Dr. Avrum Spira,

Associate Editor

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Dear Dr. Spira,

Thank you very much for the provisional acceptance of our manuscript entitled “Non-invasive Analysis of the Sputum Transcriptome Discriminates Clinical Phenotypes of Asthma” (#Blue-201408-1440OC.R2). We greatly appreciate the reviewers’ efforts. We have responded point by point to the comments and revised the manuscript accordingly. In these responses, the reviewers’ comments are in bold italics and our responses are below each comment in normal font.

**Reviewer #1:**

***C1. The authors have addressed my concerns.***

R1: We thank the reviewer for reviewing our manuscript.

**Reviewer # 2**

***Minor Comments:***

***Minor C1 There is no mention of the previous gene expression endotyping literature in the intro. While this doesn’t need to be discussed in depth, there should really be at least passing mention that there is previous work in this field (particularly if there is a comparative analysis to it), not just clustering based on clinical features.***

Minor R1: We thank the reviewer for the comment. We did mention the previous gene expression endotyping literatures (5. Woodruff et al. and 6. Peters et al.) in the introduction section in the first paragraph of page 2. In addition, we have also cited the other gene expression endotyping paper (10. Baines et al.) in the introduction section (first paragraph on page 3).

***Minor C2 There needs to be more mention of the limitations of the Th2 comparison, or only the comparison using the Th2 signature as a continuous variable should be mentioned, instead of dividing into Th2 high and low. The boxplots the authors show in the supplement now using the Th2 associated gene expression as a continuous variable show that there is a separation in these signatures between clusters. However, the authors state in the discussion that they found that only ~10% of subjects could be classified as Th2 high, which I think was determined using the same statistical techniques as in the sputum Th2 gene expression paper (Peters et al, JACI). This classification of Th2 high vs low may not have been appropriate given the differences in techniques used by the authors here and those in previous studies (array vs PCR for sputum, differences in sputum processing and RNA extraction techniques). Furthermore, heavy ICS use in this cohort may have diminished the Th2 signature expression as has been shown previously (Woodruff et al, AJRCCM), making the difference between Th2 high and Th2 low less pronounced in the cohort.***

Minor R2: We thank the reviewer for the comment. To be clear, the analysis that divided the patients in YCAAD cohort into Th2 high and low groups was not mentioned in either the manuscript or the supplement. We only included this analysis in the response to the reviewers. In the supplement, only the comparison using the Th2 signatures as continuous variable was mentioned in the supplement. In the discussion section, we claimed that the overlap between TEA cluster and Th2 high/low concept is relative weak based on the following two facts. First, the asthma patient population recruited in the Woodruff et al. did not include significant number of severe asthma patients while YCAAD cohort has recruited patients with more complete spectrum of severity. This has lead our study to find two subgroups among patients with relatively lower Th2 gene signatures, or Th2 low group based on the continuous Th2 signature comparison. In addition, the allergic inflammation pathways KEGG pathways were not a pathway that drove the TEA clustering results. These suggested that the TEA clusters are driven by biologic phenomena that are upstream or possible parallel to Th2 inflammation.

***Minor C3 It would be important to note how many subjects were taking ICS as this greatly effects gene expression in the airway and ICS use may have affected Th2 signature expression. Only ICS dose is shown in the paper currently, not how many subjects were taking ICS.***

Minor R3: We thank the reviewer for the comment. We have added the descriptive statistics for ICS use (yes or no) in Table 1A.