)


**Resource Sharing Plans:**

Acceptable


**Budget and Period of Support:**

Recommend as Requested


**CRITIQUE 3:**

Significance: 5
Investigator(s): 1
Innovation: 2
Approach: 8
Environment: 1

**Overall Impact:** Significant racial disparities exist in kidney cancer among African-Americans (AA) as compared to Caucasian-Americans.  AA have increased cancer incidence, earlier age of onset, a different subtype distribution, and worse survival.  The biological or genetic causes of this cancer health disparity are currently unknown, and no study has addressed genetic mechanisms associated with RCC racial disparity. Thus, the investigator and his team propose to test the hypothesis that (1) recurrent non-coding as well as coding germline and somatic alterations are associated with kidney cancer and 2) specific germline and somatic alterations are more common in African-Americans with kidney cancer.   This hypothesis will be tested through four specific aims.  In Aim 1, whole genome sequencing (WGS) of African-Americans with ccRCC will be performed in order to complete a missing aspect of the cancer genome atlas (TCGA).  In Aim 2, a set of coding and non-coding regions associated with kidney cancer, both in terms of somatic and germline alterations, will be assembled.  In Aim 3, genomic regions differing most between African-Americans and Caucasians with kidney cancer will be identified. In Aim 4, specific regions with either germline or somatic mutations suspected of contributing to kidney cancer racial disparity will be validated.  Strengths of the application are: (1) The investigatory team is highly experienced and qualified to conduct the proposed project.  Dr. Gerstein, the investigator, is a leader in Computational Genomics, and has served on many previous NIH-funded genomics projects (such as ENCODE & 1000 Genomes).  Dr. Brian Shuch, the co-investigator, is a urologic surgeon who serves on the Cancer Genome Atlas (TCGA) kidney cancer working group, as well as on the NCI PDQ Cancer Genetics Board.  He runs a hereditary kidney cancer program at Yale, and serves as the Director of the Genitourinary Cancer Biospecimen repository.  He is also the lead investigator on an investigator-initiated trial involving small renal tumors. (2)  The environment at Yale University is excellent. (3)  The proposal contains novel concepts and hypotheses, and the experimental approach utilizes novel bioinformatics algorithms.  (4)  The proposal addresses a high

significant health disparity. (5) There are novel bioinformatics tools within the approach. The weaknesses of the application within its experimental approach far outweigh the strengths of the application. These major weaknesses in the experimental design include: (1) the proposed hypotheses are not supported by provided preliminary data. This makes this application without a sufficient foundation or premise and it is not likely to yield useful results as currently proposed, especially since the focus is on the genome, and not on functional aspects of the renal cell carcinoma. At the very least, the current knowledge of genetic mechanisms and biological factors associated with renal cell carcinoma in the general population or even on Caucasian Americans should have been provided. (2) The experimental design lacked controls; it is not enough to use TCGA controls because these would not account for experimental variation. (3) The project failed to stratify patients into men, women and children, since mechanisms may vary according to these parameters. The project did not include ethnic DNA markers to correctly identify the ethnicity of the samples. (4) It is not clear why the team proposes to investigate germ line mutations, since the tumor samples do not normally originate from the germ line; at the very least this should be justified. (5) The success of Aim 3 is dependent on Aim 2, and Aim 4 is dependent on Aim 3. These major weaknesses in the experimental approach, and the lack of focus on the functional aspects of the disease, dampens enthusiasm and the significance of the project. Completion of this project is likely to have only a low to moderate impact on the renal cell carcinoma health disparity field.

**Protections for Human Subjects:**

Not Applicable (No Human Subjects)

**Vertebrate Animals:**

Not Applicable (No Vertebrate Animals)

**Biohazards:**

Not Applicable (No Biohazards)

**Resource Sharing Plans:**

Acceptable

**Budget and Period of Support:**

Recommend as Requested

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Footnotes for 1 R01 CA209781-01; PI Name: Gerstein, Mark Bender

NIH has modified its policy regarding the receipt of resubmissions (amended applications). See Guide Notice NOT-OD-14-074 at http://grants.nih.gov/grants/guide/notice-files/NOT-OD-14-074.html. The impact/priority score is calculated after discussion of an application by averaging the overall scores (1-9) given by all voting reviewers on the committee and multiplying by 10. The criterion scores are submitted prior to the meeting by the individual reviewers assigned to an application, and are not discussed specifically at the review meeting or calculated into the overall impact score. Some applications also receive a percentile

ranking. For details on the review process, see
http://grants.nih.gov/grants/peer_review_process.htm#scoring.

CENTER FOR SCIENTIFIC REVIEW
Cancer Health Disparities/Diversity in Basic Cancer Research
ZRG1 OBT-D (55)
04/11/2016 - 04/12/2016

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Consultants are required to absent themselves from the room
during the review of any application if their presence would
constitute or appear to constitute a conflict of interest.