Drs. Molenberghs and Tsiatis

Editors, Biostatistics

Oxford University Press

Dear Drs. Molenberghs and Tsiatis

Please find attached our original manuscript (now revised), entitled, “Mixture Models for Single Cell Assays with Applications to Vaccine Studies”.

This paper presents novel statistical methods and an associated R software package for modelling vaccine immune responses from paired count data derived from single-cell assays like flow cytometry intracellular cytokine staining or Fluidigm single-cell gene expression. These assays routinely arise in immunological studies and vaccine clinical trials and are used to measure subject-specific immune response to vaccine via antigen-challenge experiments. It is critical that statistical methods used to analyze this data maximize sensitivity and specificity, since effect sizes and sample-sizes (i.e. numbers of cells measured per subject) are generally small, while the estimated vaccine response rates are generally used as surrogates for potential vaccine efficacy and determine, in part, whether candidate vaccines progress from phase I trials.

In our paper we present a new statistical model based on a mixture of Beta-binomial or Dirichlet-multinomial (in the multivariate case) distributions that models the observed, subject-paired pre- and post-antigen challenge count data directly. Rather than testing the empirical proportions, we implement a Bayesian approach to shrink the estimated proportions towards a common mean amongst the vaccine responders and non-responders, respectively, thus sharing information amongst subjects in order to increase the sensitivity of our approach. We use the marginal likelihoods to perform model selection returning an easily interpretable posterior probability of vaccine response for each trial subject. We show that our method has greater sensitivity and specificity than current standard approaches to analyze such data and demonstrate further applications of the method on novel single-cell assays including Fluidigm single-cell gene expression as well as multivariate extensions.

Knowledgeable referees for this work may be:

1. Martha Nason ([mnason@niaid.nih.gov](mailto:mnason@niaid.nih.gov)), Biostatistics Research Branch,NIAID

2. George Luta ([gl77@georgetown.edu](mailto:gl77@georgetown.edu)), Biostatistics, Georgetown University

3. Lori Dodd ([doddl@mail.nih.gov](mailto:doddl@mail.nih.gov)), Biostatistics Research Branch, NIAID

4. Cliburn Chan ([cliburn.chan@duke.edu](mailto:cliburn.chan@duke.edu)), Biostatistics, Duke University School of Medicine

We would like to suggest Dr. Naisin Wang or Dr. Alex Lewin as Associate Editors to handle our manuscript.

We believe our work represents an innovative application of statistical methodology to address the problem inferring response to vaccine from single cell assay data in clinical trials and will be a good fit for your journal. We welcome the opportunity to share our results with its readership. We thank you for your consideration of our work.

Sincerely,

Greg Finak, PhD

Vaccine and Infectious Disease Division

Fred Hutchinson Cancer Reasearch Center

Seattle, WA, 98101

gfinak@fhcrc.org