Study Protocol and Statistical Analysis Plan

Study Title: Exploratory Investigation of Changes in Gene Transcription and Immunophenotypes Following Mepolizumab Treatment for Asthma

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Study Population

Study subjects: This study involved twenty adult (≥18yo) individuals with a diagnosis of severe eosinophilic asthma (SEA) who have been prescribed mepolizumab during their course of routine clinical care. Children (<18yo) will be excluded from this study.

Inclusion criteria:

1. Diagnosis of SEA by a health care provider
2. History of an absolute eosinophil count ≥ 300/mm^3
3. Prescription for mepolizumab provided during course of routine clinical care but mepolizumab not yet started

Exclusion criteria:

1. Age <18 years old
2. Pregnancy
3. Currently using or have used within 3 months of the initial baseline visit any biologic or immunomodulatory therapy with the exception of #3
4. Currently using or any prior use of rituximab
5. History of upper/lower respiratory tract infection or asthma exacerbation within the previous four weeks of the first baseline visit
6. Any prior history of malignancy, autoimmune disease, or immune deficiency
7. Any other significant medical issue as determined by the principal investigator

Methods

Each study subject had sequential whole blood samples obtained at four time points. The first time point was prior to receiving the initial dose of mepolizumab. Thereafter, blood samples were obtained at 4 weeks, 8 weeks, and 12 weeks post-initiation of mepolizumab (prior to each subsequent dose of mepolizumab). Blood obtained was isolated for PBMCs to undergo immunophenotyping by mass cytometry (CyTOF) and to undergo RNA sequencing.

Data Collection

In addition to usual demographical variables and record of meeting inclusion criteria, each research subject had the following primary data collected:

1. Differentially expressed gene transcripts identified by RNAseq from PBMCs
2. CyTOF mass cytometry (50 test immune phenotype panel including cell surface markers, transcription factors, and cytokines) performed on PBMCs isolated from peripheral blood
3. Laboratory values in accordance with clinical guidelines and standard of care such as CBC with differential, liver function studies, etc.
4. Record review of allergy testing, pulmonary function testing, etc. in accordance with clinical guidelines and standard of care
5. Record review of cllinical course and associated therapies such as corticosteroids, leukotriene modifiers, etc

Study Endpoints

Subject participation ended after the fourth blood sample was obtained. Primary analysis was descriptive of data collected. Secondary analysis involved associations between changes in gene transcripts and immunophenotypes and the respective clinical correlates.

Data analysis

Our institution sequencing core has developed MAP-RSeq - a comprehensive computational pipeline for secondary analysis of RNA- Sequencing data. MAP-RSeq uses a variety of freely available bioinformatics tools along with in-house developed methods. The main goal of the MAP-RSeq workflow is to align, assess and provide multiple genomic features from transcriptomic sequencing data for further tertiary analysis.

For CyTOF data, FCS files were exported and analyzed using Cytobank (Cytobank, Inc., Mountain View, CA). Doublets and debris will be excluded using standard methods. Non-hierarchical clustering was performed using viSNE. Expression levels were displayed as median intensities in all viSNE plots.