We are trying to understand at a basic level why some patients with the virus don’t show any symptoms at all, while others need to be hospitalised and some sadly die.
Summary

• Our initial high throughput in-depth analysis of over 60 hospitalised Covid-19 patients and over 50 controls has revealed:

  1. An immunological signature of Covid-19 disease
  2. Several discrete components of that signature that track with severity

Potential importance

• SARS-CoV-2 virus is not the threat to global stability; that threat stems from the seriousness of the disease that the virus has the potential to cause

• Understanding the basis for serious disease symptoms may permit new approaches to diagnosis and therapy that may considerably reduce the risk that infection by SARS-CoV-2 would lead to severe, potentially fatal disease.
Scene setting

- Despite most exposures being pauci-symptomatic, Covid-19 has been the most socially-transformative infectious disease in living memory - Millions infected; hundreds of thousands dead or only gradually recovering from severe disease - Precipitous burden on health care.

- The virus has not gone away – major rises in global infections continue to be reported, and may “re-surge” in autumn. Effective anti-virals and vaccination are still in development.

- Serious symptoms have been associated with underlying morbidities, age and gender, but are there other factors?

- Serious symptoms are clinically associated with lymphopenia, CRP, and some other metrics, but what mechanisms and processes do these reflect?
Hypothesis for Covid-IP

• The immune system lies at the heart of the host-pathogen relationship and may track with and/or underpin disease course.

Key questions

• Can we identify biologically meaningful markers or ‘immune signatures’ that predict disease course at the front door?

• Can we identify ‘immune signatures’ in patients who do well? This will guide us toward “correlates of protection”, informing vaccine design and helping to predict the likely efficacy of current or proposed medications that can be re-purposed?

• What is the relative contribution to sustained protection of T cells versus antibody production?

• What is the nature of covid-19 lymphopenia and what are its implications - an impact on innate→adaptive switch?
The Covid-IP Pipeline *

Day 1, 3, 9
Co-ordination with other studies

Serology; IgG/IgM [S/RBP/N]
ELISA- Dr Katie Doores
LIPS – Professor Pärt Peterson (Tartu)

Legendplex assay – plasma cytokines, inc. IL-6, IL-8, IP10, IL10
Additional analytes, including Tryptophan metabolites.
Autoantibodies

8 panel flow cytometry on fresh specimens to measure cell types, cell status, and cell cycling

Individual CD4 and CD8 T cells are sorted and DNA extracted for T cell Receptor repertoire sequencing

Research Nurse Collections

Plasma

Leukocytes

Biobank storage for future projects

Heparin

PAXgene for RNA

*facilitated by the King’s College Infectious Diseases Biobank
The COVID-IP cohort:
hospitalized SARS-CoV-2 infected patients, ~80% of whom recovered without intensive care, and 20% of whom declined, with ~10% declined irreversibly.

Clinical conditions were classified according to a combination of local clinical practice and WHO classifications.
What we have learned

This is a preliminary data-set, made broadly available in the hope that it can guide and/or inform others internationally. More formal peer-reviewed data releases will follow, respectful of compliance with appropriate scientific standards.
T cells are one of the major components of the **adaptive immune system**.

**CD4+ Helper T (Th) cells** help to shape, activate and regulate the adaptive immune response by activating other immune cells, releasing cytokines, and helping B cells produce antibodies.

**CD8+ Cytotoxic T cells** recognise and directly kill virus-infected cells.

Covid-19: both Helper and Cytotoxic T cells are diminished with disease, with some link to severity.
Covid-19: T cell lymphopenia is selective: T helper (Th) cell subsets are differentially affected

The impact on CD4+ Helper T cells is selective
Th1, Th17.1 cells are diminished, while Th2 and Th17 are relatively unaffected
Covid-19: Other CD4⁺T cell subsets are also differentially affected

Naïve T (Tₐ) cells have not yet encountered their specific, and antigen are usually resting

Effector Memory T (Tₑᴹ) cells will interact with host cells to carry out their immune function

Central Memory T (Tₑᴹ) cells act as a reservoir of our response to secondary infections

CD₄ₑᴹRA - terminally differentiated effector memory cells - are temporarily exhausted

Reduced levels of CD₄ naïve and CD₄ₑᴹ in Covid-19, while CD₄ₑᴹ and CD₄ₑᴹRA cells are largely protected
Covid-19: The impact on CD8$^+$ T cells is also selective.

- **Naive T (T$_N$) cells** have not yet encountered their specific antigen and are usually resting.
- **Effector Memory T (T$_{EM}$) cells** will interact with host cells to carry out their immune function.
- **Central Memory T (T$_{CM}$) cells** act as a reservoir to our response to secondary infections.
- **T$_{EMRA}$ - terminally differentiated effector memory cells** are temporarily exhausted.

As for CD4 cells, CD8$_{naive}$ and CD8$_{EM}$ cells are reduced in Covid-19; but no clear link to severity.
Covid-19: remaining T cells, although low in numbers, are actively traversing the cell cycle and this is linked to severity.
Covid-19: a collapse of Vδ2 T cells is a disease signature, that is particularly striking in some patients.

γδ TCR+ T cells express a γδ-TCR rather than the αβ-TCR on the cell surface, and are a predominant lymphoid population in body surfaces such as the lung which is targeted by SARS-Cov-2.

There is a collapse in numbers of the predominant blood γδ cells type (Vδ2+). Conversely, Vδ1+ T cells show increased representation and their active cycling is severity associated (not shown).
Innate immunity markers
Loss of basophils from the blood appears as a biomarker of COVID-19 severity

Basophils assessed via flow cytometry [gated as non-PMN based on ssc and then CD3neg, CD19neg, HLADRneg, CD14neg, CD123+]

Basophils are potentially disease-relevant because of their homing to the lung, wherein Covid-19 disease is manifest, and because of their implication in regulating blood coagulation
In sum, several biomarkers of COVID-19 severity are emerging:

- Cell cycling of CD4_{EM}, CD4_{CM}, CD8_{EM}, Vδ1 cells
- Depletion of specific CD4 and CD8 T cell subsets
- Basophil counts crashing
- Neutrophil counts increasing
- Crash in plasmacytoid dendritic cells
- Sustained IL6, IP10, IL10
- CRP

Other major changes, e.g. including plasmablast expansion, major changes in monocyte populations; reductions in CD5⁺ B cells, change in DC phenotypes, and production of antibodies compose a disease-associated signature but are not obviously linked to severity.
Take home messages

• Phenotyping ➔ define perturbations in immunity ➔ design a ‘panel’ of descriptive markers tied to severe disease ➔ prospectively evaluated as a predictive score for severity ➔ clinical endpoint e.g. decision aid for early ICU admission

• Phenotyping ➔ insight into disease pathology ➔ ongoing investigation into what drives T lymphopenia and how this impacts on memory responses ➔ informs Vaccine design

• Data will be freely available on www.immunophenotype.org.

• Applications alongside GSTT clinical trials e.g. phenotyping before and after therapeutic interventions (e.g. IL7, anti-IL6) ➔ mechanism of action
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