Sub-study: ISS 11066

Sub-study involves participants who are suitable for study inclusion and who have provided Informed Consent in the 'Choosebetweenamab' trial are already being randomised (1:1) using to receive either open label Mepolizumab or Omalizumab for 6 months. Site personnel are unblinded to the randomisation process. From the blood collected in the 'Choosebetweenamab' study at baseline white blood cells are isolated by fractionation with red blood cell removal. The leukocyte populations will undergo single cell transcriptomic sequencing (scRNA-seq) procedure. This process will label each cell with unique barcodes for downstream de-multiplexing in the bioinformatics pipeline. Libraries will be sequenced using Illumina NovaSeq 6000 system. Data generated will be compared to clinical treatment effect for each of these patients in the 'Choosebetweenamab' trial. This will develop a gene based prediction algorithm that could be later translated into a protein-based assay. Transcriptional analysis at the single cell level will enable this biomarker prediction to be narrowed down to individual cell types. This data will provide a biomarker signature in patients with severe allergic/eosinophilic asthma before undergoing treatment, with the aim to predict response to Mepolizumab or Omalizumab, non-response to either, and treatment effect.

Data analysis plan

All bioinformatics and statistical analysis will be performed by the CReDITSS service unit at HMRI. Data will be initially processed to get count matrices by CellRanger. Cell populations will be identified by clustering. We will then perform differential expression analysis on all cells in a given cluster (cell type). Biomarkers to predict response, non-response and treatment effect as in the 'Choosebetweenamab' trial.

The clinical parameters for comparison to the single cell gene expression data will include:

Change in ACQ5 after 6 months of treatment, adjusted for baseline ACQ5.

Number of Exacerbations, requiring change in oral corticosteroids, with either a course of prednisone for at least 3 days or, for those subjects on maintenance OCS, an increase in dose of at least 50% for at least 3 days.

Time to first exacerbation

Number of admissions to hospital and /or ED presentations

Reduction in dose of regular OCS

Reduction in total OCS use during the 6 month treatment period
Changes in spirometry (FEV1 or FVC)
Change in blood eosinophil count
FeNO
Proportion continuing on Australian PBS treatment (successful treatment)
Adverse events

Treatment non-response: After 6 months of treatment subjects will be clinically assessed to determine if the treatment has succeeded or failed. The following criteria will deteremine whether the treatment has failed:
1.No improvement in ACQ5 of at least 0.5 (minimum clinically important difference) from baseline, or
2.No reduction in regular prednisone dose or intermittent prednisone usage by at least 15% or
3.An intolerance to the agent or the emergence of clinically significant side-effects.

For each treatment arm, we will compare the scRNA-seq data for each patient to each of the clinical treatment effect outcomes. For the continuous outcomes variables (e.g. ACQ5, FEV1 etc) we will employ a generalised linear regression model with a normal response distribution and identity link function. This model will explore the differential gene expression from the scRNA-seq datasets associated with the defined clinical outcomes. The model will be fitted in certain cases with an ANCOVA framework (i.e. adjusted for baseline). The model will include fixed effects for group and baseline eosinophil count. The treatment effects will be estimated as baseline-adjusted, least-square mean differences at 6 month follow-up. The generalised linear model provides unbiased estimates of treatment effect under the assumption that data are missing completely at random. Sensitivity analyses such as multiple imputation and pattern mixture modelling will be used to investigate the robustness of conclusions to different missing data mechanisms. Once specific molecular biomarkers have been defined for specific clinical outcomes the analyses will rank them using receiver operating characteristic (ROC) curves. We will also test the data using random forest models (minimum log2-fold change, with n-fold cross-validation), support vector machine, and LASSO classifiers trained on the transcriptomes of single cells. These models will be used to identify and rank the most discriminatory molecular markers (ranked by ROC curves) for each cell type between responders (continuation on PBS treatment) and non-responders to therapy, as well as specific positive treatment effects. The output from this analysis will

consist of a genelist for each therapy that predicts treatment effect. For the categorical variable of ‘occurrence of any adverse reactions’ we will run logistic regression modelling for the transcriptomes of each clustered cell type across each patient.