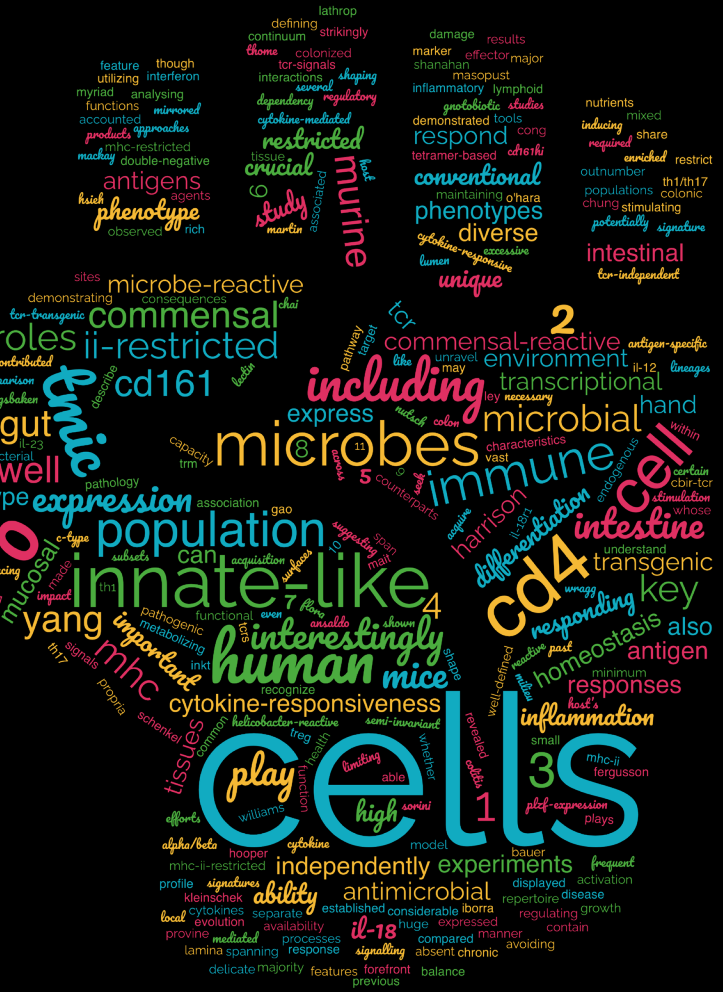
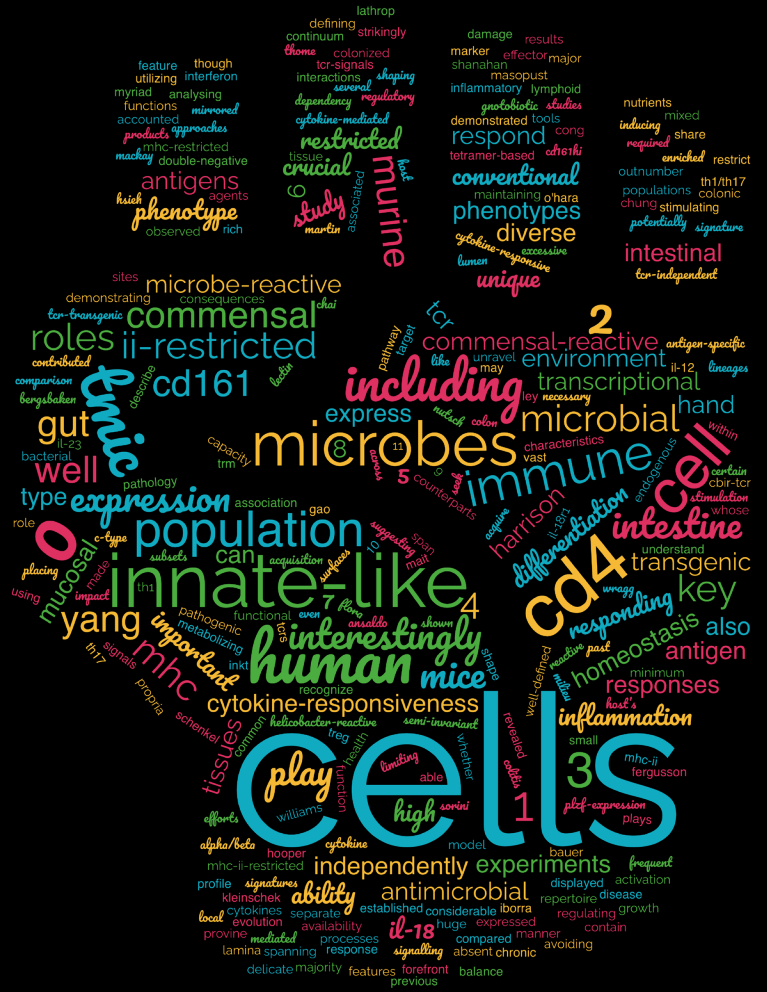


Immune protection and Pathology: New tales from new tools



Immune protection and Pathology: New tales from new tools

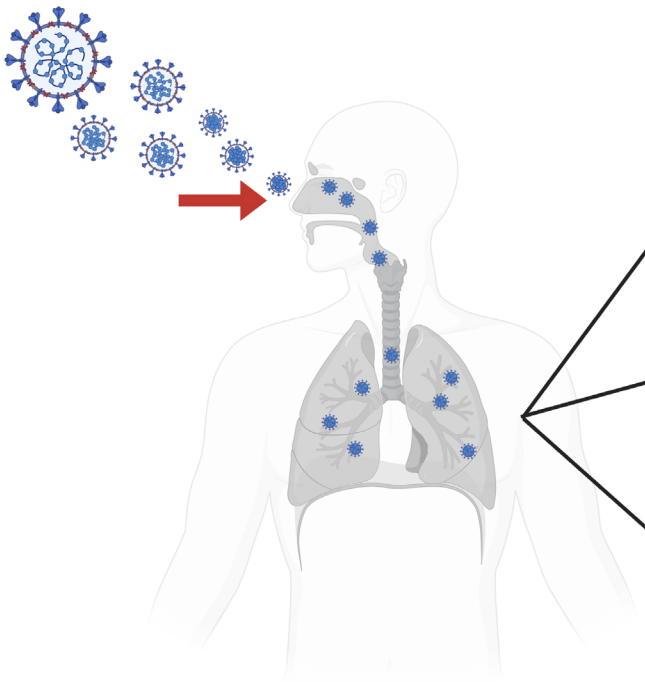
Towards an Atlas of Infectious Diseases

Talk outline

- What we can learn from detailed studies of the host response to infections.
- How new broader immune tools can be applied (Covid, gut inflammation).
- Where this could go in the future.

Immunology and COVID-19 outcomes

Sars-CoV2 infection



OUTCOME

asymptomatic

mild:
flu-like symptoms

severe:
pneumonia, death

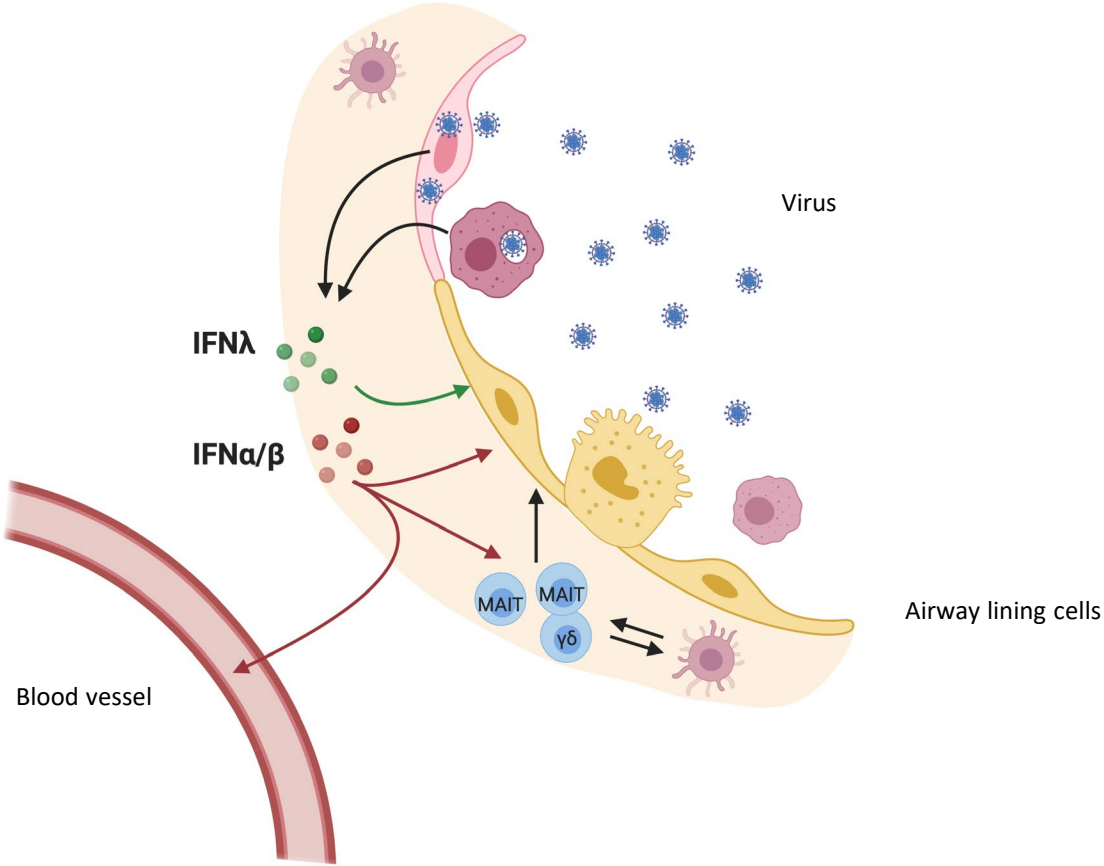
Factors determining outcome

- age, sex

- underlying diseases

- quality and magnitude of the immune response?

Front line workers – innate immunity



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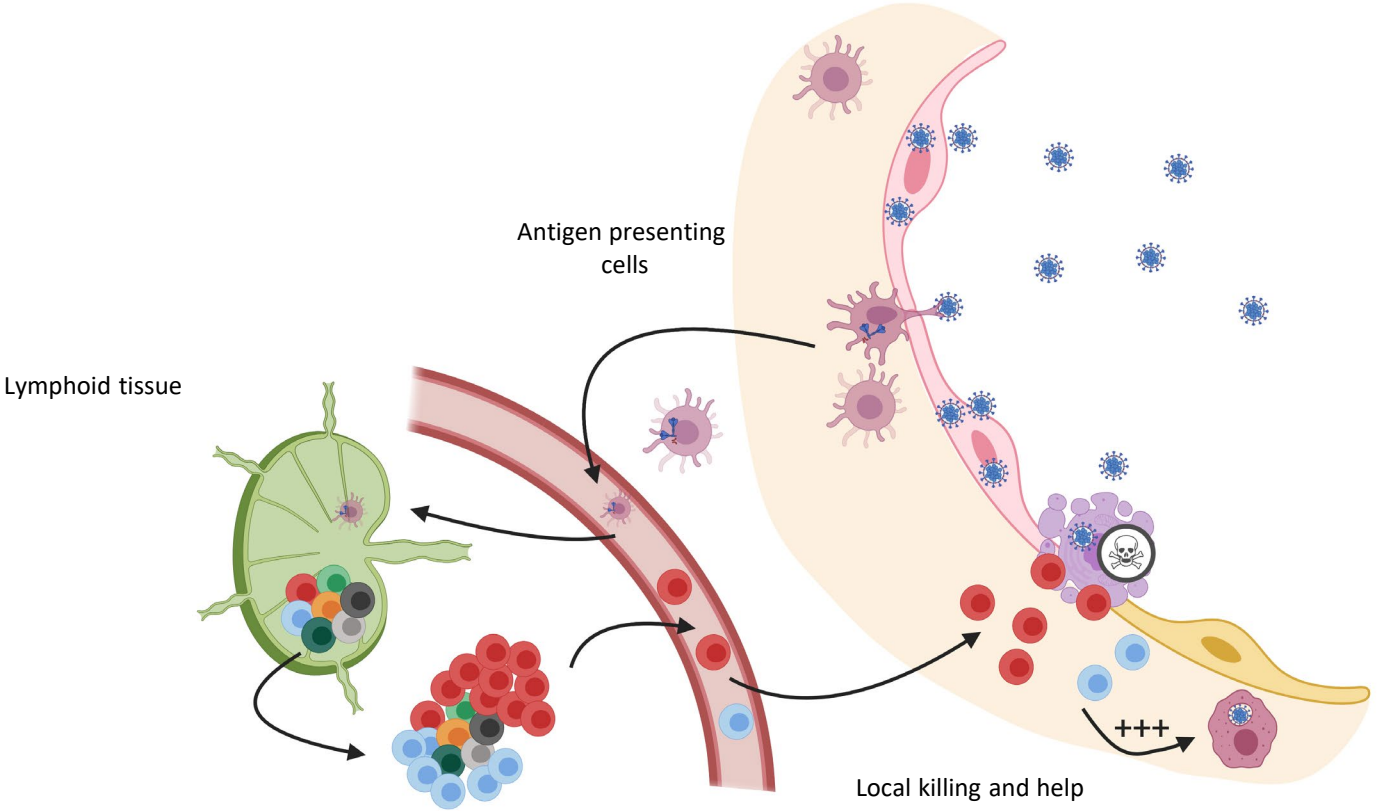
Local heroes– the T cell response



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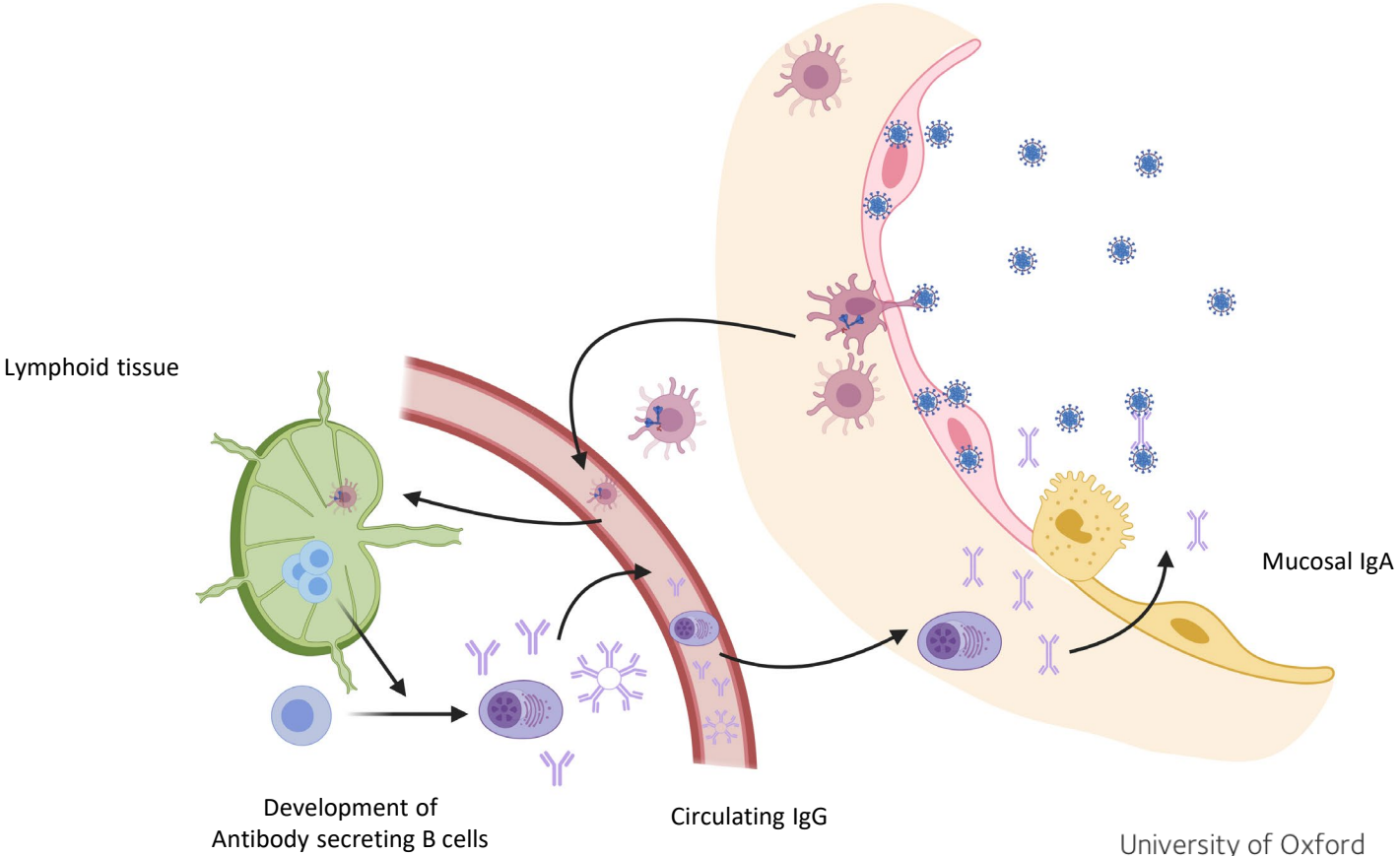
Broader cover– the B cell response



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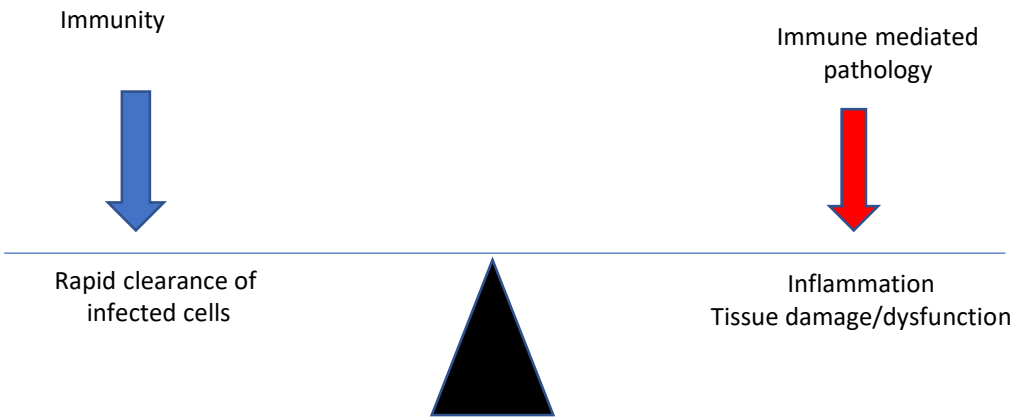
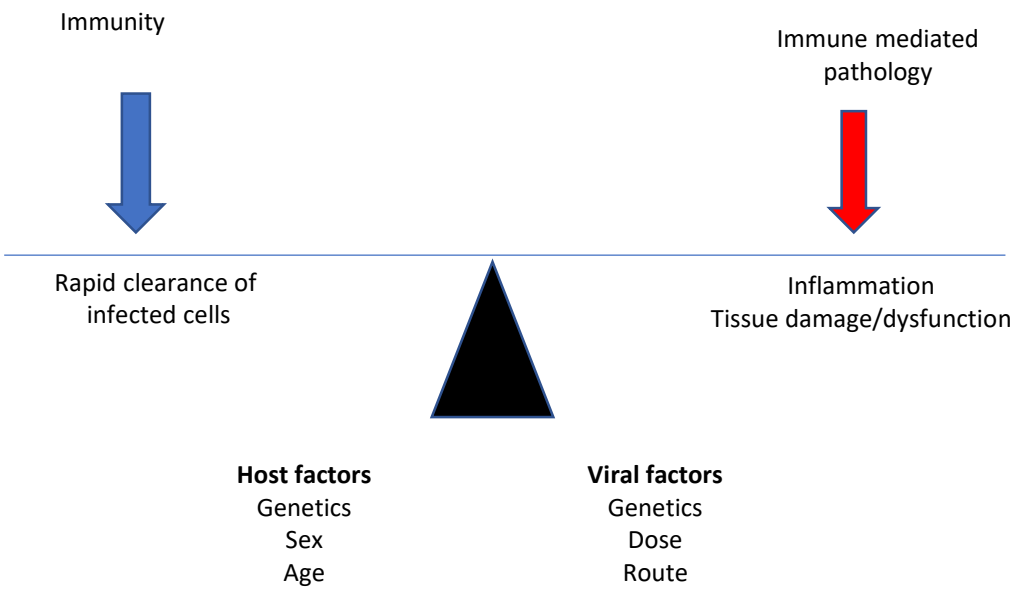
Setting the balance between immunity and immune pathology



Setting the balance between immunity and immune pathology

HIV
Hepatitis C
Hepatitis B
Influenza
SARS
LCMV (model)
etc



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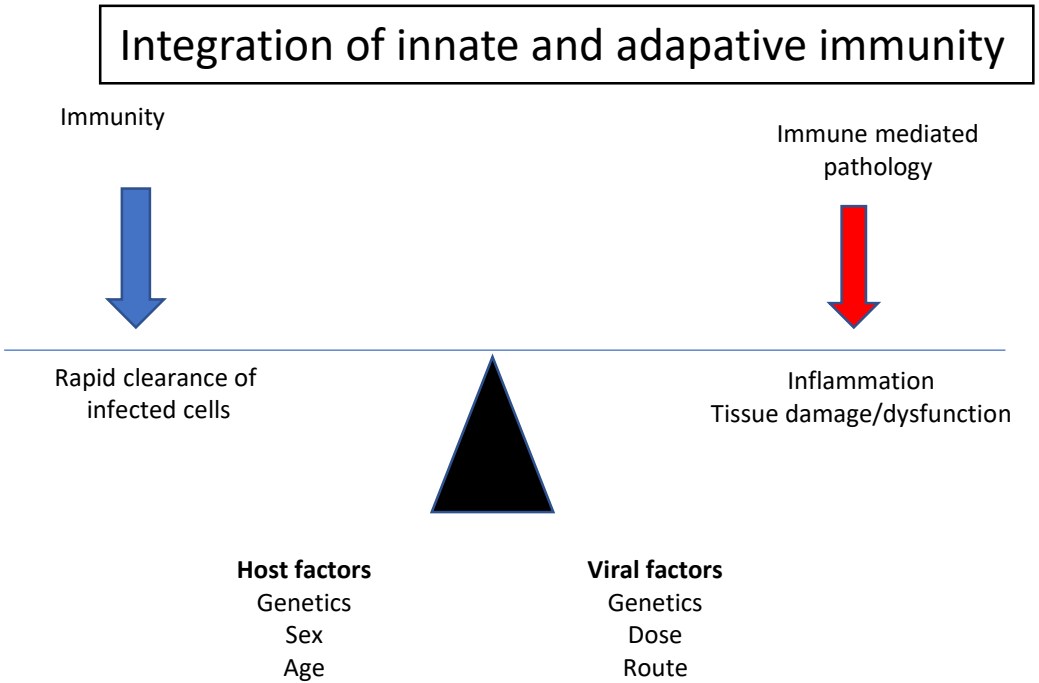
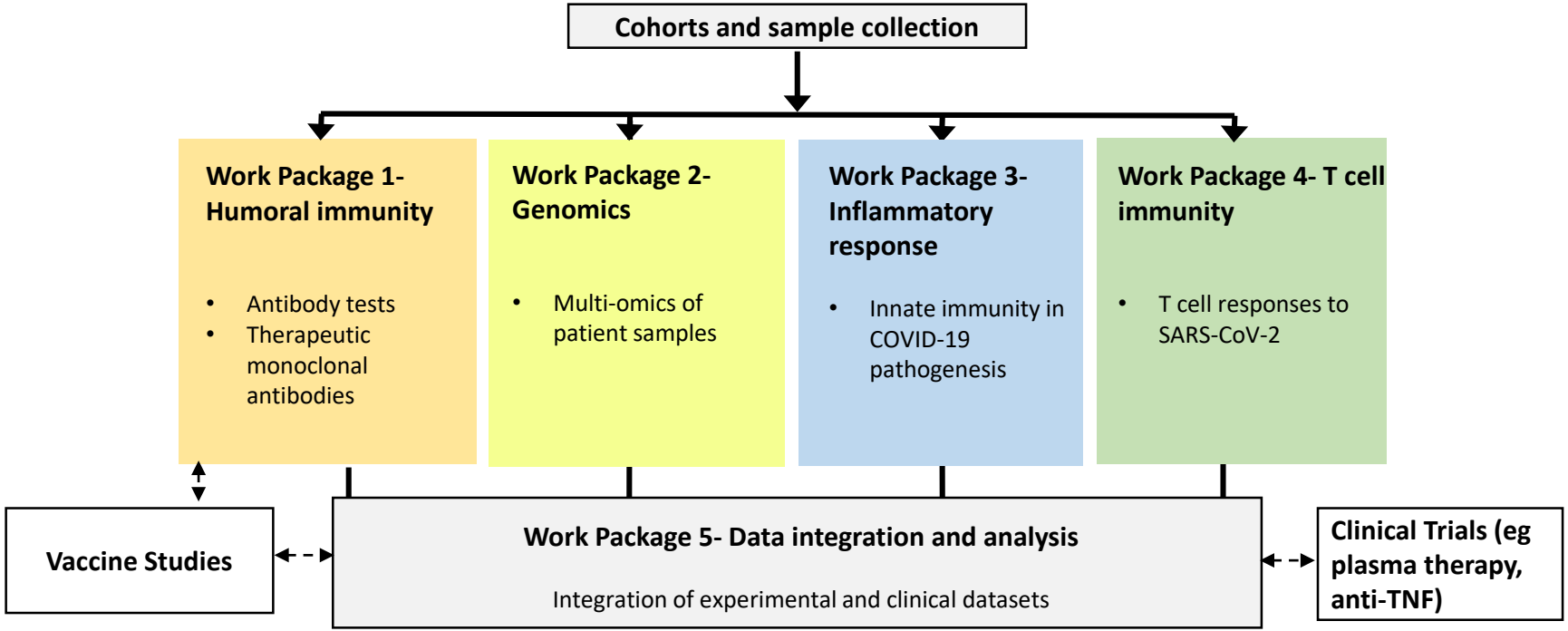


Oxford COVID-19 immunology work packages



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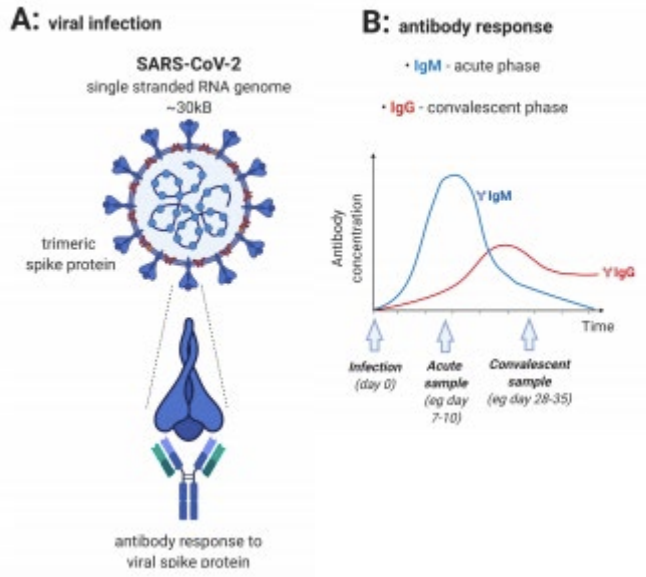
Work Package 1- Humoral immunity

Understanding and utilizing the human antibody response to SARS-CoV-2

Objectives:

- Development of **sensitive and specific antibody tests** (ELISAs) that can be utilized at scale (high throughput)
- Development of assays to detect **neutralizing antibodies** to SARS-CoV-2
- Understanding the **prevalence of infection** across populations
- Development of **monoclonal antibodies**



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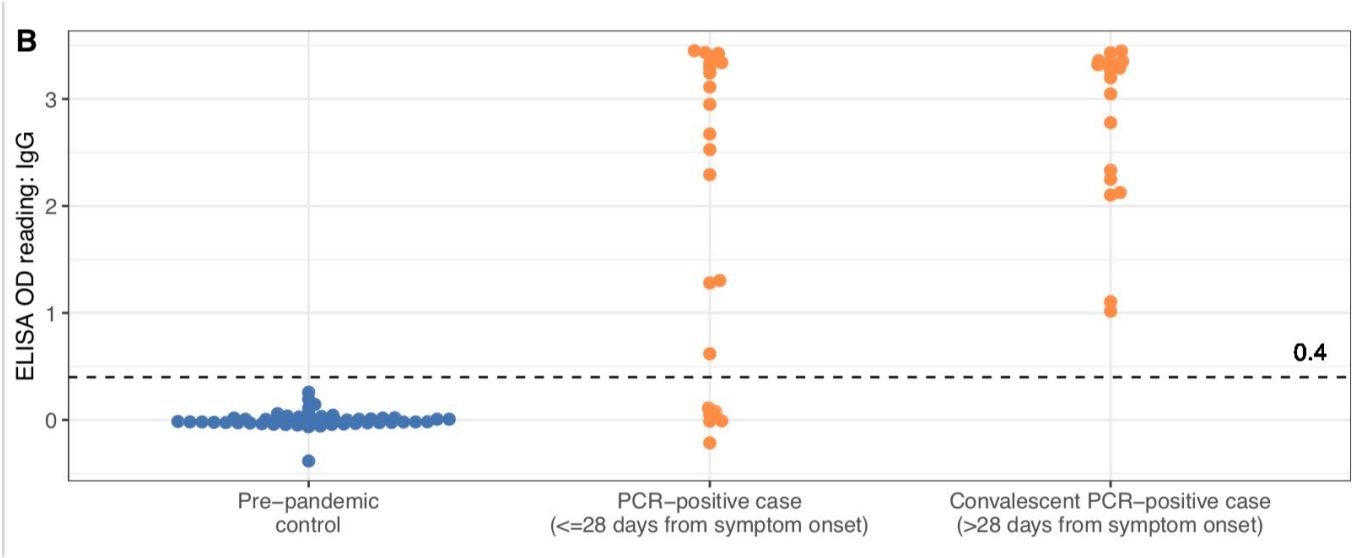

Antibody testing for COVID-19: A report from the National COVID Scientific Advisory Panel

Emily R Adams, Mark Ainsworth, Rekha Anand, Monique I Andersson, Kathryn Auckland, J Kenneth Baillie, Eleanor Barnes, Sally Beer, John Bell, Tamsin Berry, Sagida Bibi, Miles Carroll, Senthil Chinnakannan, Elizabeth Clutterbuck, Richard J Cornall, Derrick W Crook, Thushan De Silva, Wanwisa Dejnirattisai, Kate E Dingle, Christina Dold, Alexis Espinosa, David W Eyre, Helen Farmer, Maria Fernandez Mendoza, Dominique Georgiou, Sarah J Hoosdally, Alistair Hunter, Katie Jeffrey, Paul Klenerman, Julian Knight, Clarice Knowles, Andrew J Kwok, Ullrich Leuschner, Robert Levin, Chang Liu, Cesar Lopez-Camacho, Jose Carlos Martinez Garrido, Philippa C Matthews, Hannah McGivern, Alexander J Mentzer, Jonathan Milton, Juthathip Mongkolsapaya, Shona C Moore, Marta S Oliveira, Fiona Pereira, Elena Perez Lopez, Timothy Peto, Rutger J Ploeg, Andrew Pollard, Tessa Prince, David J Roberts, Justine K Rudkin, Veronica Sanchez, Gavin R Screaton, Malcolm G Semple, Donal T Skelly, Jose Slon-Campos, Elliot Nathan Smith, Alberto Jose Sobrino Diaz, Julie Staves, David Stuart, Piyada Supasa, Tomas Surik, Hannah Thraves, Pat Tsang, Lance Turtle, A Sarah Walker, Beibei Wang, Charlotte Washington, Nicholas Watkins, James Whitehouse

doi: https://doi.org/10.1101/2020.04.15.20066407

Neutralising antibodies to SARS coronavirus 2 in Scottish blood donors - a pilot study of the value of serology to determine population exposure

Craig Thompson, Nicholas Grayson, Robert Paton, José Lourenço, Bridget Penman, Lian Ni Lee, Valerie Odon, Juthathip Mongkolsapaya, Senthil Chinnakannan, Wanwisa Dejnirattisai, Matthew Edmans, Alexander Fyfe, Carol Imlach, Kreepa Kooblall, Nicholas Lim, Chang Liu, Cesar Lopez-Camacho, Carol-Anne McInally, Narayan Ramamurthy, Jeremy Ratcliff, Piyada Supasa, Beibei Wang, Alexander J Mentzer, Marc Turner, Calum Semple, John Kenneth Baillie, ISARIC4C Investigators, Heli Harvala, Gavin Screaton, Nigel Temperton, Paul Klenerman, Lisa Jarvis, Sunetra Gupta, Peter Simmonds

doi: https://doi.org/10.1101/2020.04.13.20060467

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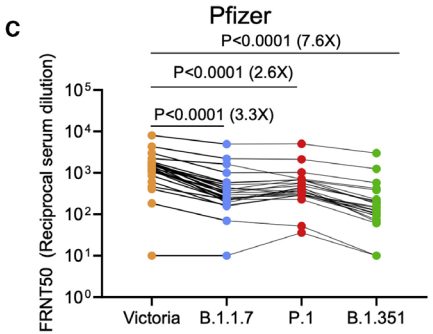
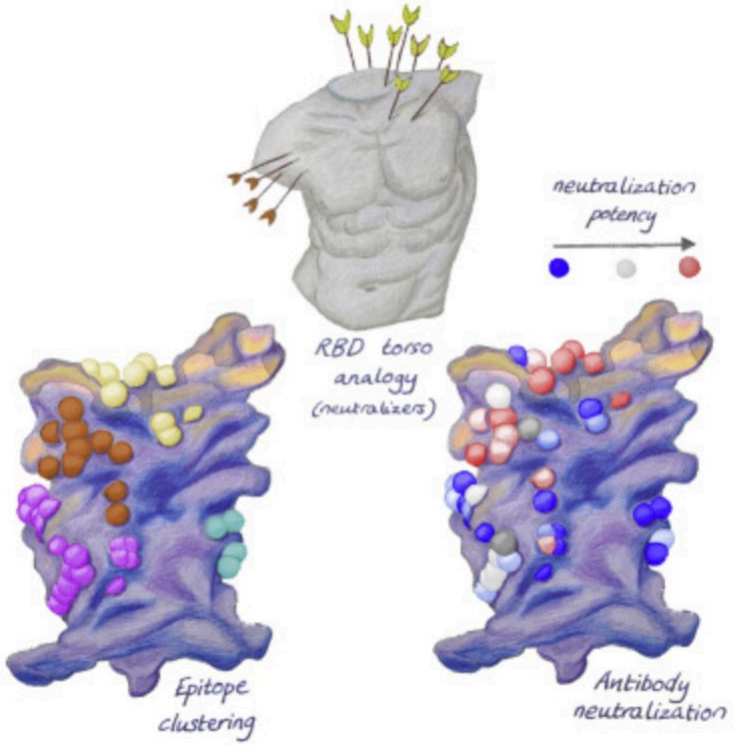
Ability of antibodies to deal with variants



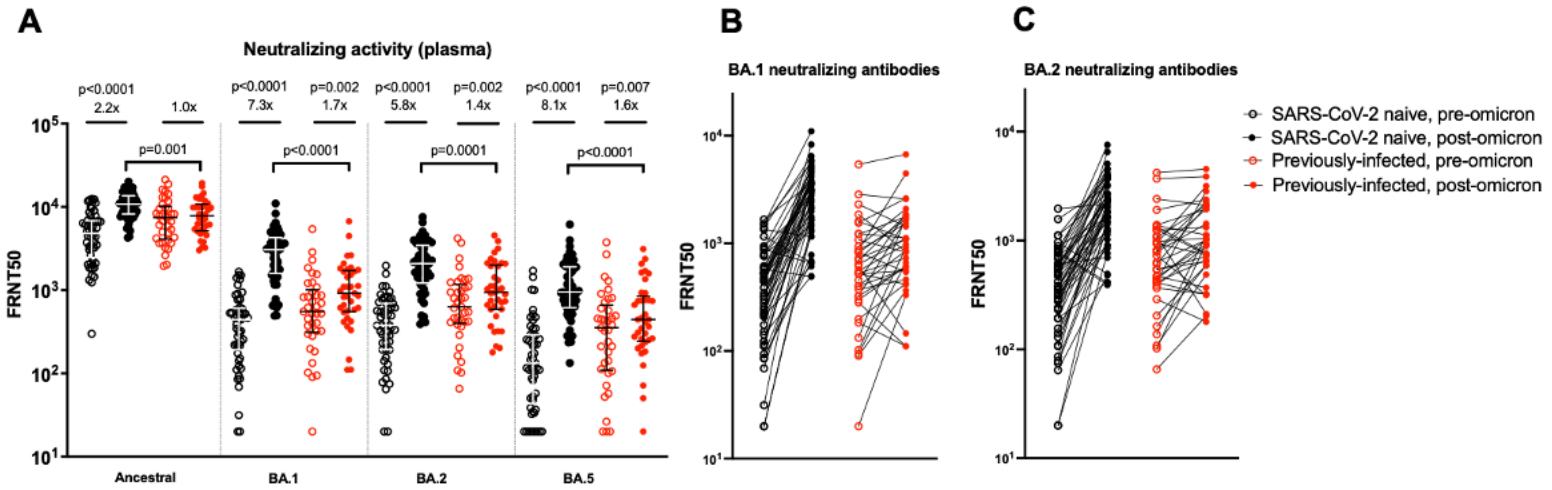
Good neutralization but impacted by mutation…

- Supasa et al Cell 2021
- Zhou et al Cell 2021
- Dejnirattisai et al Cell 2021
- Liu et al Cell 2021



Variation affects the binding of antibodies at very specific sites in the spike protein

Current status of antibodies in UK HCW: pre and post omicron wave



Hornsby et al, submitted

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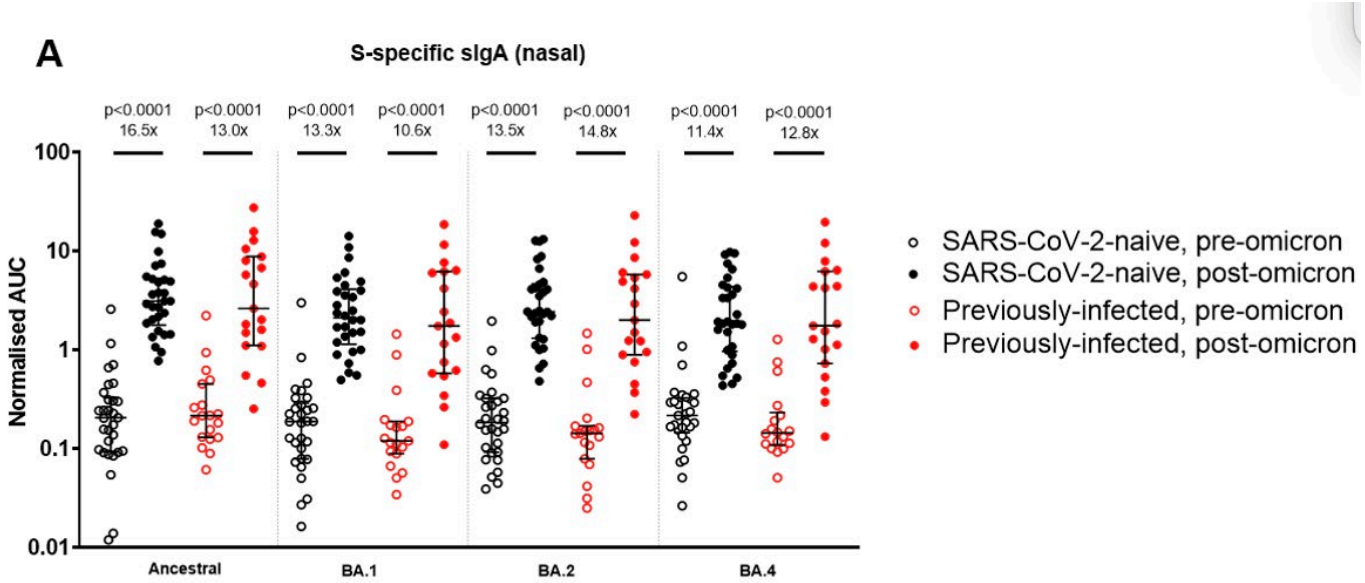


Importance of looking mucosally

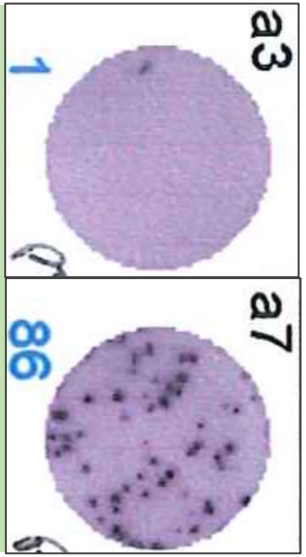
Hornsby et al, submitted

Work Package 4- T cell immunity

Define T cell responses to SARS-CoV2 to support developments in diagnostics, vaccination and therapeutics

Objectives:

- Define **immunogenic and protective** regions of SARS-CoV2
- Track antiviral T cells in blood and lung during acute infection and **define correlates of protection for vaccination**
- Define associations between T cell responses and clinical outcomes and risk groups (eg multimorbidity, ageing, bacterial co-infection)



Control

COVID19

University of Oxford
IMMUNOLOGY NETWORK

PITCH Consortium

Protective **I**mmunity from **T** cells to **C**ovid-19 in **H**ealthcare workers

Aim to provide evidence of the mechanisms of immunity to underpin vaccine effectiveness data from SIREN

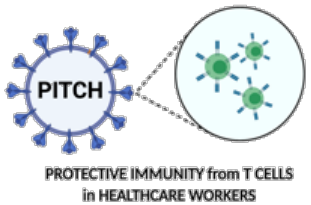
Extension of the UK SIREN Study (Antibody, PCR & Vaccine Efficacy in 50,000 healthcare workers)

Dept of Health & Social Care Funded

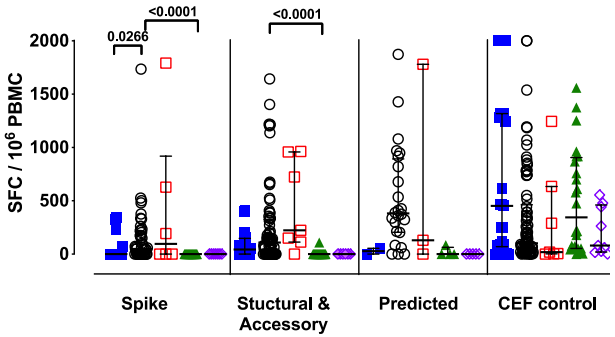
- Prospective longitudinal cohort study in 5 sites
  - Oxford (Paul Klenerman, Susie Dunachie, Ellie Barnes, Philippa Matthews, Chris Conlon, Katie Jeffrey)
  - Liverpool (Lance Turtle)
  - Sheffield (Thushan de Silva, Sarah Rowland Jones)
  - Birmingham (Alex Richter)
  - Newcastle (Chris Duncan, Rebecca Payne)

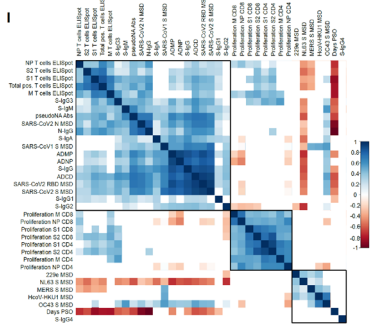
  PHE (Susan Hopkins, Meera Chand, Victoria Hall)

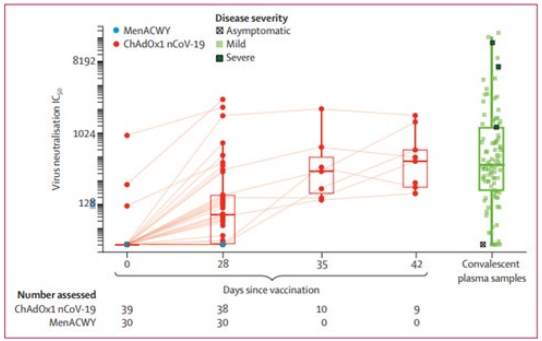
- 2074 Healthcare workers recruited to date



Baseline characterisation of T cell immunity to mild disease







Assay development. T cells can help control Covid-19 even without antibodies

- Ogbe et al Nature Comms

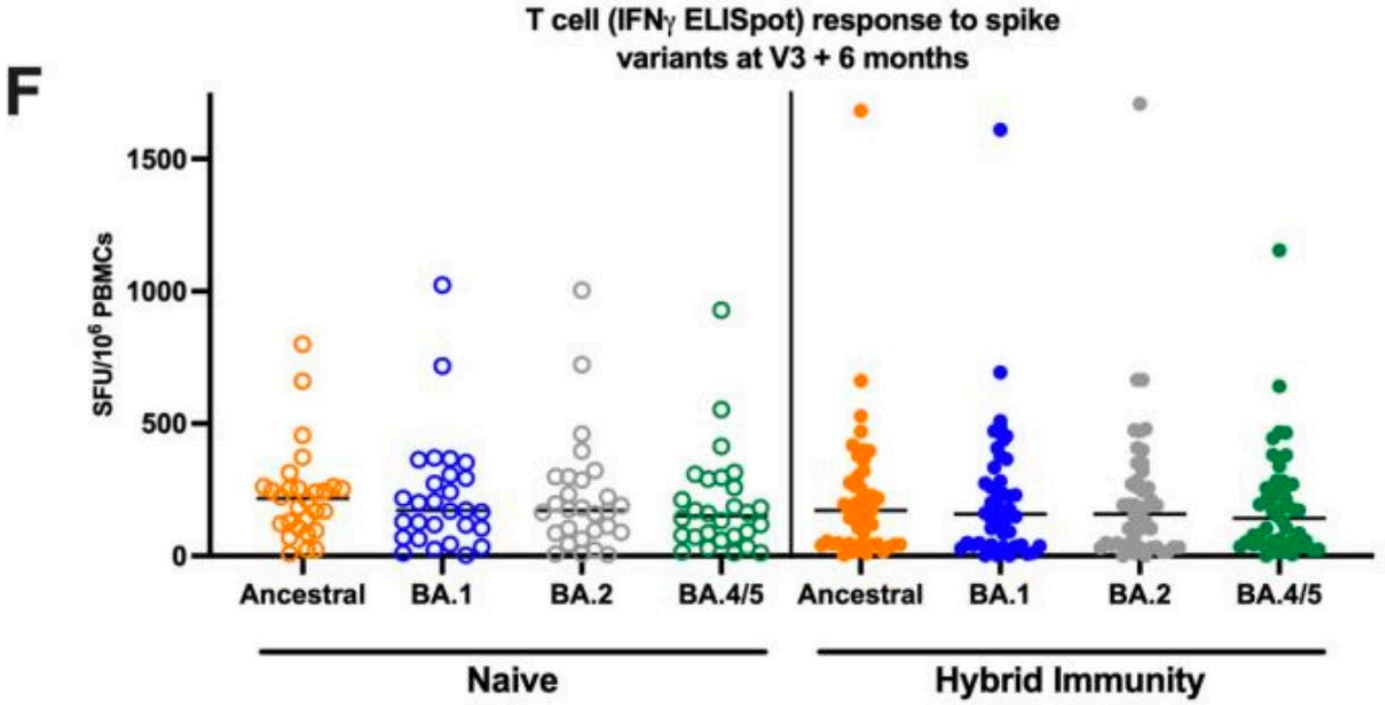
Baseline immunity predicts durability at 6 months

- Tomic et al Nature Comms

Responses to natural infection as comparator to ChAdOx trial

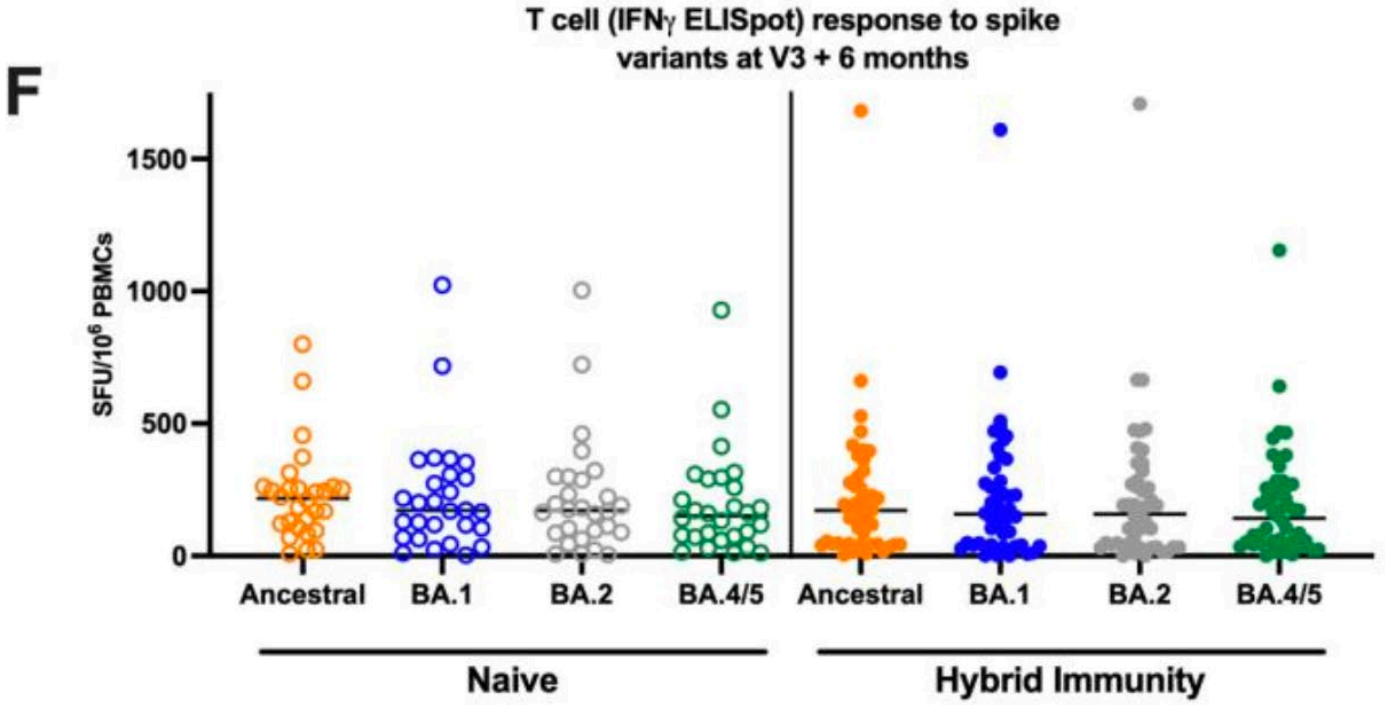
- Folegatti et Lancet 2020

Later characterisation of T cell immunity post vaccine



Moore et al, in press 2023

Later characterisation of T cell immunity post vaccine



Explore further with SEACOVARIANTS grant in SE Asia

Work Package 2- Genomics

Generating a Blood and Tissue COVID-19 Multi-omic Atlas

Objectives:

- Define the **underlying processes** which drive **distinct outcomes** for patients with COVID-19
- Define how host genetics controls these changes at a **single cell level**
- Look deeply into the **lung and respiratory tract** to address the underlying disease that we need to treat

Study design

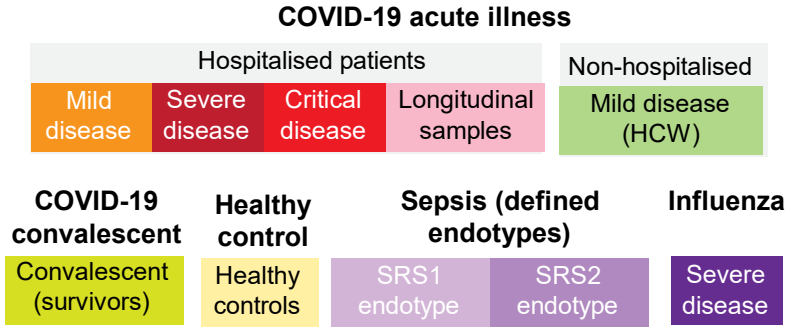
Study initiated at outset of pandemic in UK

Patients recruited and samples processed through established eCRF and protocols working with critically ill patients (sepsis immunomics study); coenrolment (ISARIC)

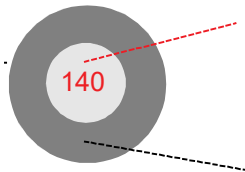
In depth clinical phenotyping, use of electronic health record, SEND

Single centre recruitment - nimble, reactive and harmonised approach

Cases for core set acute illness discovery samples recruited 16th March -27th April 2020

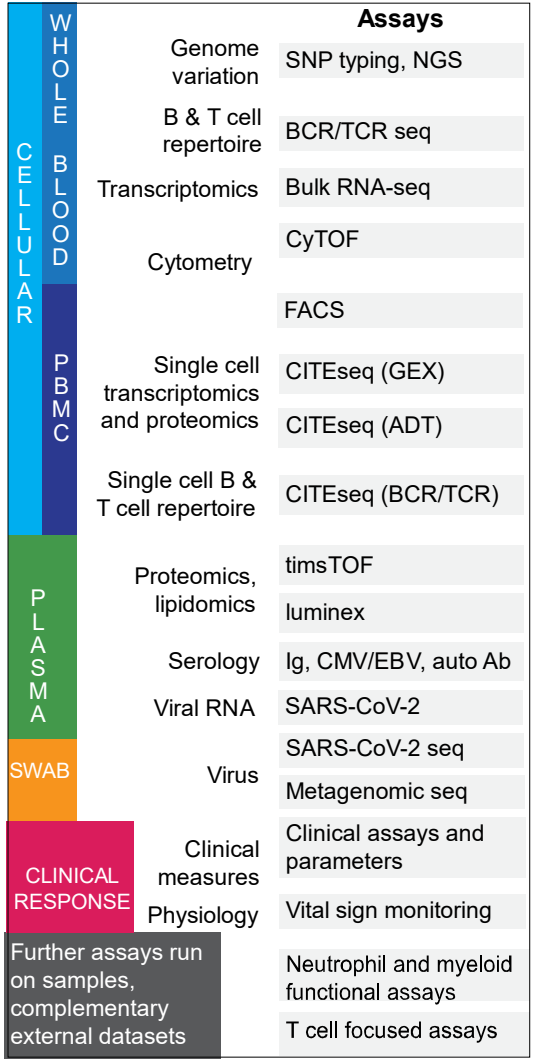


- *serial sampling in acute illness paired with convalescent*
- *spectrum of disease severity and time from symptom onset*
- *matched comparator groups*



Core set of 140 discovery samples (n=80 hospitalised COVID, n=12 in comparator groups)

Additional samples (including convalescent) to increase sample size and enable validation



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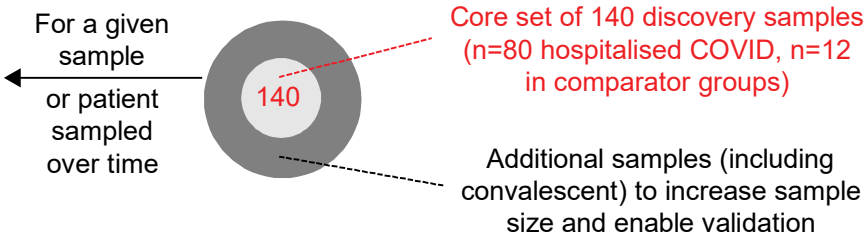
COVID-19 acute illness

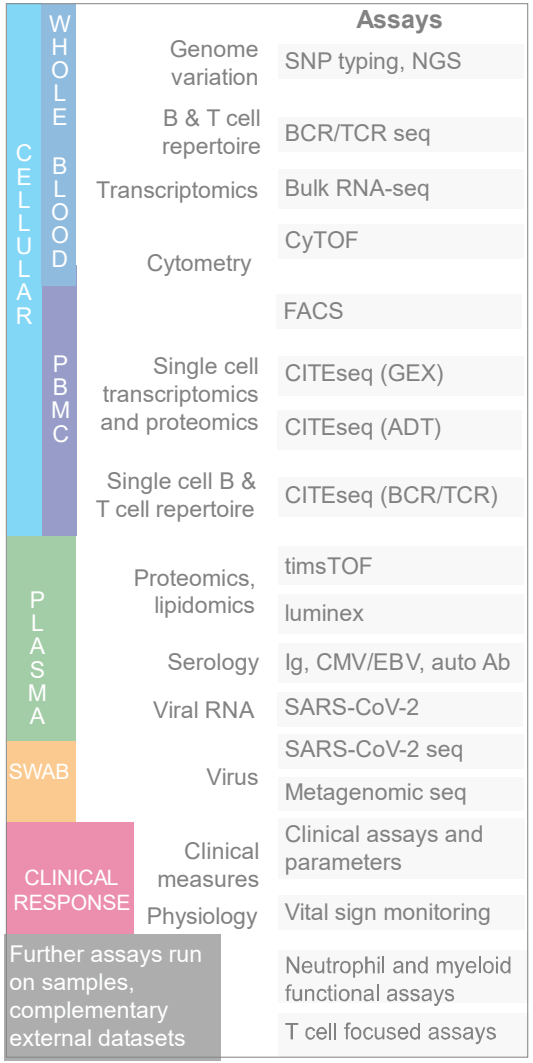
Hospitalised patients				Non-hospitalised
Mild disease	Severe disease	Critical disease	Longitudinal samples	Mild disease (HCW)

COVID-19 convalescent	Healthy control	Sepsis (defined endotypes)		Influenza
Convalescent (survivors)	Healthy controls	SRS1 endotype	SRS2 endotype	Severe disease

- *serial sampling in acute illness paired with convalescent*
- *spectrum of disease severity and time from symptom onset*
- *matched comparator groups*



COMBAT, Cell 2022



Genetic analysis

Bulk RNA-seq analysis

CyTOF analysis

FACS analysis

CITE-seq pre-processing

CITE-seq integration & alignment

CITE-seq clustering & differential modelling

Repertoire analysis

timsTOF analysis

luminex analysis

Primary Analysis Teams
PI leads, typically 3-6 team members (all grades), open calls within COMBAT

Data generation, analysis & management

Initial sample processing - CCVTM/WIMM

Experimental work - WHG/WIMM/TDI/Kennedy

H&S - working with blood from COVID-19 cases for lab/data generation

Data generation at unprecedented scale (single cell resolution); breadth of assays and platforms (immune, multi-omic); novel method development

Multiple data generation and analysis teams involving 110+ researchers, 30+ PIs, 6 institutes across Medical Sciences and Maths

Raw & processed data deposited; access to clinical data and deposited datasets

Data Management Team
- sample ID system, metadata, deposition, data warehousing, high peformance compute workspace
- governance, data security and sharing within COMBAT, Oxford Immunology Consortium and community (data releases & deposition)

All data inputed for integration and analysis

Integrative Data Analysis Team
- maximising multi-modal data types

Data visualisation | **Systems biology** | **Machine learning** | **Modelling**

Data Mining Team

External datasets

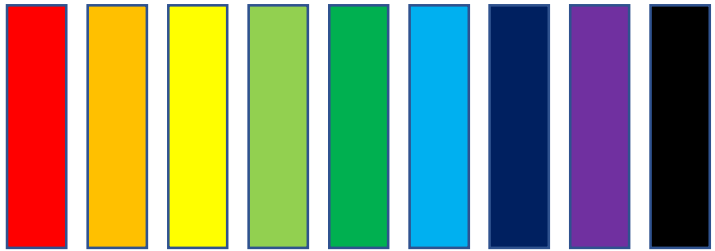
Clinical Phenotyping Team
- case definitions, data quality control, interface with local clinical teams

Cohorts & sample collection

Project manager

Dealing with multi-omics data

"Unbiased" approach



Multiple parallel datasets

Carefully collected clinical cohort

Splitting

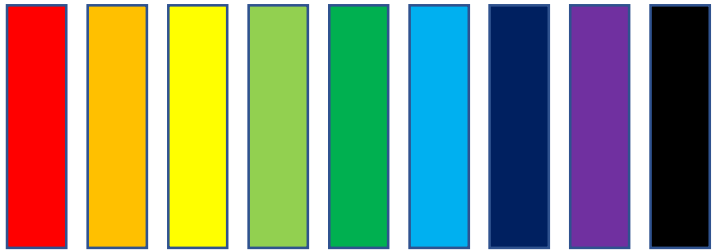
Lumping

Explore specific hypotheses
Functional studies
Classic route to mechanisms

Data integration
Tensor deconvolution
New route – **new hypotheses**

Dealing with multi-omics data

"Unbiased" approach



Multiple parallel datasets

Carefully collected clinical cohort

Splitting

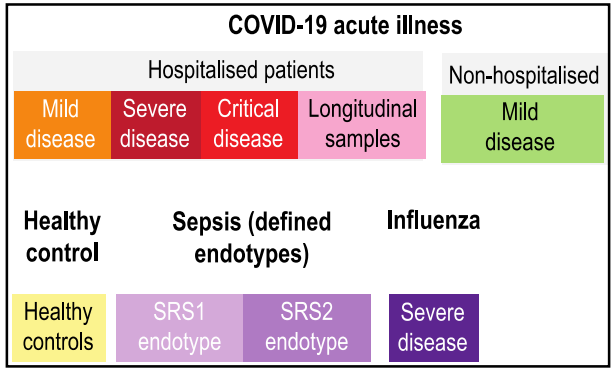
Lumping

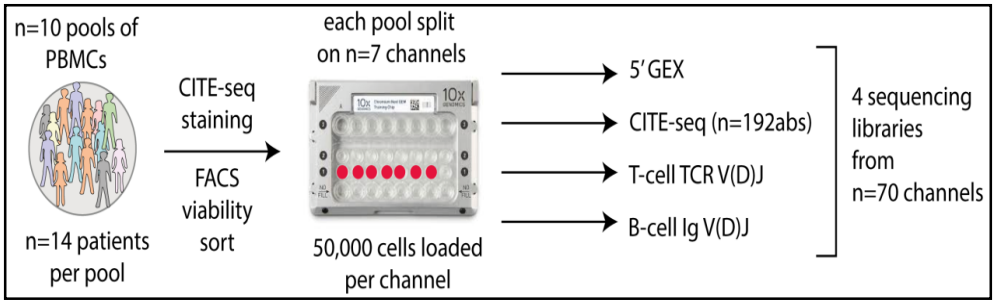
IL6 pathway
GM-CSF pathway
AP-1 signalling

Explore specific hypotheses
Functional studies
Classic route to mechanisms

Data integration
Tensor deconvolution
New route – **new hypotheses**

COVID-19 – COMBAT and CITEseq

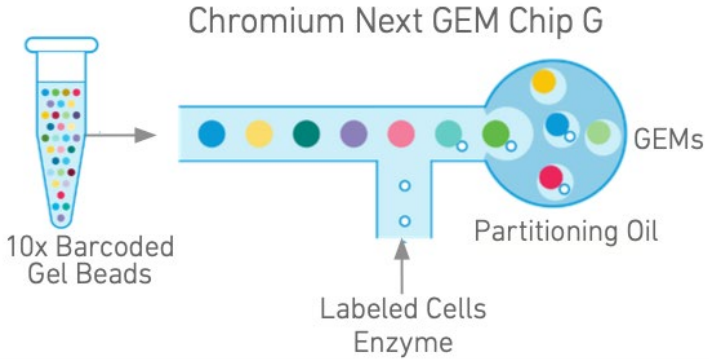




836, 148 cells analysed
Around 130 distinct clusters annotated

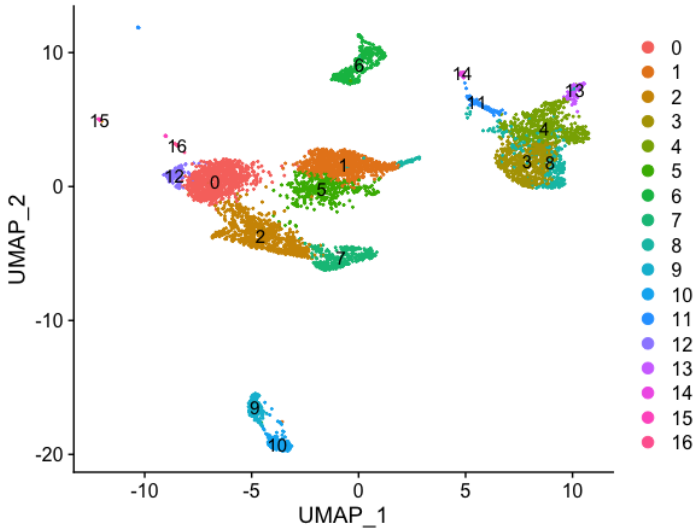
Single cell RNASeq Step 1 – GEM generation and cell barcoding

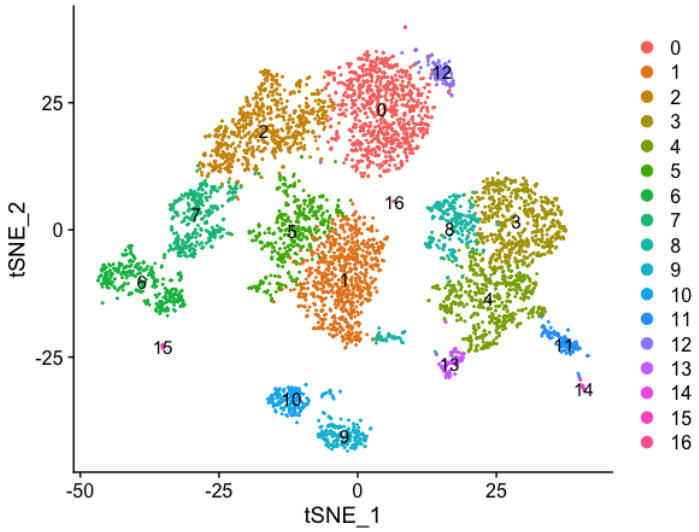
- Cells are combined with Gel Beads, master mix, and partitioning oil → nanolitre-scale GEMs (Gel Beads-in-emulsion)
- Cells delivered at limiting dilution – majority (~90-99%) of GEMs contain no cell, while remainder mostly contain a single cell



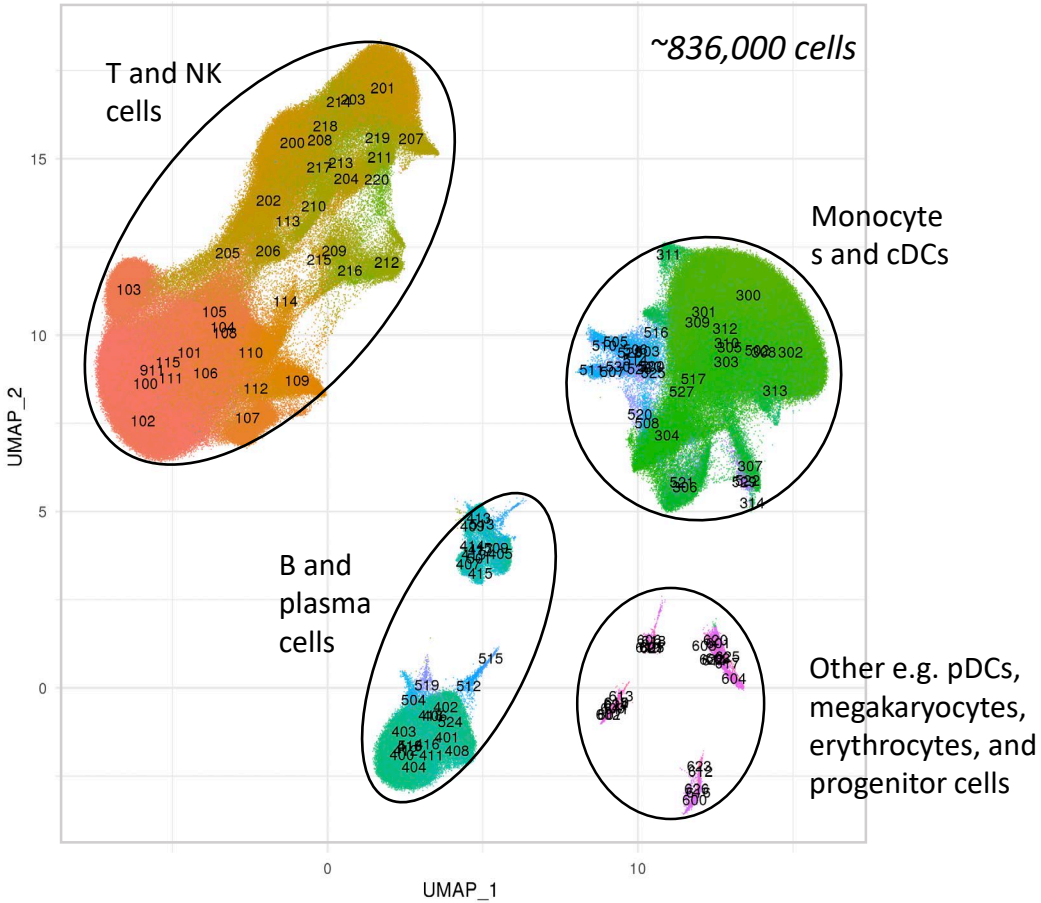
Final key steps of a general scRNA-seq analysis workflow

12. Visualise clusters – UMAP, tSNE

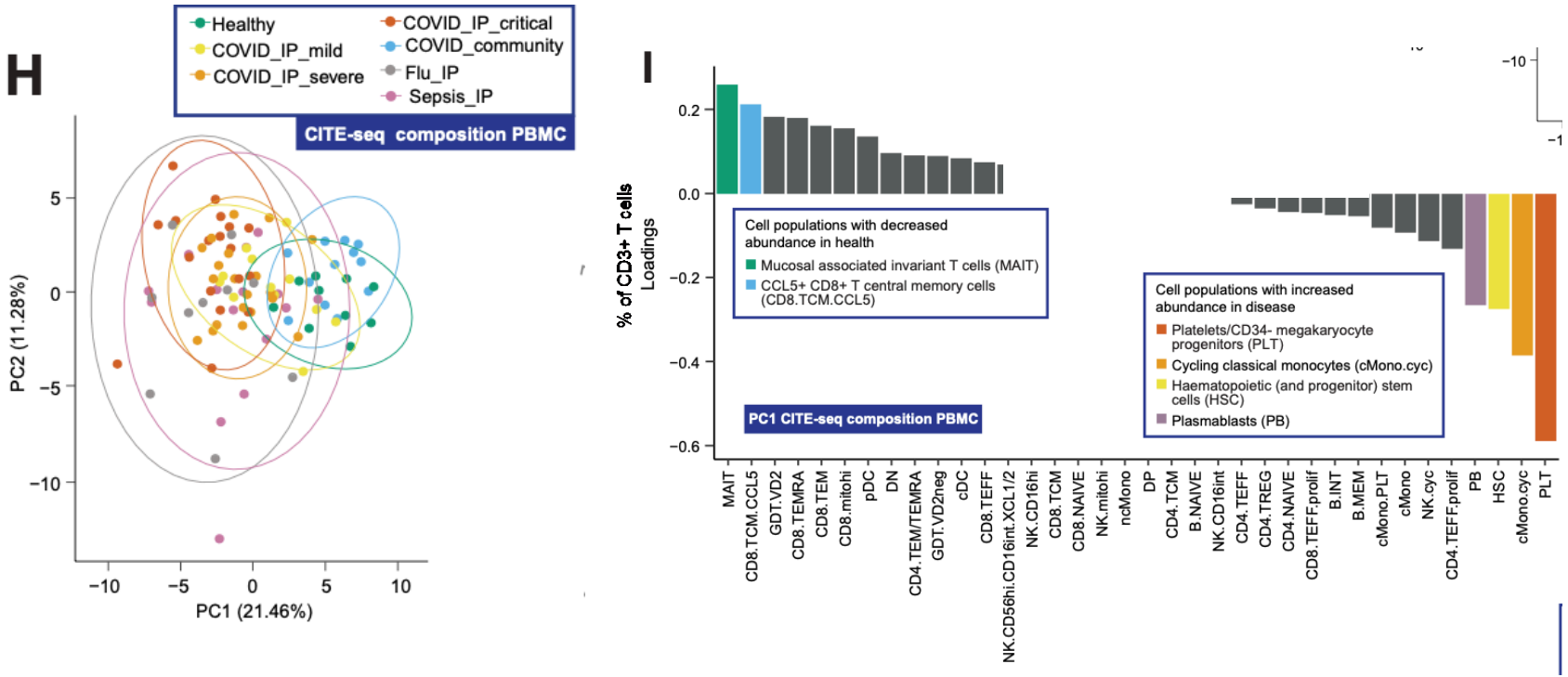




UMAP of all PBMCs

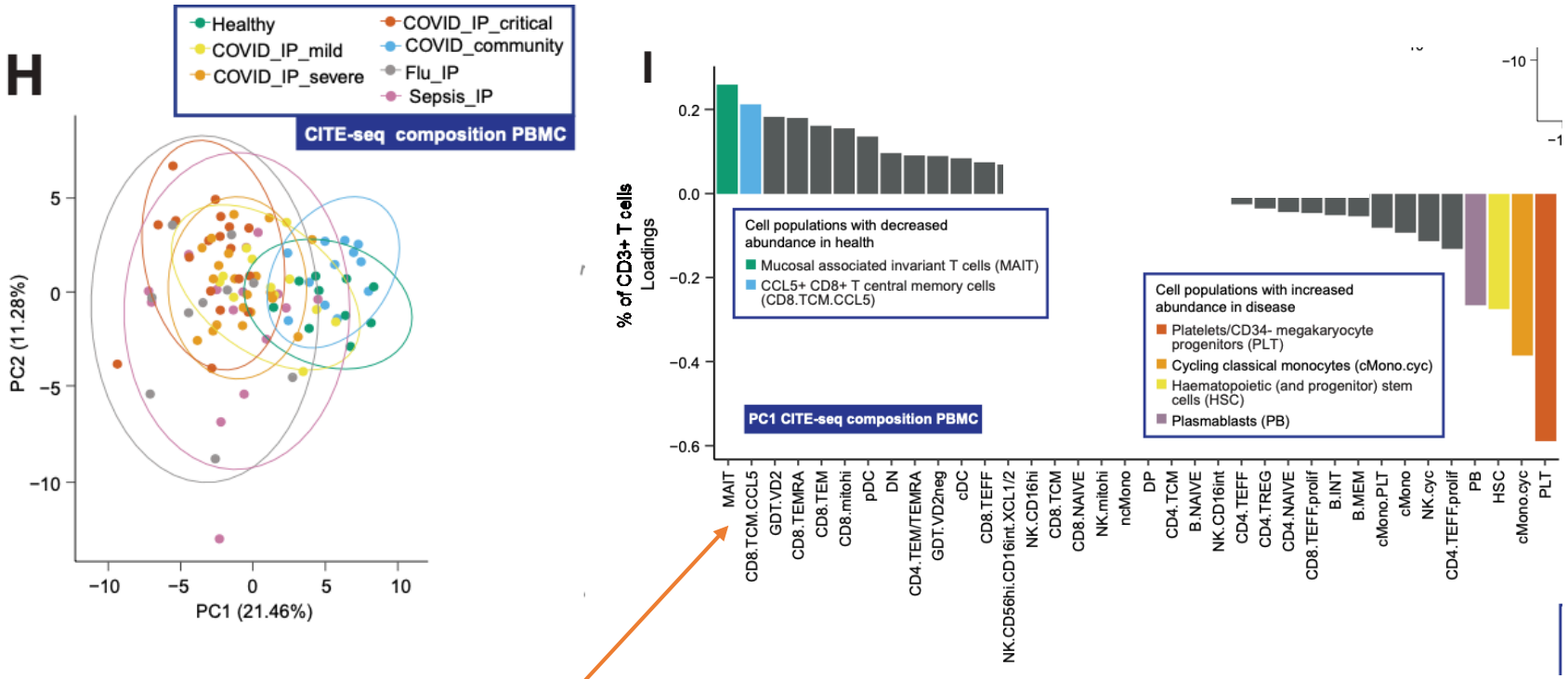


Linking scRNASeq and clinical outcomes: Loss of specific peripheral blood cell subsets in severe COVID-19/flu



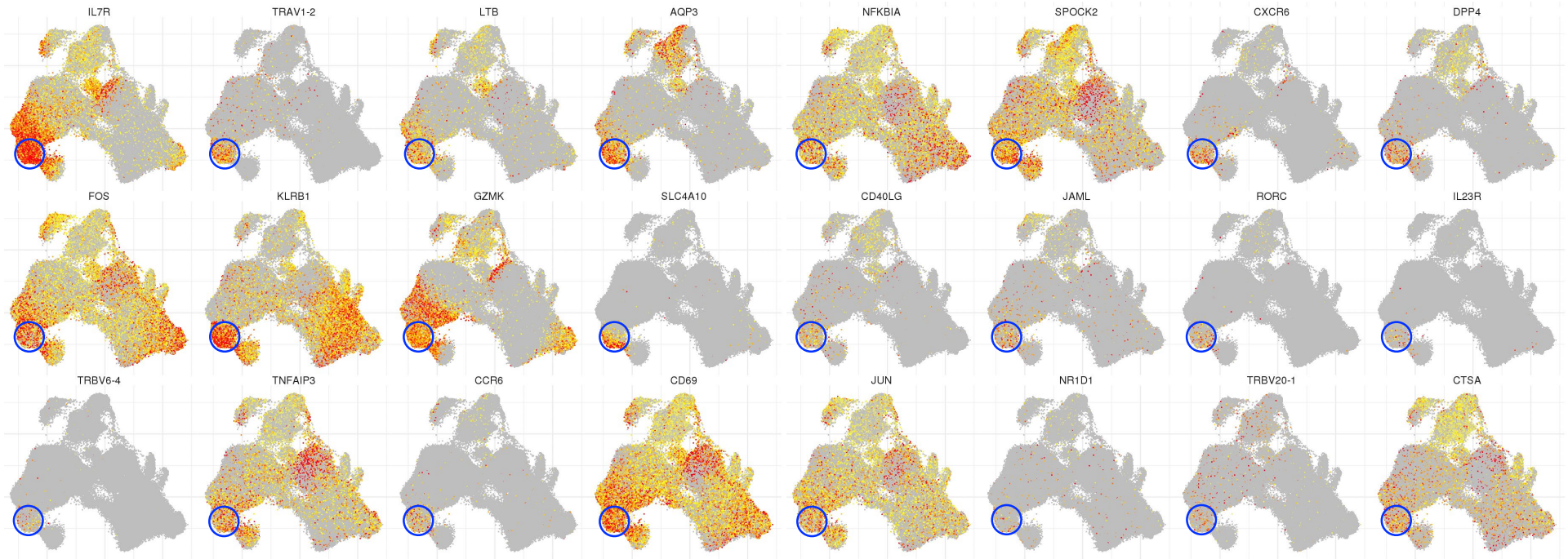
Data from COMBAT consortium

Loss of specific T cell subsets in severe COVID-19/flu



Data from COMBAT consortium

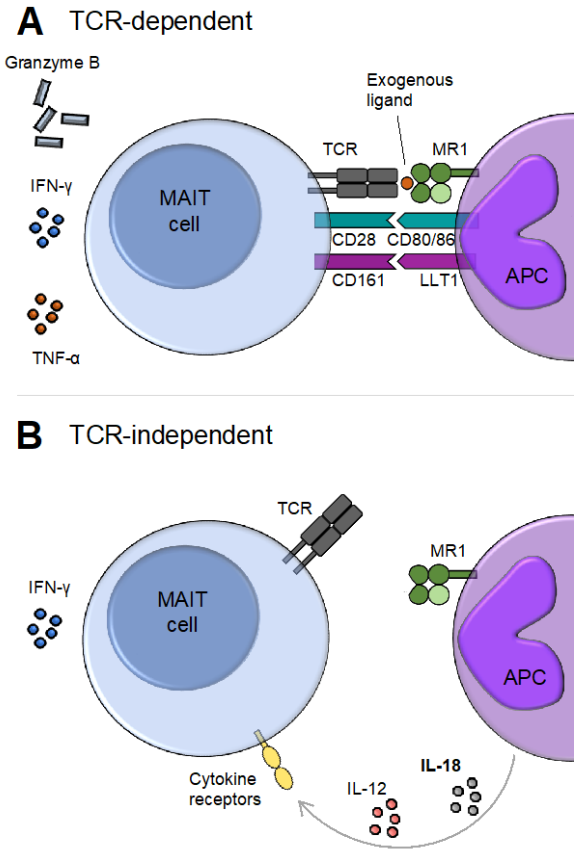
Example - **Genes upregulated** on MAIT cells



Upregulated genes include C-type lectin domain-containing proteins (*KLRB1)* cytokine and chemokine receptors (*IL7R, IL23R, CXCR6, CCR6*), transcription factors (*RORC, FOS, JUN*), *TRAV1-2*, and the activation markers and *DPP4 (*also MERS-CoV receptor).

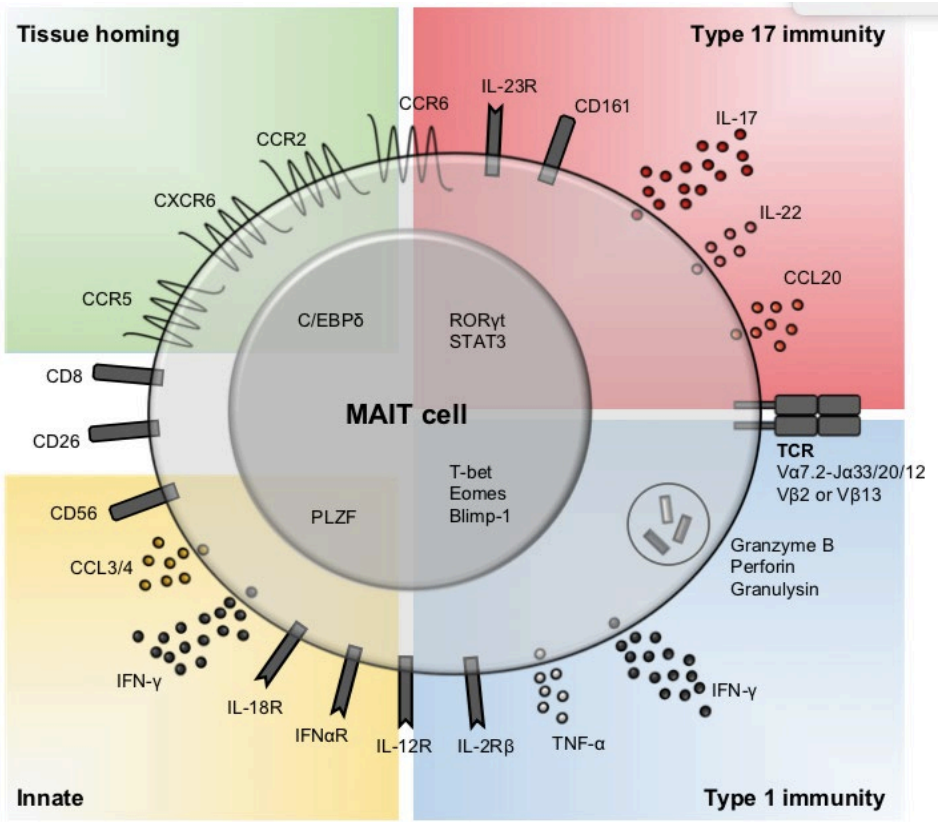
MAIT cells: innate-like T cells that display TCR-dependent and –independent activation

- Semi-invariant αβ TCR
- Recognize riboflavin metabolites presented by MR1 (microbe derived)
- Predominantly $CD8^+$ with a stereotyped phenotype
- Mixed Tc1/Tc17 response
- Potently activated by cytokines independent of TCR



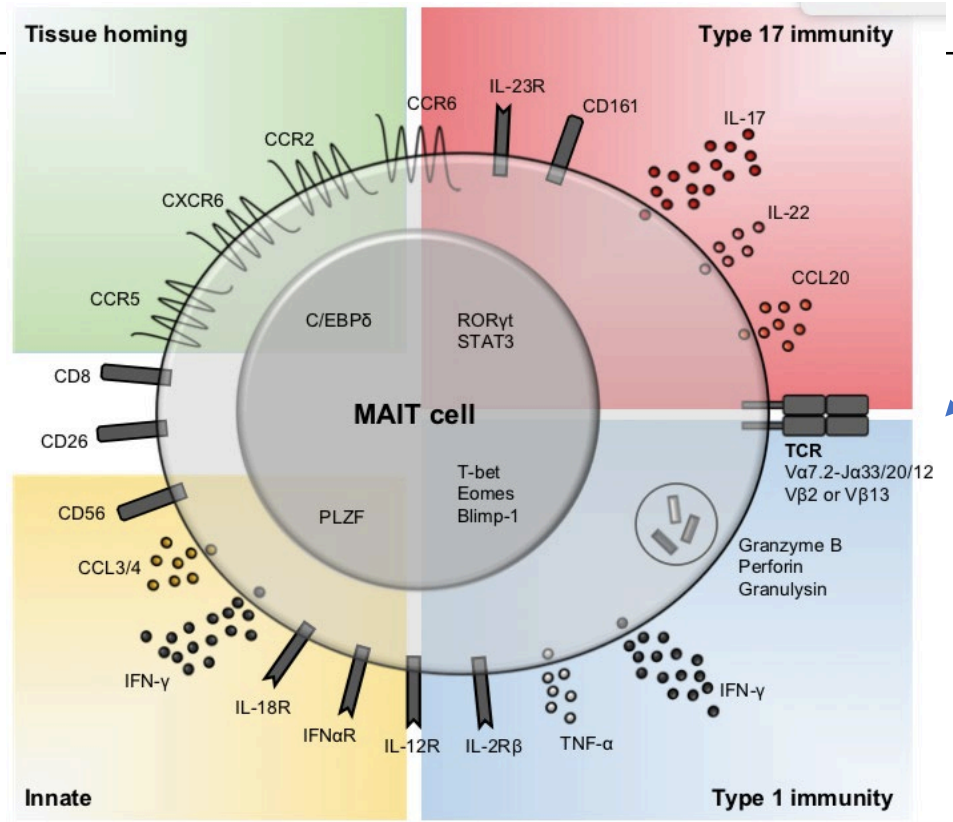
Modified from: Garner, Klenerman, and Provine, *Front Immunol,* 20

Mucosa-Associated Invariant T cells (MAITs)



Nick Provine (Ann Rev Immunol 2020)

Mucosa-Associated Invariant T cells (MAITs)

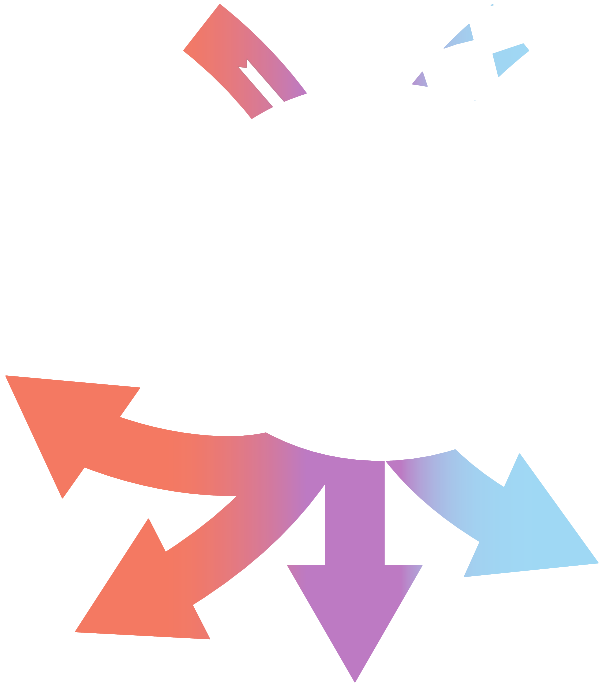


Respond to Microbial Ligand

Respond to viruses

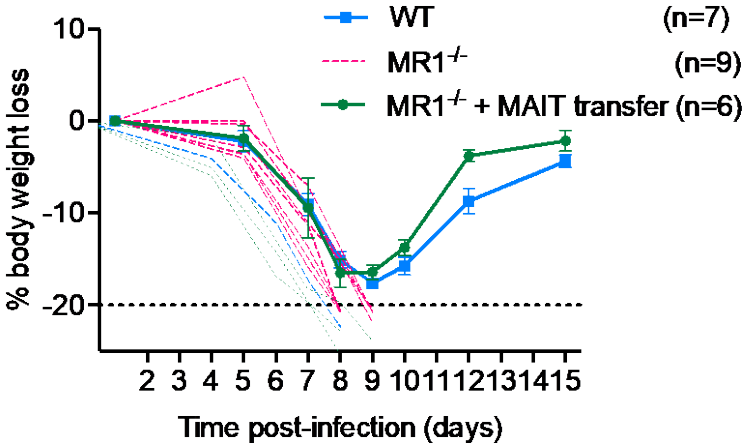
Nick Provine (Ann Rev Immunol 2020)

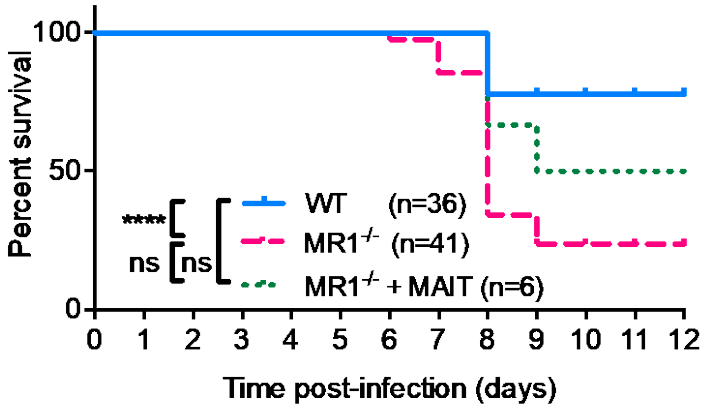
MAIT cell effector responses are governed by the integration of inflammatory and TCR signals



Provine and Klenerman,
Ann Rev Immunol, 2020

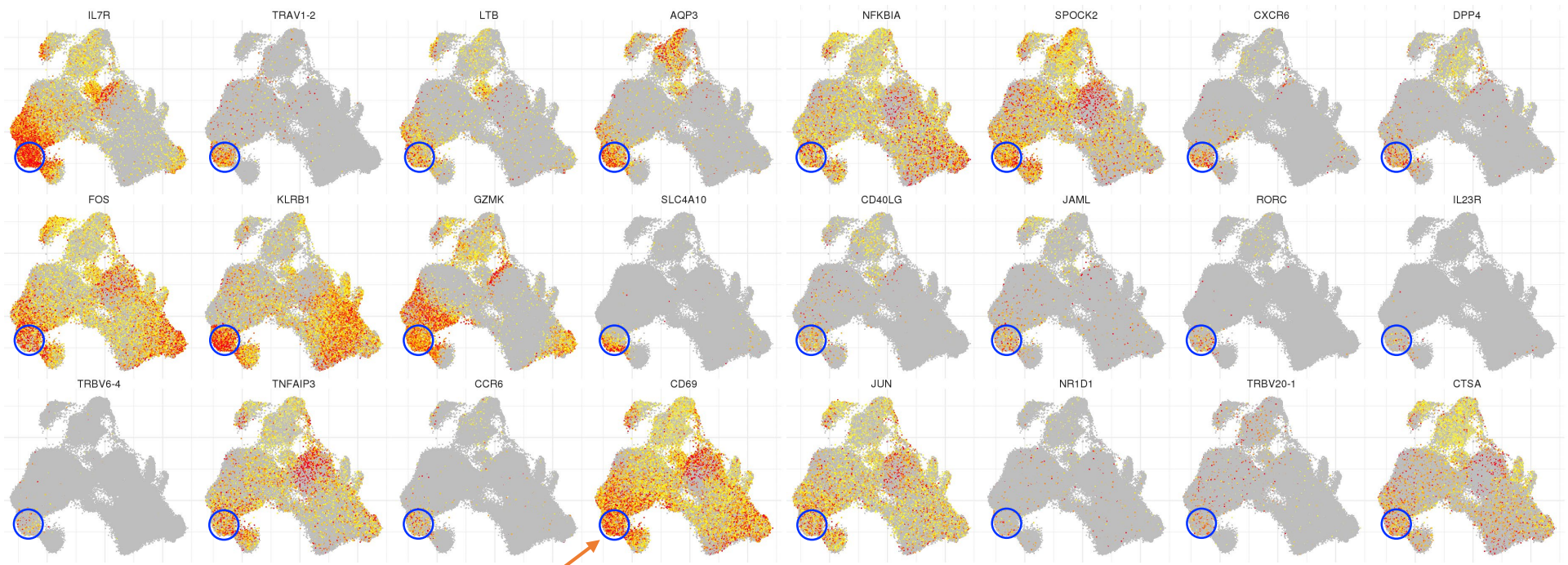
Do MAIT cells offer protection against severe influenza challenge (PR8)?





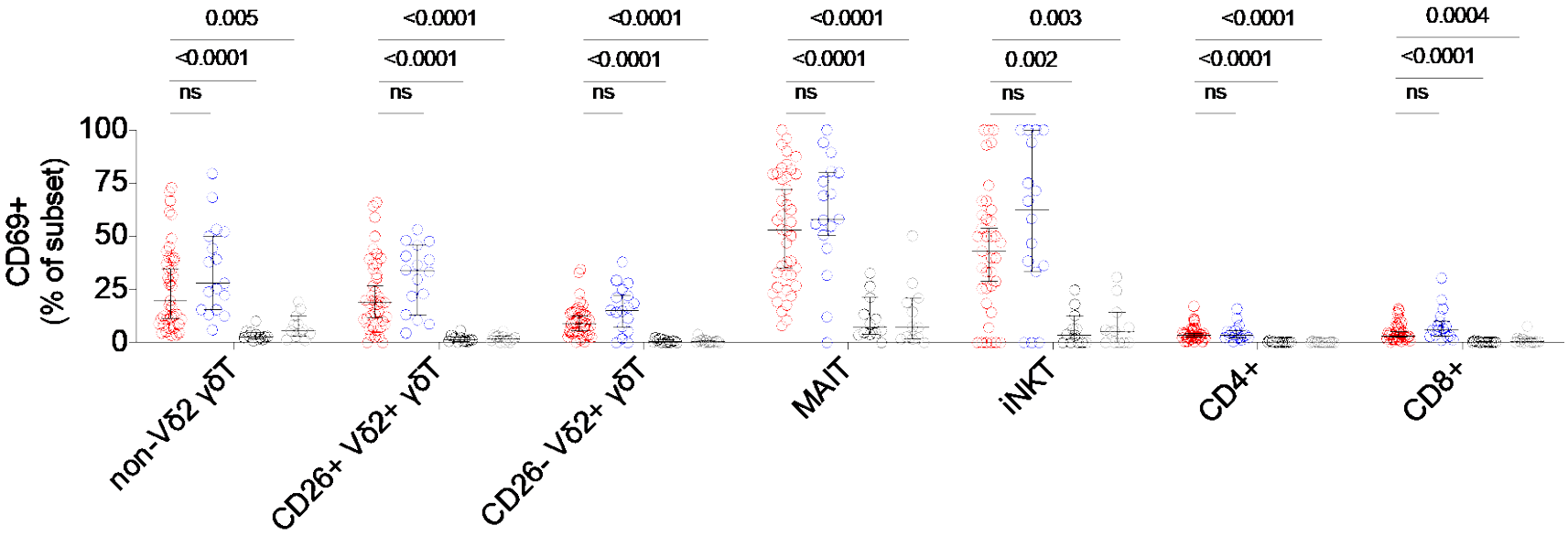
Van Wilgenburg/Loh Nature Comms 2018

Example - **Genes upregulated** on MAIT cells



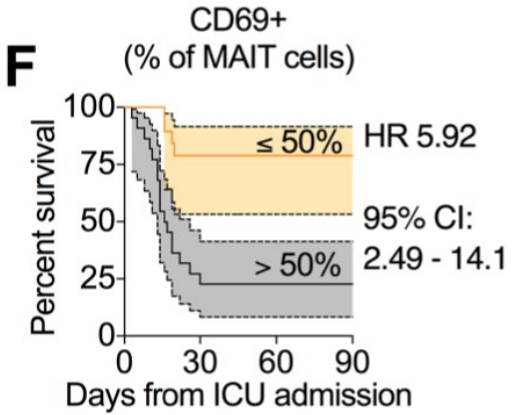
Upregulated genes include C-type lectin domain-containing proteins (*KLRB1)* cytokine and chemokine receptors (*IL7R, IL23R, CXCR6, CCR6*), transcription factors (*RORC, FOS, JUN*), *TRAV1-2*, and the activation markers and *DPP4 (*also MERS-CoV receptor). Also **strong activation (CD69)**

T cells: Association with death in ICU



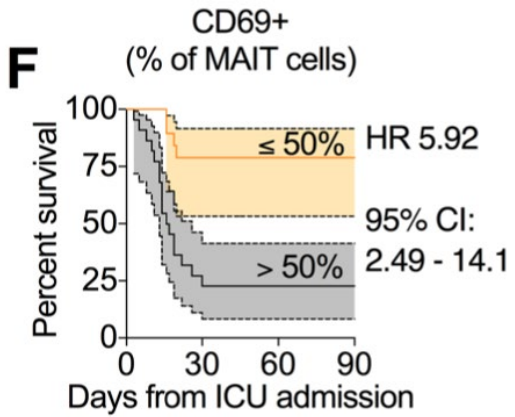
Youngs, Provine et al,
PLOS Pathogens Sept 2021

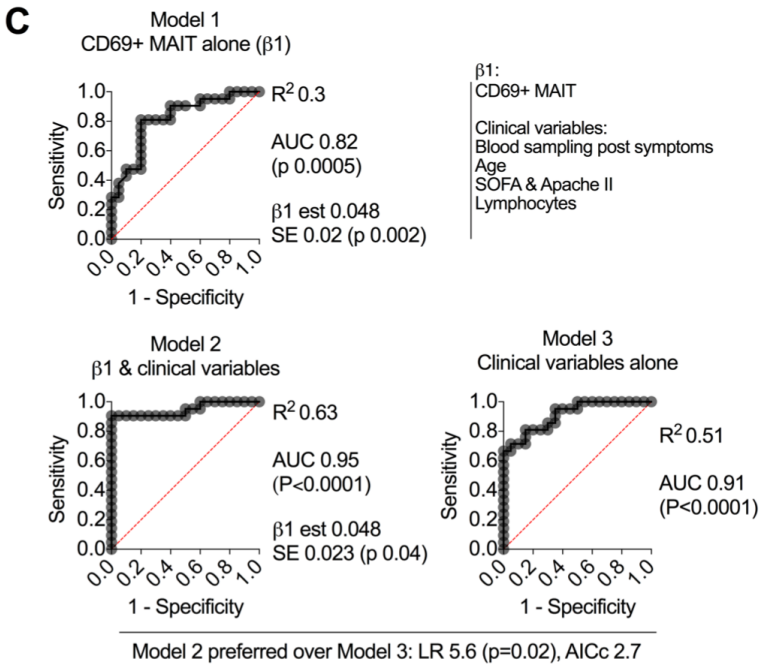
T cells: Association with death in ICU



Youngs, Provine et al,
PLOS Pathogens Sept 2021

T cells: Association with death in ICU





Youngs, Provine et al,
PLOS Pathogens Sept 2021

Single-cell RNASeq and multi-omics approaches

- Allow an “unbiased” screen
- Allow exploration of cellular functions/pathways to block
- Allow interrogation of antigen-specific responses
- Can be used in tissue samples

Single-cell RNASeq and multi-omics approaches

- Allow an “unbiased” screen
- Allow exploration of cellular functions/pathways to block
- Allow interrogation of antigen-specific responses
- Can be used in tissue samples
- But…
- Are expensive
- Need computational input (getting easier)
- Need a simple question and a cohort to address this in
- Lack any spatial data

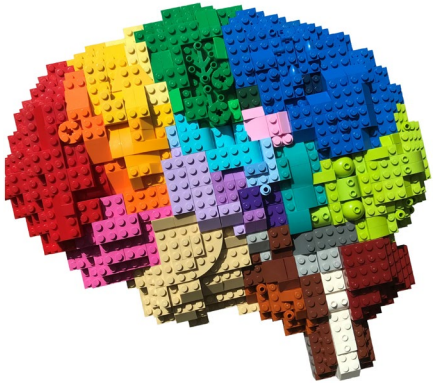
Bulk

Single-cell RNA-seq

Credit Bo Xia

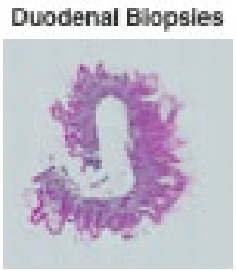
Spatial transcriptomics

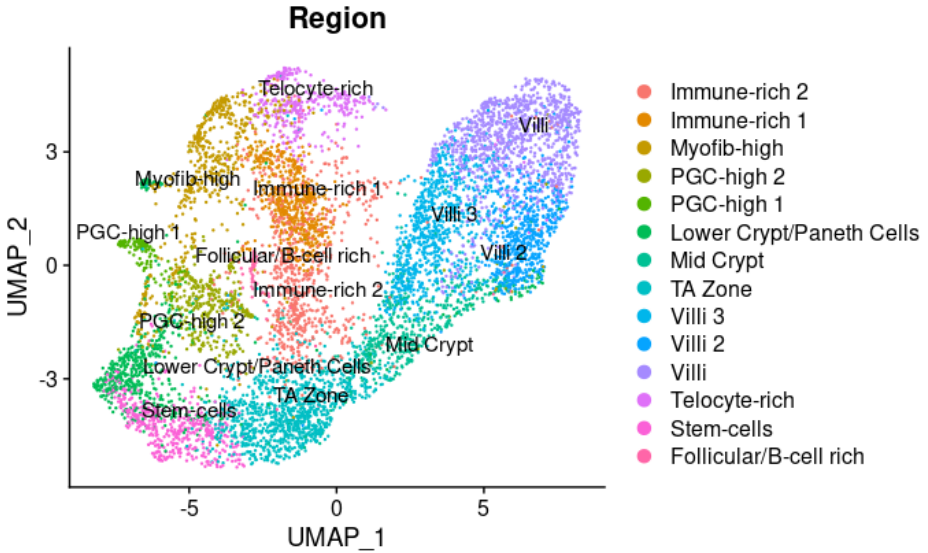
Original organ



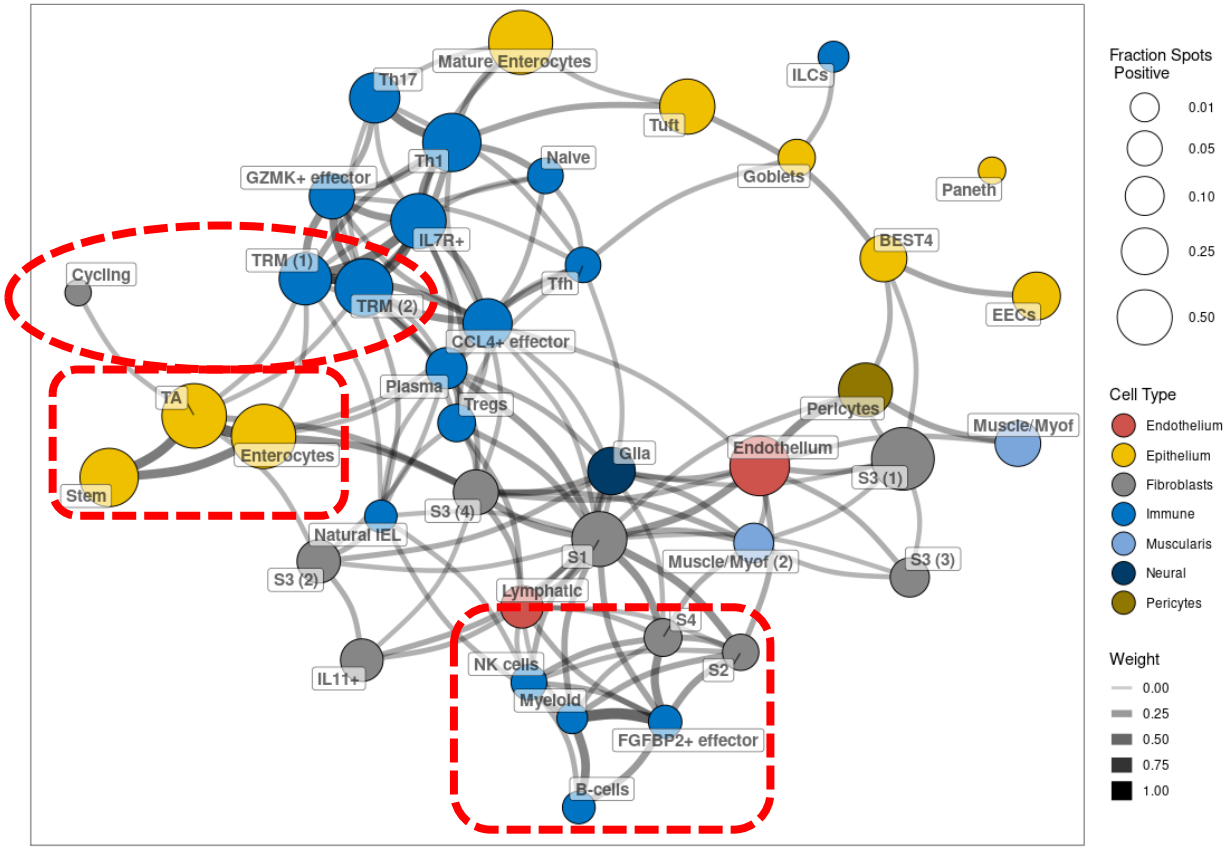
Development /pseudotime

Intestinal spatial transcriptomics: example of coeliac disease

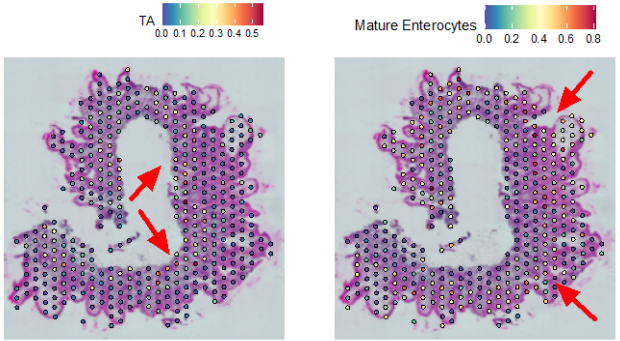




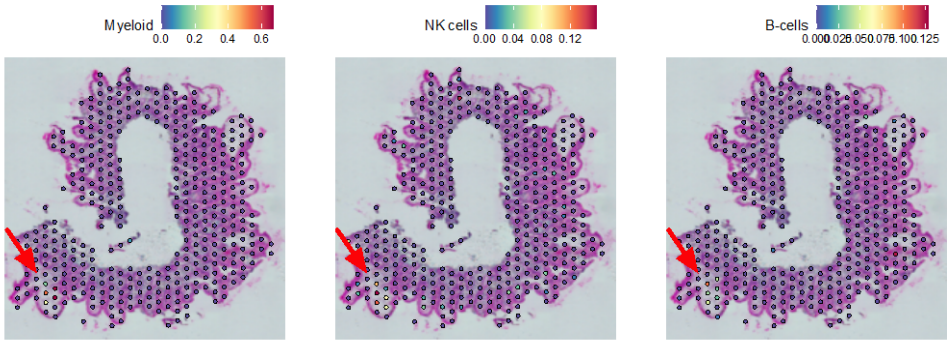
Combine single cell and spatial datasets to define **cellular networks**



Integration with scRNAseq data reveal presence of highly localized immune cell structures in the gut

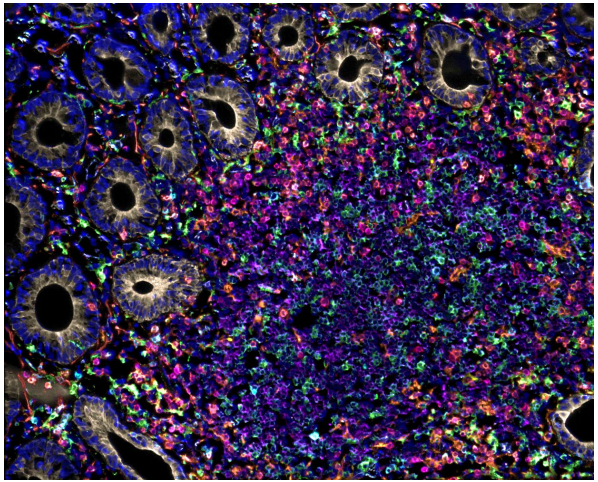


Transit-amplifying cells located at crypt bases
Mature enterocytes in surface villi



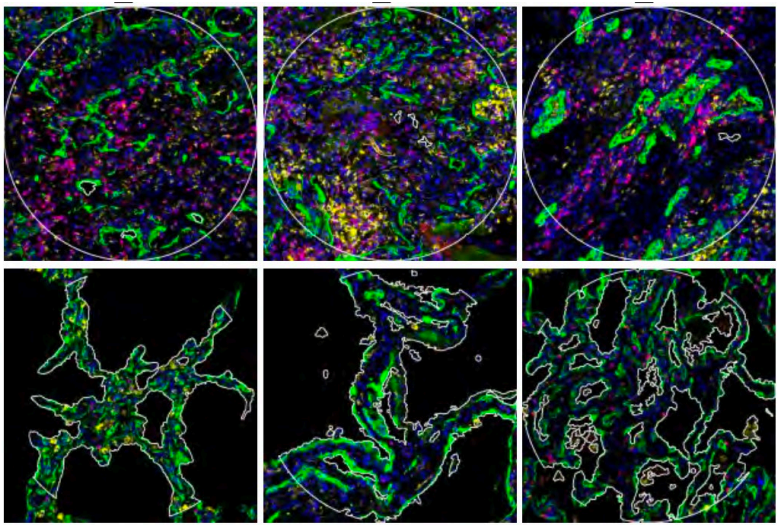
Highly localised myeloid signals in biopsy sections
Associated with NK cell and B cell signatures

Rapid development of spatial analytic tools



High content imaging
(20+ stains)

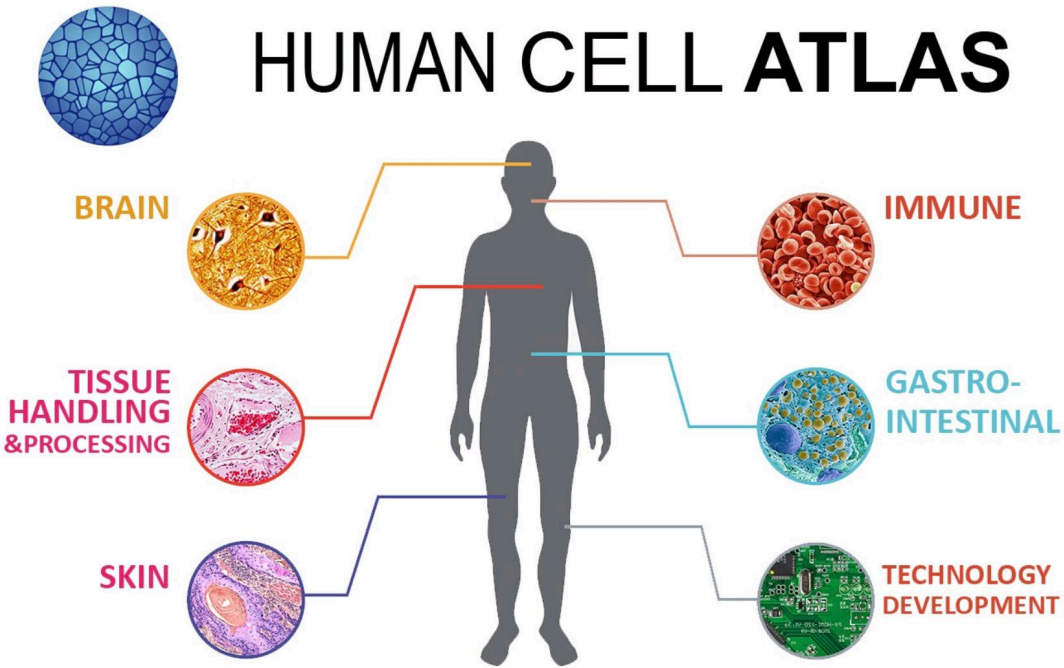
(Kate Powell)



Combined staining and
single-cell resolution transcriptomes

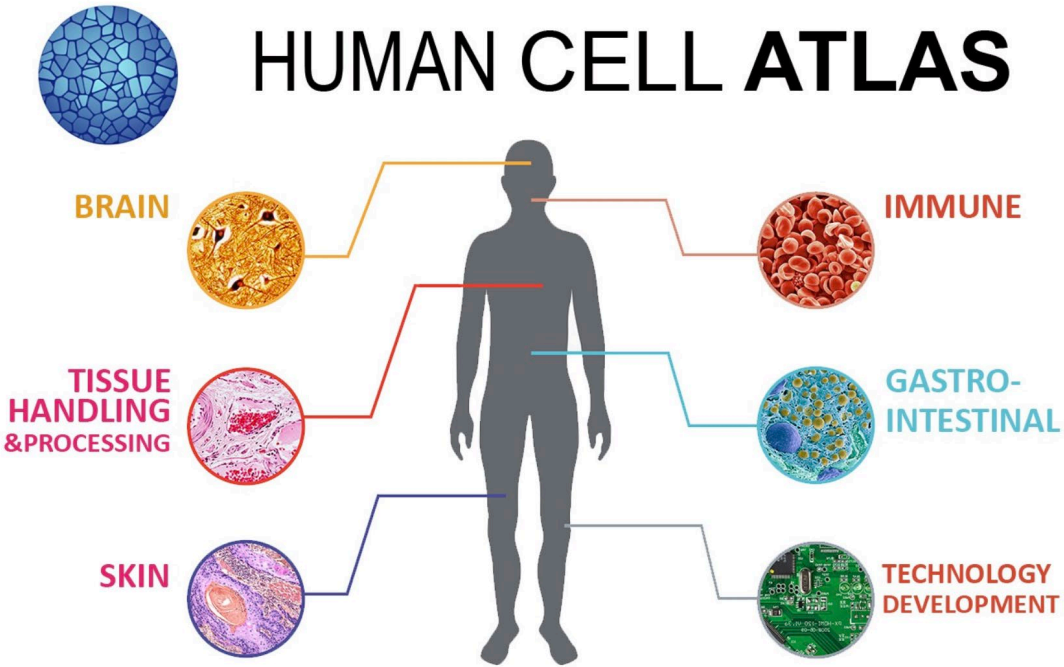
(Fadi Issa: nanostring)

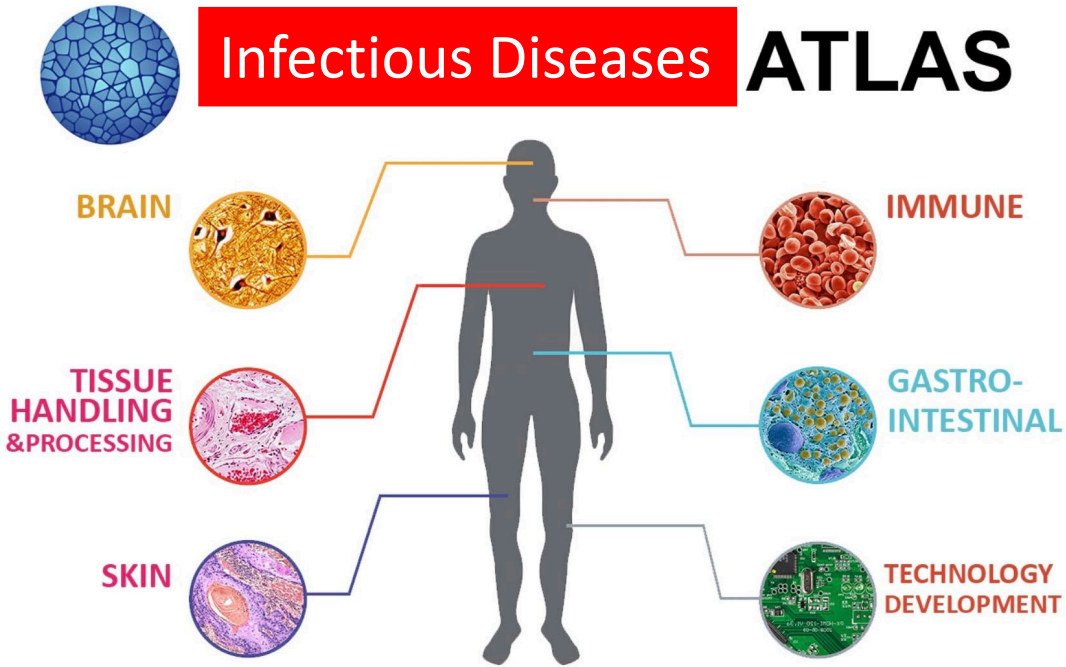
Where next?


HUMAN CELL ATLAS
BRAIN
IMMUNE
TISSUE HANDLING &PROCESSING
GASTRO-INTESTINAL
SKIN
TECHNOLOGY DEVELOPMENT

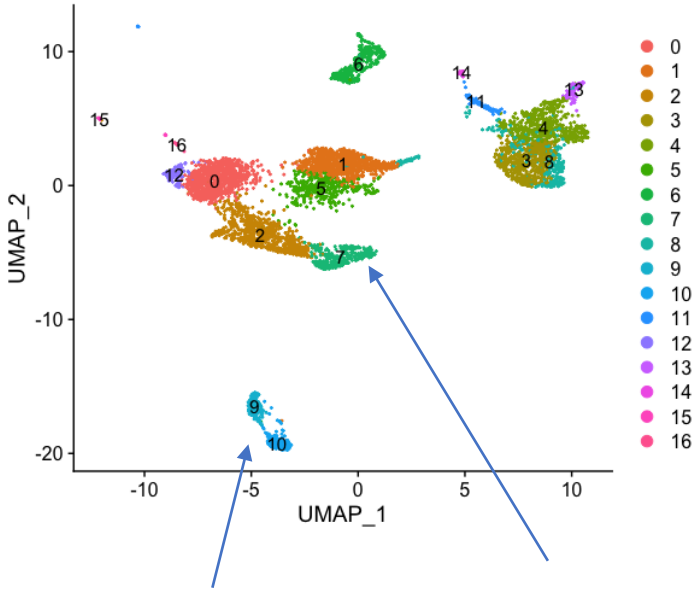
Where next?

V1.0 = Healthy tissues



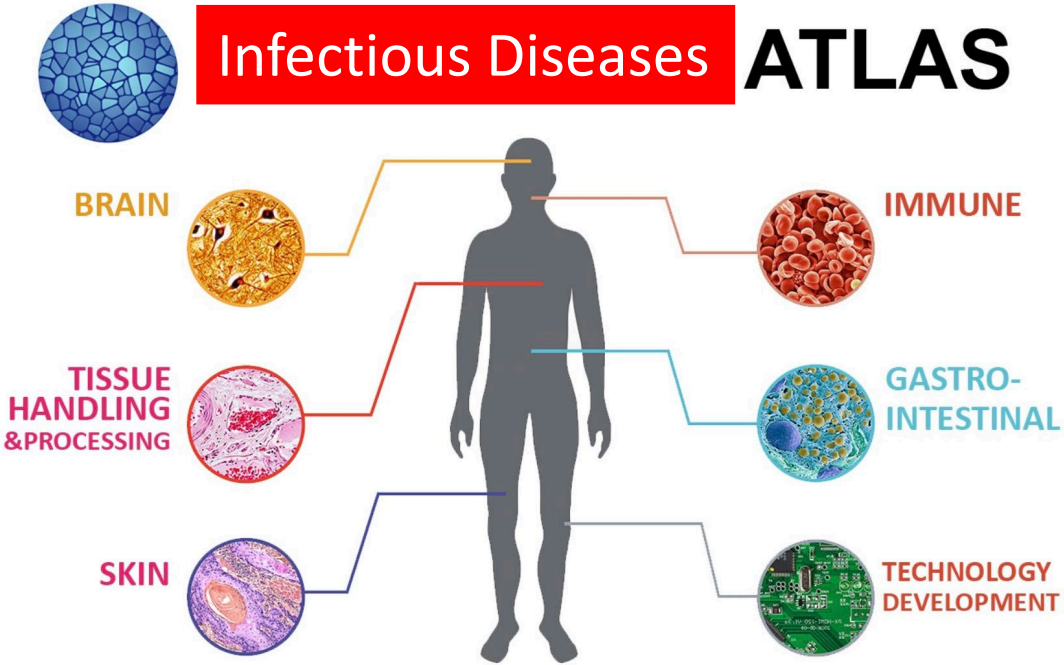

Infectious Diseases ATLAS
BRAIN
IMMUNE
TISSUE HANDLING &PROCESSING
GASTRO-INTESTINAL
SKIN
TECHNOLOGY DEVELOPMENT

For a given disease/site

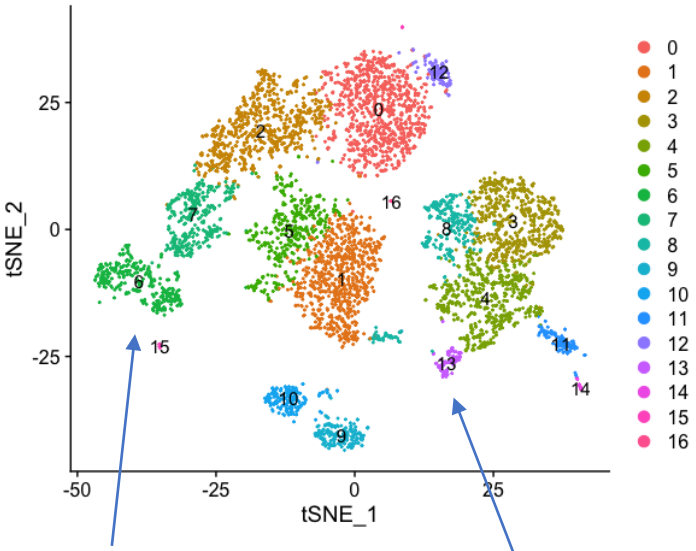


MAIT

Plasma cells

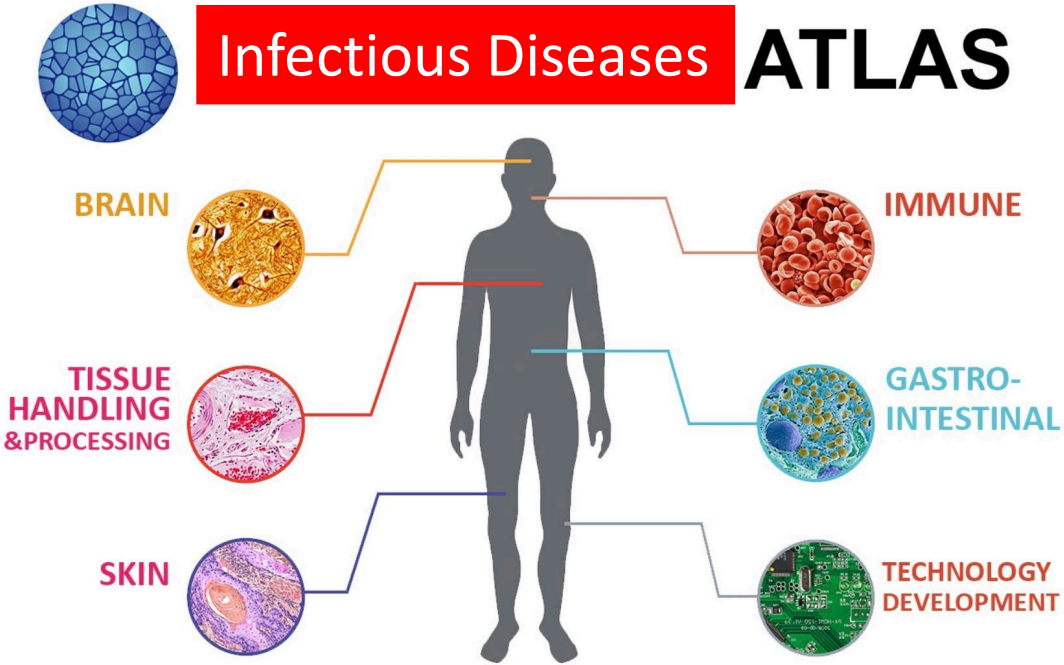


Across diseases –
define fundamental processes

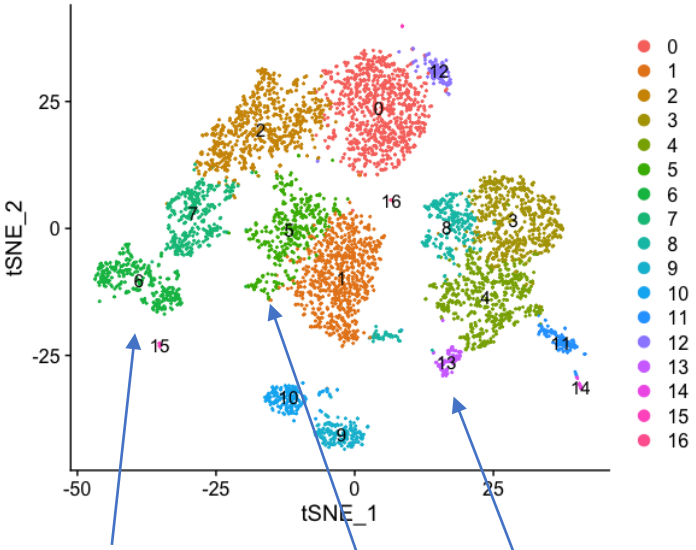


TB meningitis

Melioid



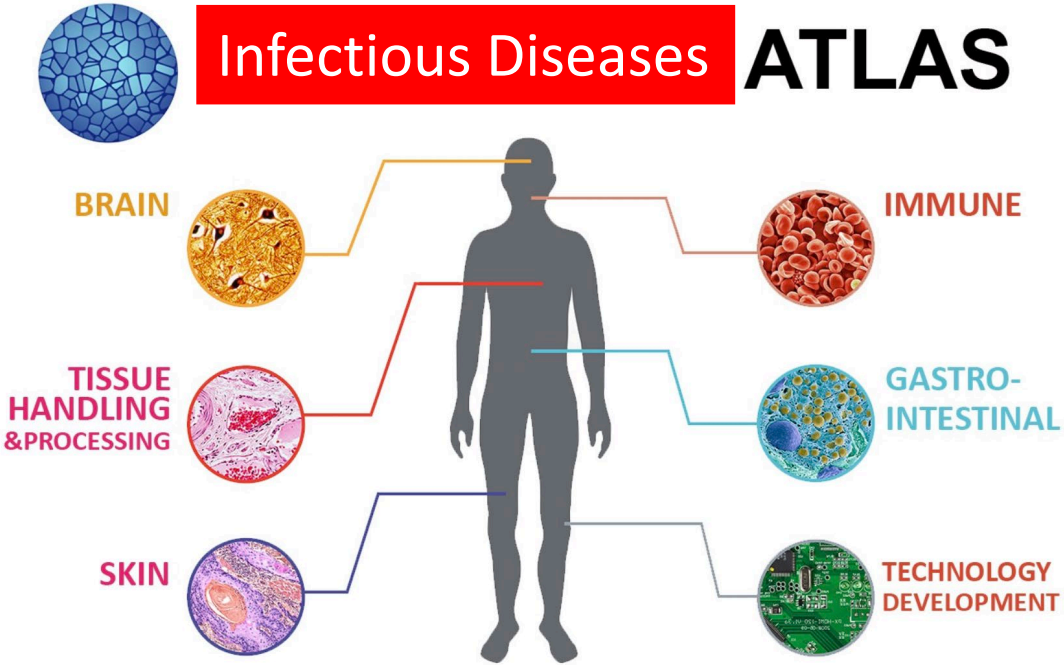
Across diseases –
define fundamental processes
- Accelerate treatments



Covid-19

Melioid

Disease X



Medawar pathogen research

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Tom Malone
Azim Ansari
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Ellie Barnes
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Matt Pace
Ane Ogbe
Emily Adland
Helen Brown
Philip Goulder
Hema Mehta
Ali Amini
Nicholas Provine
(and many more!)

Oxford University Hospitals NHS
NHS Trust

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Anni Jamsen
Siobhan Gardiner
Síle Johnson
Bea Simmons
Tim James
Stavros Dimitriadis

University of Oxford medical students

oxford vaccine group

Christina Dold
Adriana Tomic
Daniel O'Connor
Andrew Pollard
(and many more!)

PITCH
PROTECTIVE IMMUNITY from T CELLS in HEALTHCARE WORKERS

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Jem Chalk (Database developr)

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Sue Dobson (U. Liverpool)
Christopher Duncan (U. Newcastle)
Sian Faustini (U.Birmingham)
Rebecca Payne (U. Newcastle)
Alex Richter (U. Birmingham)
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Dan Wootton (U. Liverpool)

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NIHR | Oxford Biomedical Research Centre

the HUO FAMILY FOUNDATION

wellcome

UKCIC UK Coronavirus Immunology Consortium

UK Research and Innovation

NIHR | National Institute for Health Research

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Department of Zoology

MATHEMATICAL INSTITUTE

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