STOP-COVID19: Superiority Trial Of Protease inhibition in COVID-19

Multi-centre prospective, randomized placebo-controlled trial in patients presenting to hospital with PCR confirmed COVID-19 (SARS-CoV-2 infection). Planned Sample Size was 300 across the UK. Treatment duration was 28 days, with 29 days follow-up.

## Study rationale and hypothesis:

COVID-19 can cause substantial morbidity and mortality.Mortality in acute respiratory distress syndrome (ARDS) correlates directly with the extent of neutrophilia in the lung. Both human clinical data and murine studies demonstrate a key role for neutrophils in ARDS. Neutrophil proteases and particularly neutrophil elastase are believed to be central to the neutrophil induced lung damage.

Neutrophil elastase, proteinase-3 and cathepsin-G are activated during neutrophil maturation in the bone marrow through dipeptidyl peptidase 1 (DPP1; also known as cathepsin C), which removes the N-terminal dipeptide sequence of neutrophil serine proteases allowing active enzymes to be packaged into granules prior to release of neutrophils into the circulation. Brensocatib (INS1007, formerly AZD7986) is an orally delivered selective, competitive, and reversible inhibitor of DPP1. Brensocatib has been shown to inhibit neutrophil serine protease activity in blood in both animal models and healthy volunteers and in the airways of patients with the chronic respiratory disease Bronchiectasis.

**We hypothesised that Brensocatib, by blocking damaging neutrophil proteases, would reduce the incidence of acute lung injury and acute respiratory distress syndrome (ARDS) in patients with COVID-19, thereby resulting in improved clinical outcomes.**

## Study overview:

In total 144 participants were enrolled in Dundee, and 17 enrolled in Sheffield, both of these sites obtained longitudinal research blood samples from participants whilst hospitalised, for exploratory analyses to better understand Brensocatib mechanisms of action. Participants were also invited to return for sampling a day 29 if discharged, resulting in larger sample numbers at day 29 than at intermediate timepoints. Isolated peripheral blood neutrophils across various timepoints have been processed and analysed with LC-MS, utilising Spectronaut software for protein identification.

## Major questions for initial proteomic data analysis:

-Does treatment with Brensocatib alter the COVID-19 peripheral blood neutrophil proteome across the study period, compared with Placebo treatment?

-Do factors such as age, gender, or time from symptom onset influence responses to Brensocatib within the neutrophil proteome?

-If possible, the above would be performed in the study population who completed the trial (completed=1), and then would also be performed subsequently as an intention-to-treat analysis, including all participants (both completed=1 and completed=0)

# Data Analysis Group Quotation Reference: 150422-JC03

Project Title: Neutrophil/PBMC proteomic analysis

## 1. PI Details

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## 2. Background

From May 2020 we carried out a trial of dipeptidyl peptidase-1/Cathepsin-C inhibitor in hospitalised patients with COVID-19. In total enrolled 144 participants in Dundee. We have data from around 250 neutrophil samples across various timepoints which have been processed and analysed with LC-MS, subsequently utilising Spectronaut software for protein identification. Comparisons of drug vs. placebo groups at 4-5 timepoints is requested, with possible sub-analyses for e.g. age and gender, time from symptom onset. Batch correction across 3-5 batches would also be needed, as significant batch effects were found in other analyses of the proteomics data. If possible we would also do the same thing for the PBMC samples later, however the neutrophil data would be a first priority.

## 3. Description of Proposed Work

This is a large and complex experiment, so would be better to cost in stages. The first stage would include:

* overview of the data
* quality control
* multidimensional analysis: finding important and non-important factors, dealing with batch effects
* differential abundance on selected conditions (e.g. day 29 vs day 1, drug vs placebo at a given time point)
* multivariate differential abundance on time + treatment + batch, etc. This might not work if data are not “clean”, e.g., if timing behaviour is complex (i.e. not a simple trend)
* simple timing analysis, identifying proteins consistently changing up or down
* providing a simple interactive tool to look at the above results