BOSTON UNIVERSITY
Microbiology | Boston University School of Medicine
Biomedical Engineering | College of Engineering
University of Oxford
MEDICAL SCIENCES DIVISION
UNIVERSITY OF OXFORD

Systems Immunology: an intro to multi-omics data integration and machine learning

In this interactive course, we will learn how to use machine learning for biological and biomedical data integration and knowledge discovery.

March 6th and 7th 2023, Oxford

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Training course - overview

Part I – SIMON, pattern recognition and knowledge extraction platform (March 6th 2023)

- Machine learning and AI – what is all the fuss about?
- What is SIMON?

Theoretical part (9:30-10:30am) ~1h

- Case study – example 1 (dealing with missing values, overfitting, model performance)

Case study (10:30-11:30am) ~1h

- Perform SIMON analysis using provided dataset
- Performance metrics, evaluation and selection of high-quality models

Hands-on (11:30-1:30pm) ~2h

Part II – Exploratory analysis (March 7th 2023)

- Feature selection: scoring and elimination

Hands-on (9:30-10:30am) ~1h

- Feature processing methods to avoid '*curse of dimensionality*'

Theoretical part (10:30-11am) ~0.5h

- Case study – example 2 (multi-omics data integration)

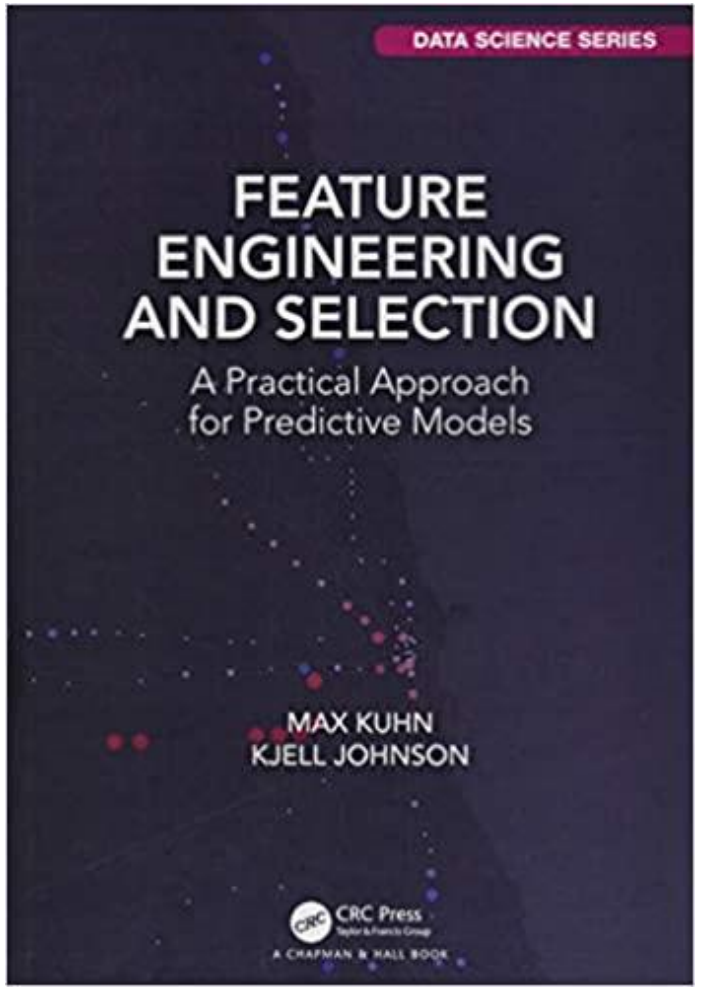
Case study (11-11:30am) ~0.5h

- Case study – example 3 (unsupervised ML + practical demonstration)

Hands-on (11:30am-1:30pm) ~2h

- Discussion about project-specific problems

The art of feature engineering and selection

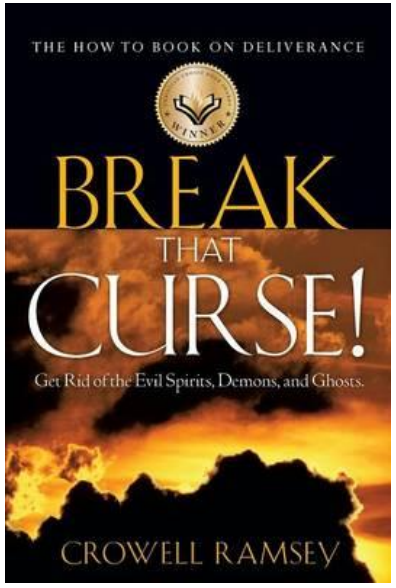


Free book online:
https://bookdown.org/max/FES/

Max Kuhn and Kjell Johnson;
Chapman & Hall/CRC Data
Science Series: 2019

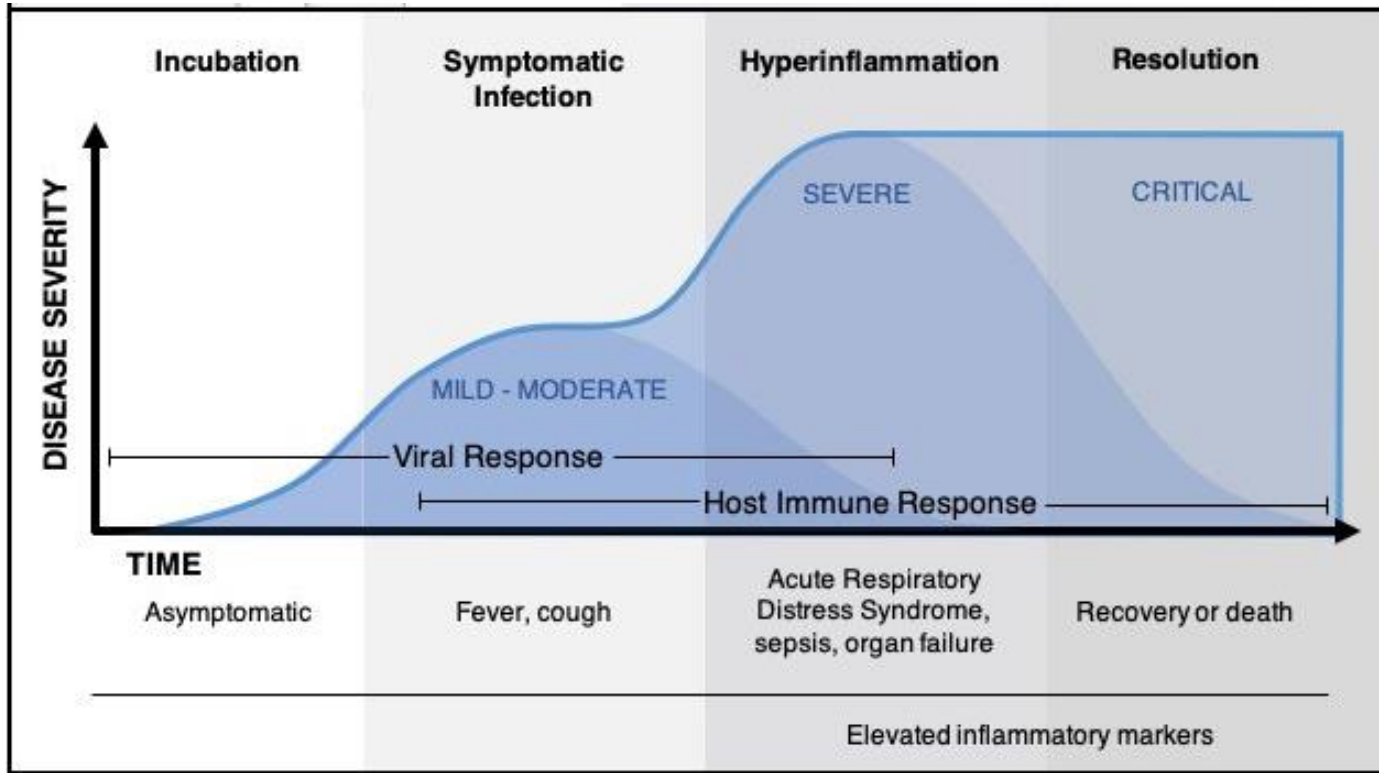
Part I. How to avoid the curse of dimensionality: Multi-omics data integration

SIMON to the rescue: COMBATing COVID-19



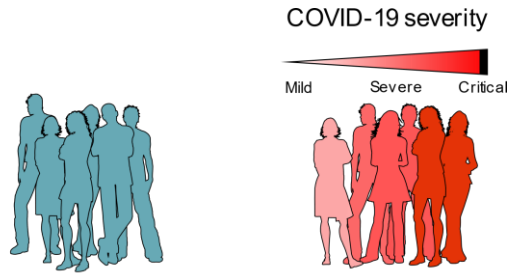
COMBAT
Oxford COVID-19 Multi-omic Blood ATlas

Understanding COVID-19

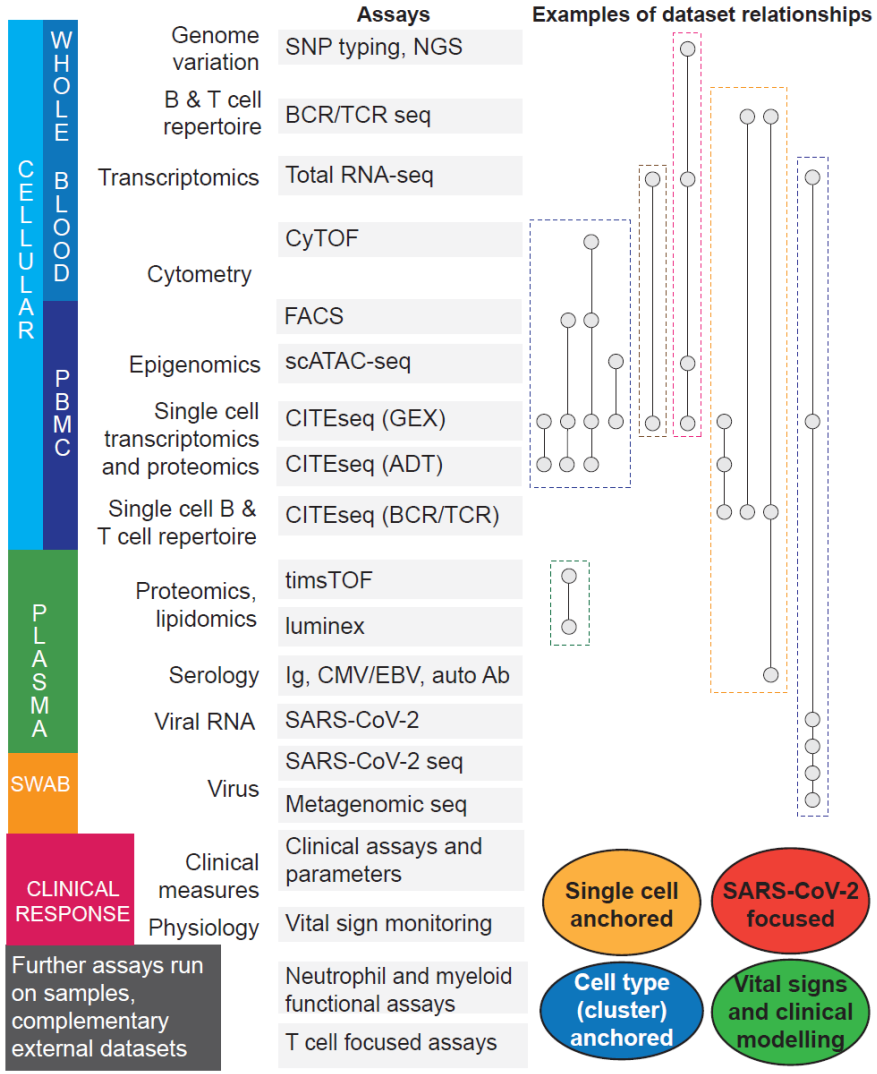


Oberfeld B. et al, SnapShot: COVID-19, Cell, 2020

5-10% of patients with COVID-19 progress to severe disease

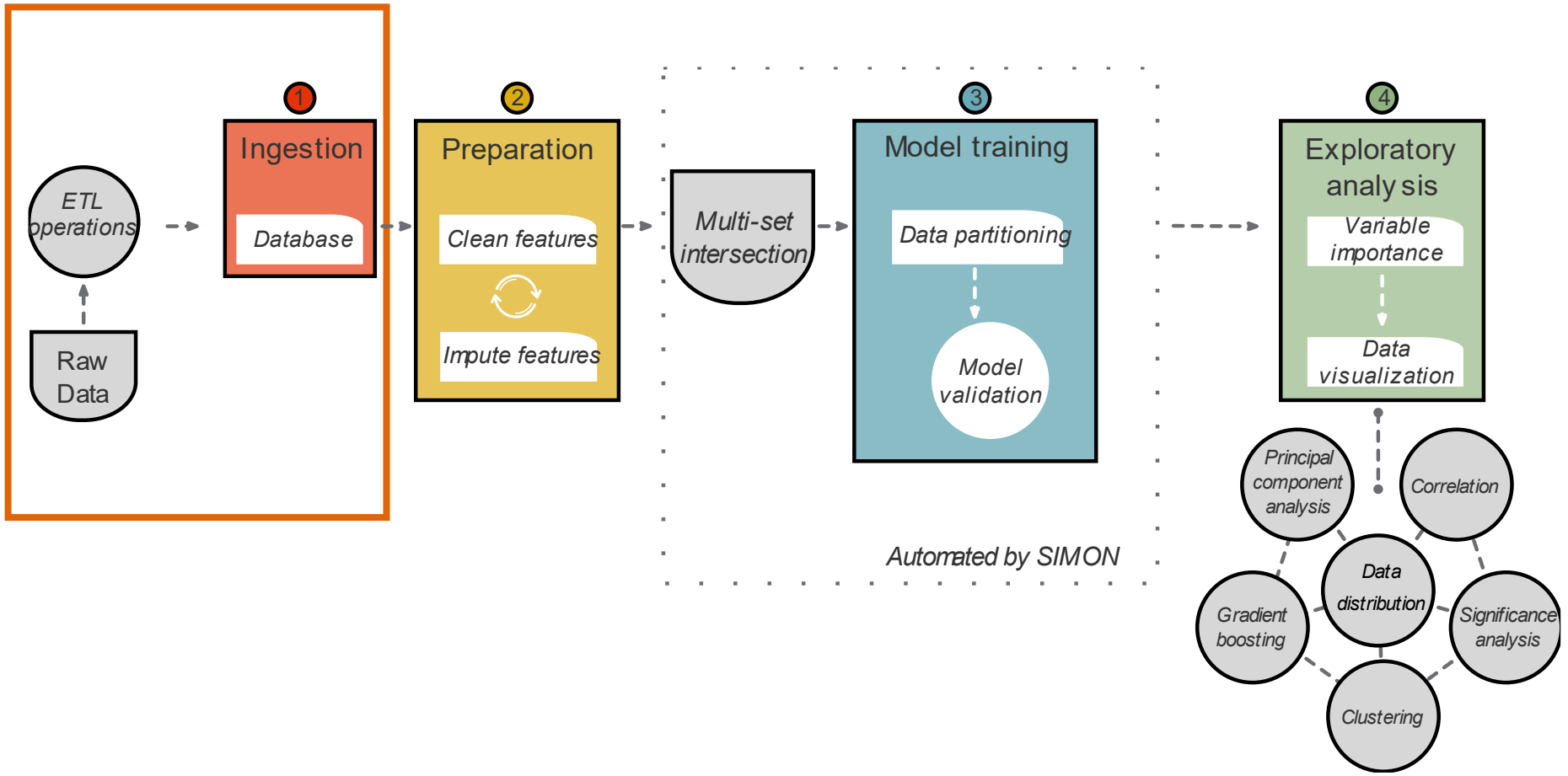


Immunology research at Oxford aimed to COMBAT COVID-19



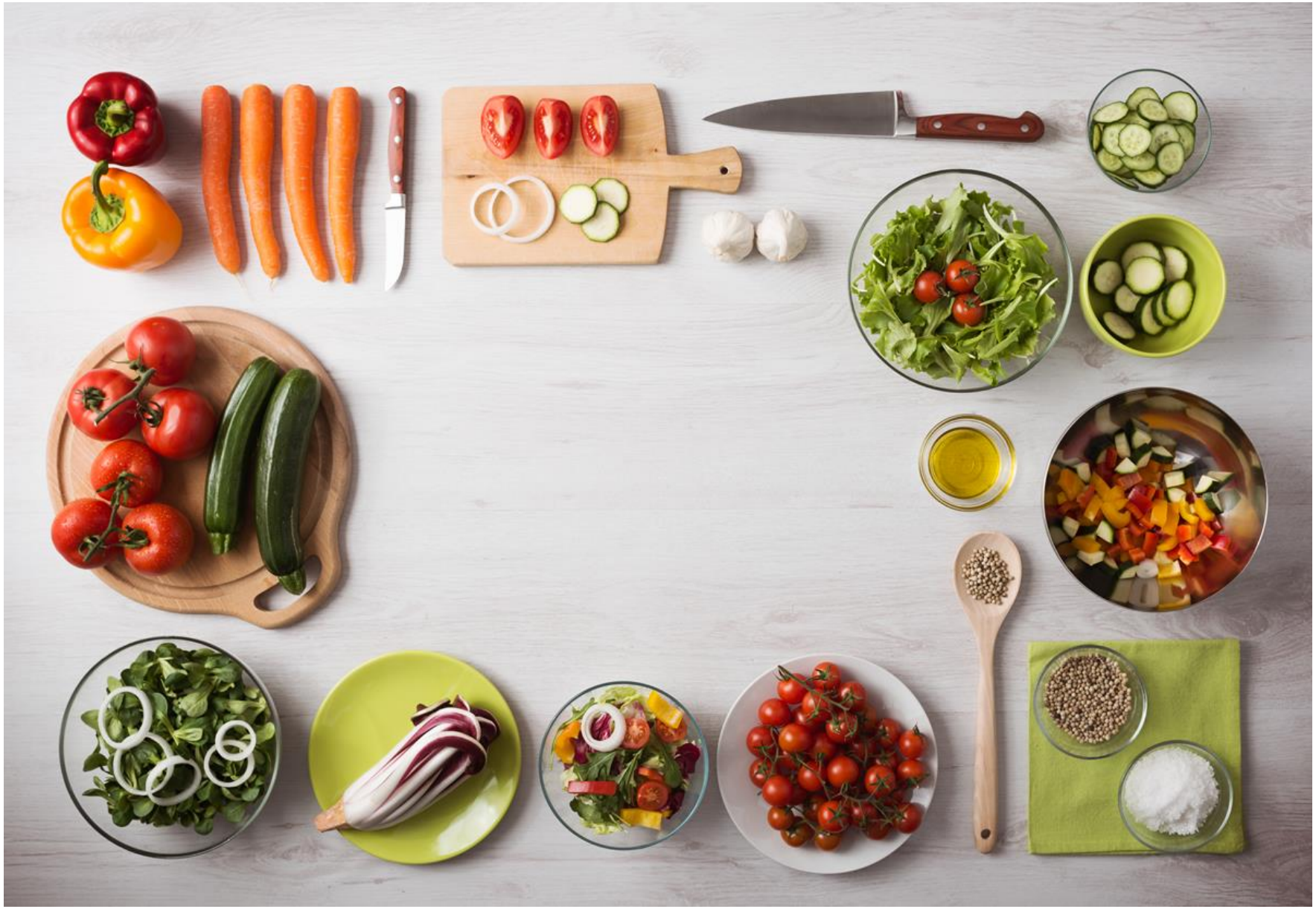
COMBAT
Oxford COVID-19 Multi-omic Blood ATlas

SIMON - Knowledge data discovery process



Tomic et al, JI, 2019 & Tomic et al, Patterns, 2021

‘Everything in its place’



'Everything in its place'

CyTOF

Cell frequencies and numbers in the granulocyte depleted samples
(8 files)

Iron measurement

Two measurements
(1 file)

GSA

Chr3p21 COVID19-associated GWAS region and ABO type
(2 files)

Mass Spec

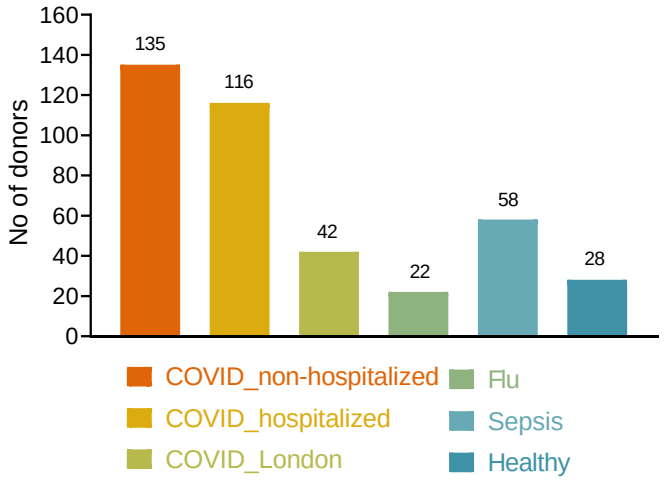
Processed intensity matrix
(1 file)

FACS

Cell frequencies, numbers and clusters
(3 files)

CLINICAL dataset

Total number of donors: 401
COVID_non-hospitalized: 135
COVID_hospitalized: 116
COVID_London: 42
Flu: 22
Sepsis: 58
Healthy: 28



RNAseq

Processed gene expression data (log cpm) and PCs COVID samples
(2 files)

Luminex

Concentrations and fluorescence intensities
(2 files)

CITEseq

Pseudobulk residuals broad and narrow
(2 files)

Data cleaning and generation of new variables

Pre-processing steps for each assay:

- **Data cleaning**

  replace special with alpha-numeric characters (+ → pos)

  data should only be numeric (replace ‘no data’ or ‘nd’ → NA)

  adding prefix/sufix to same parameters with different measurements

  (e.g. freq_cell subsets and Luminex parameter_intens)

- **Generating new features**

  hospitalization (yes or no)

  ventilation status (none or ventilated)

  oxygenation status (normal or abnormal)

  days_sample_taken_from_max_disease (days max disease – days

  sampling)

  Sampling (before or after max disease)

  Disease (recovered – convalescent samples vs ongoing)

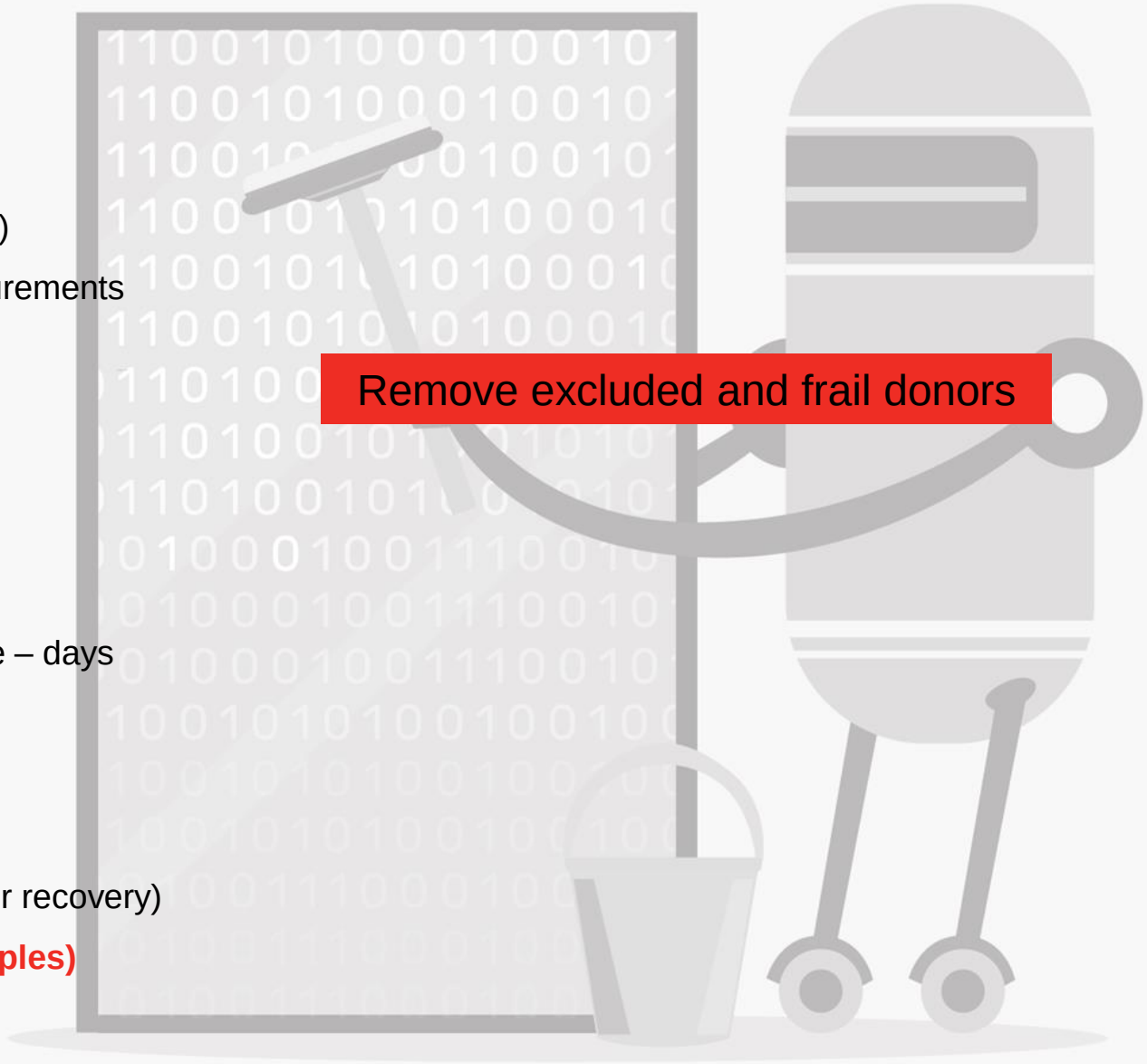
  Disease progress – for longitudinal samples (deterioration or recovery)

  Sampling order (first sample taken or second/third samples)

  sampling_from_max_disease

  Sampling after symptom onset (<6d - early and >6d – late)

Written as factors



COMBAT
Oxford COVID-19 Multi-omic Blood ATlas

428 samples → 268 donors

CyTOF

Total: 117 donors
COVID hospitalized: 58
COVID non-hospitalized: 11
COVID London: 0
Flu: 11
Sepsis: 37
Healthy: 11

Focusing on depleted samples

FACS

Total: 116 donors
COVID hospitalized: 57
COVID non-hospitalized: 13
COVID London: 2
Flu: 11
Sepsis: 23
Healthy: 10

Luminex

Total: 137 donors
COVID hospitalized: 72
COVID non-hospitalized: 22
COVID London: 0
Flu: 0
Sepsis: 43
Healthy: 0

Iron measurement

COVID hospitalized: 15

CLINICAL dataset

Total number of donors: 401
COVID_non-hospitalized: 135
COVID_hospitalized: 116
COVID_London: 42
Flu: 22
Sepsis: 58
Healthy: 28

GSA

Total: 124 donors
COVID hospitalized: 60
COVID non-hospitalized: 12
COVID London: 0
Flu: 0
Sepsis: 41
Healthy: 11

Mass Spec

Total: 229 donors
COVID hospitalized: 69
COVID non-hospitalized: 100
COVID London: 0
Flu: 0
Sepsis: 38
Healthy: 22

Focusing on processed samples

RNAseq

Total: 116 donors
COVID hospitalized: 57
COVID non-hospitalized: 13
COVID London: 0
Flu: 0
Sepsis: 36
Healthy: 10

CITEseq

Total: 116 donors
COVID hospitalized: 57
COVID non-hospitalized: 13
COVID London: 2
Flu: 11
Sepsis: 23
Healthy: 10

Focusing on pseudobulk residuals (broad & narrow)

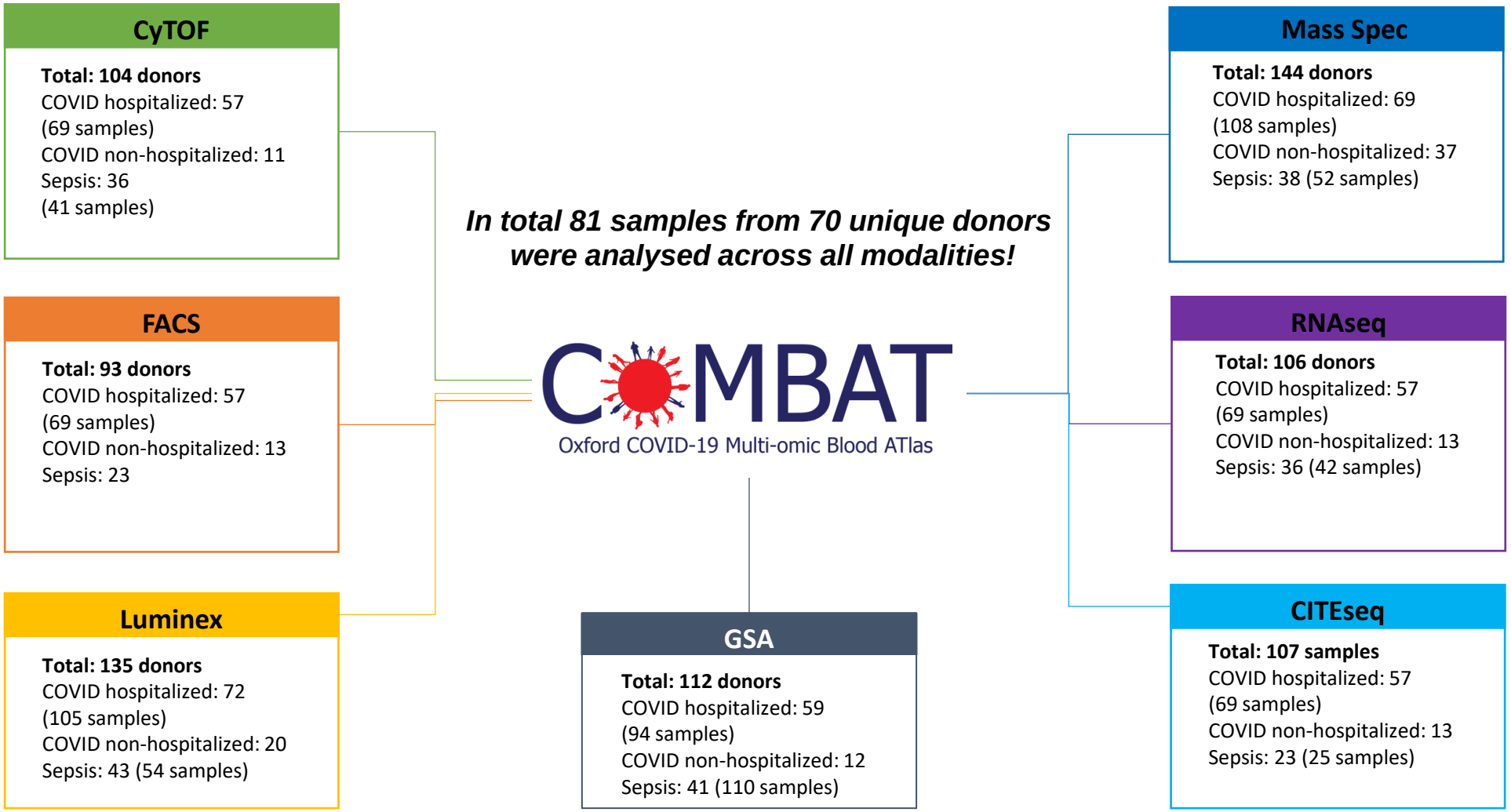
COMBAT dataset: 428 samples → 268 donors

Remove convalescent (covid, sepsis, hcw), Covid_London, flu and healthy volunteers

281 samples → 167 donors

Which samples were analysed across modalities?

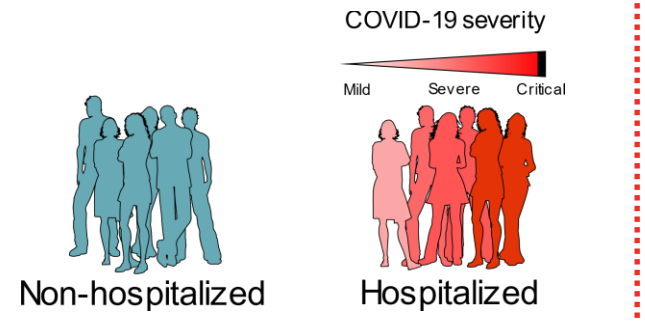
Samples and unique donors analysed across modalities

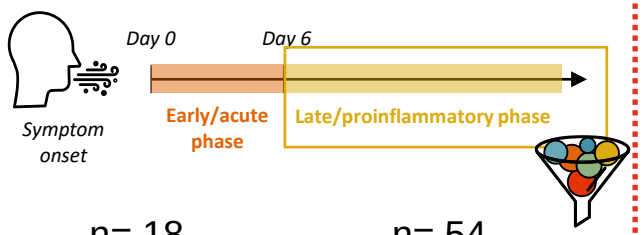


Knowledge discovery using COMBAT dataset

Outcome 1

Difference between Sars-CoV2-infected individuals?





n= 18 n= 54

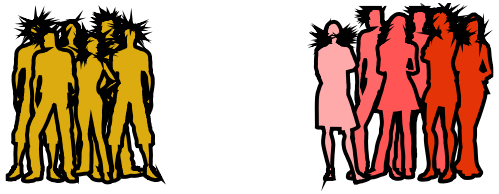
Integration of Mass Spec and Luminex (no GSA)

n= 5 n= 47

Integration of CyTOF, FACS, RNAseq, CITEseq and GSA

Outcome 2

Difference between hospitalized Sars-CoV2-infected and sepsis patients?



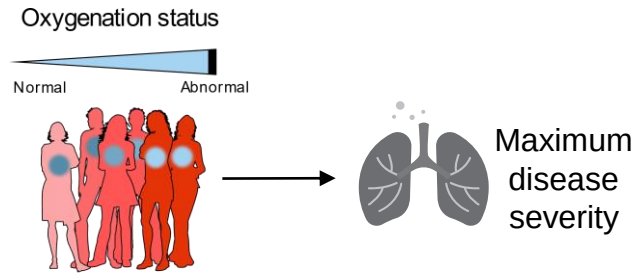
Sepsis
n= 15

COVID-19
n= 53

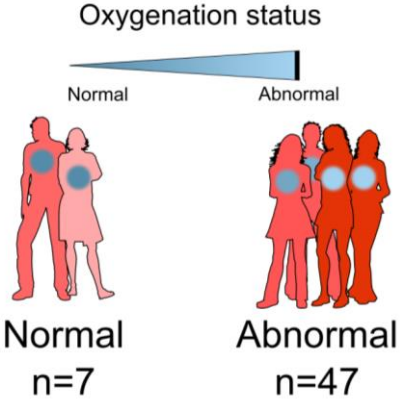
Use entire COMBAT dataset

Outcome 3

Difference between hospitalized Sars-CoV2-infected patients?



Hospitalized COVID-19
n= 53

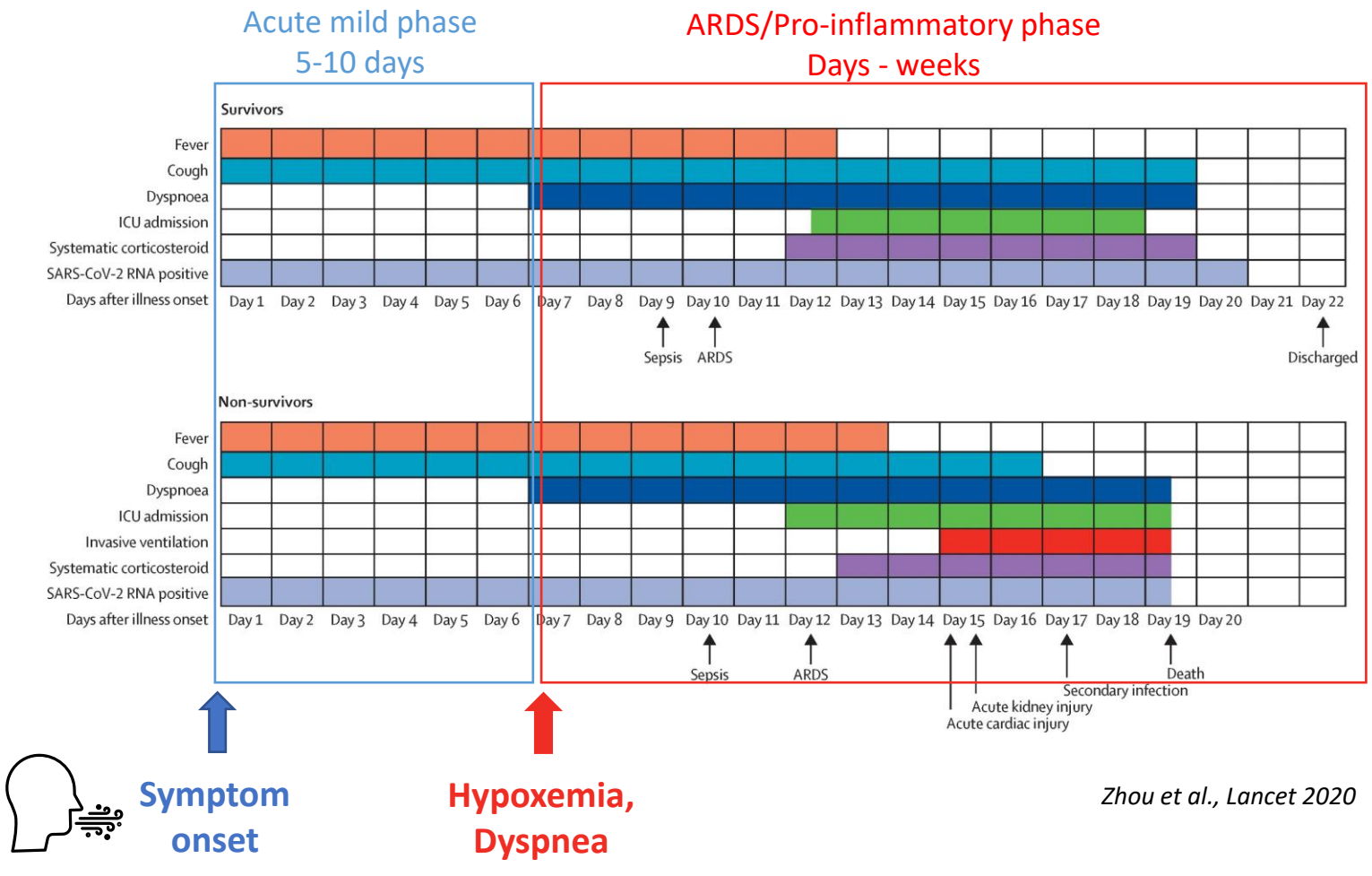


Normal
n=7

Abnormal
n=47

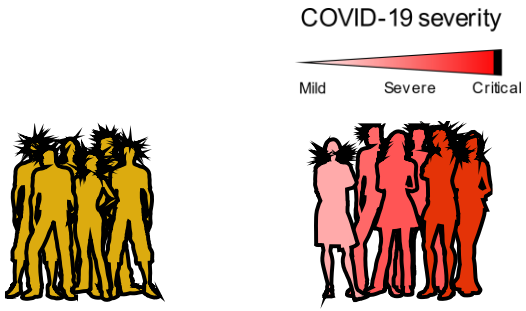
Use entire COMBAT dataset

Knowledge discovery using COMBAT dataset



Zhou et al., Lancet 2020

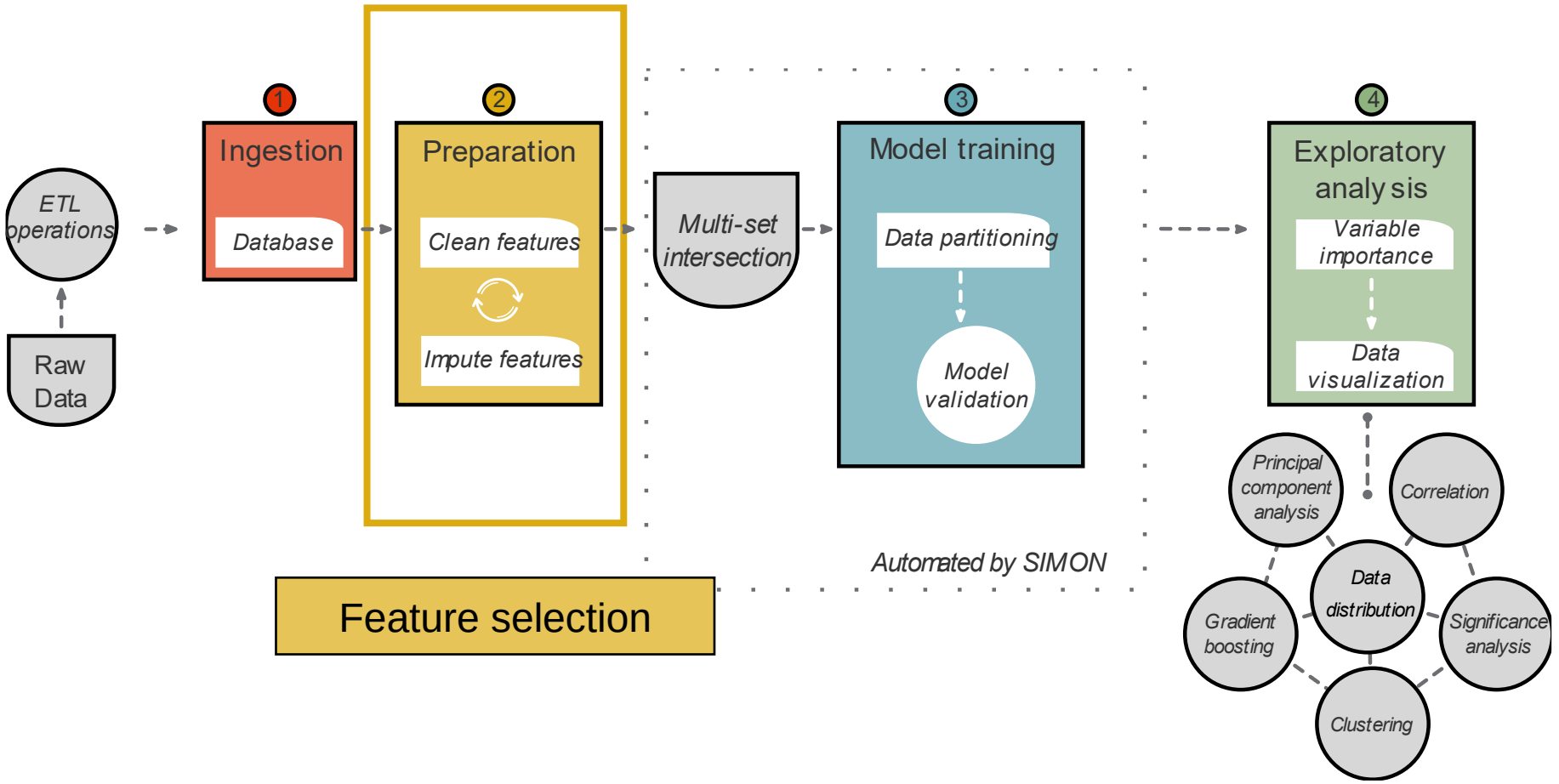
Difference between hospitalized SARS-CoV-2-infected and sepsis patients?



Sepsis
n= 15

COVID-19
n= 53

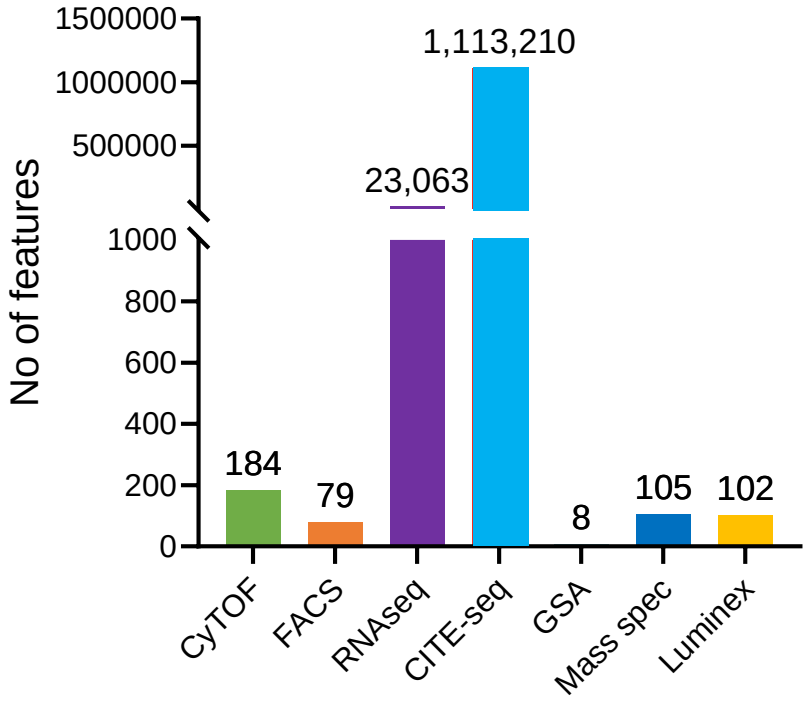
SIMON - Knowledge data discovery process

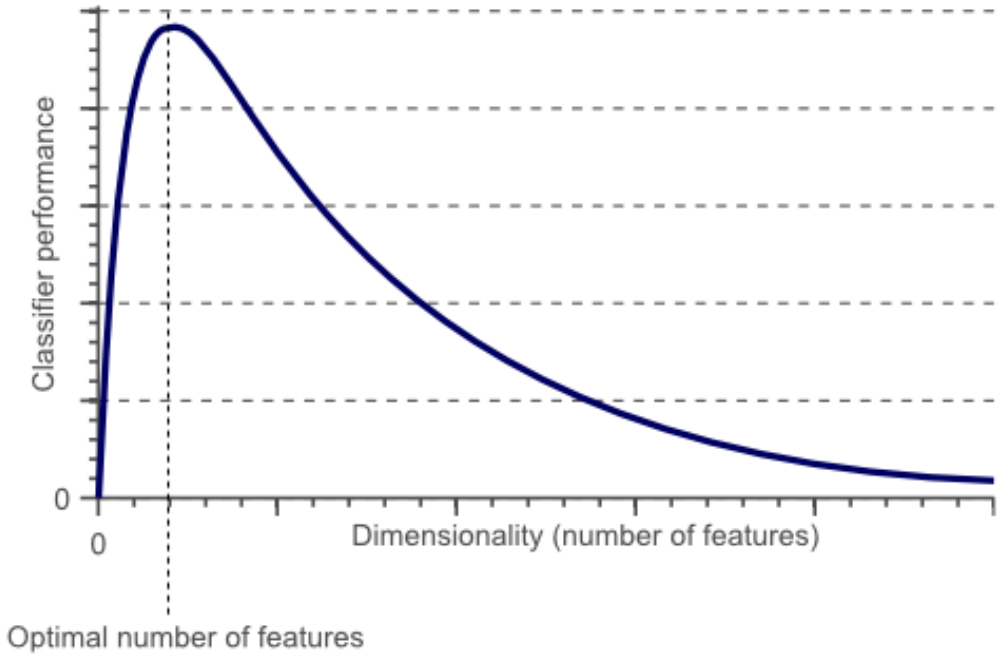


Tomic et al, JI, 2019 & Tomic et al, Patterns, 2021

‘Curse of dimensionality’

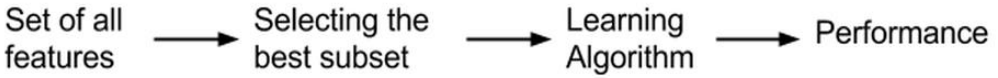
COMBAT
Oxford COVID-19 Multi-omic Blood ATlas





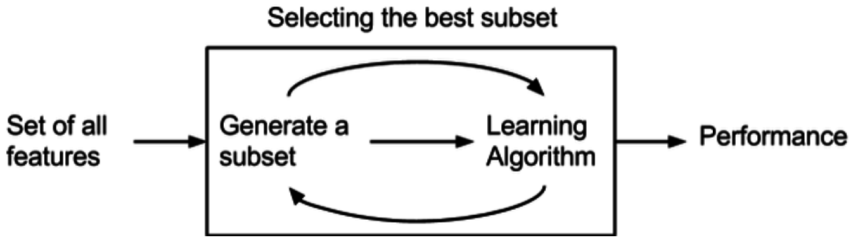
Feature selection

1. ***Filter methods*** - use a proxy measure to score a feature subset

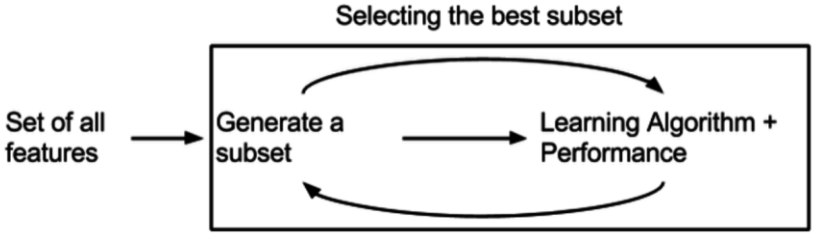


e.g. significance test, correlation

2. Wrapper methods - use a predictive model to score feature subsets



3. Embedded Methods - catch-all group of techniques which perform feature selection as part of the model construction process (e.g. LASSO)



SIMON - Feature selection process

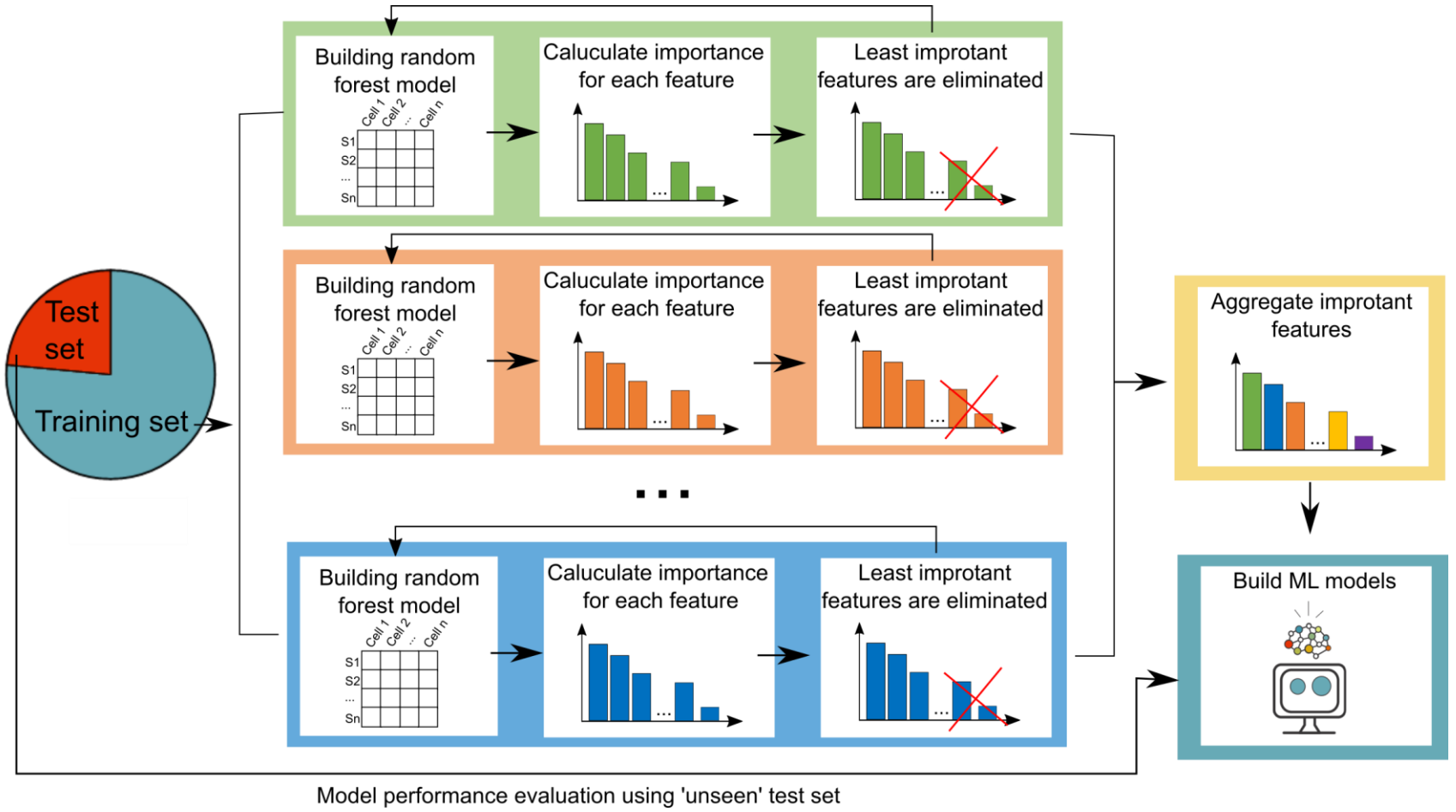
1. Dataset partitioning

2. Feature selection on training set within each assay (recursive feature elimination)

3. Merging selected features

4. Machine learning using SIMON

5. Model performance on test set



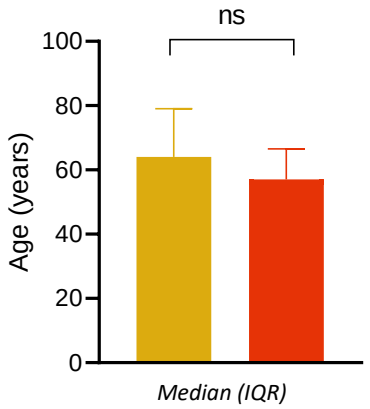
Difference between hospitalized SARS-CoV-2-infected and sepsis patients?

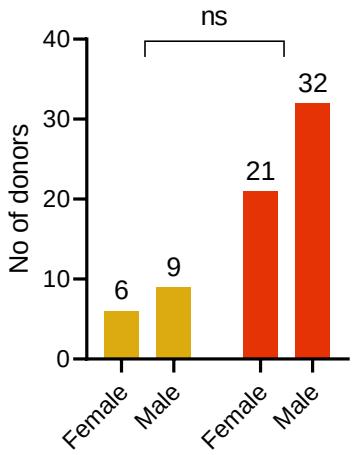
Sepsis

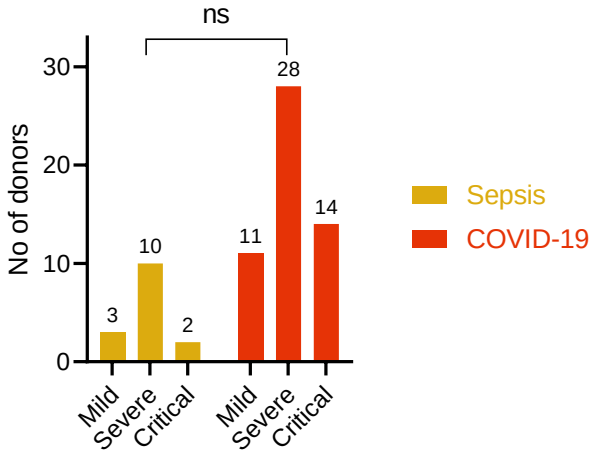
n= 15

COVID-19

n= 53





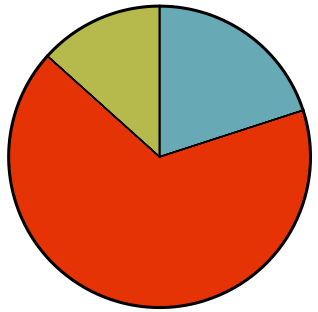


Demographic data of hospitalized COVID-19 and sepsis patients

Characteristics	Sepsis (n=15)	COVID-19 (n=53)	*P value*
Age (y)			0.0969[a]
Mean ± SD	63.6 ± 17.2	56.6 ± 14.2	
Median (IQR)	64 (52-79)	57 (47.5-66.5)	
Gender			0.3305[b]
Female (%)	6 (40%)	21 (40%)	
Male (%)	9 (60%)	32 (60%)	
Disease severity			0.1250[b]
Mild (%)	3 (20%)	11 (21%)	
Severe (%)	10 (67%)	28 (53%)	
Critical (%)	2 (13%)	14 (26%)	

Abbreviation: IQR, interquartile range
[a]Two-tailed Mann-Whitney test
[b]Two-way ANOVA

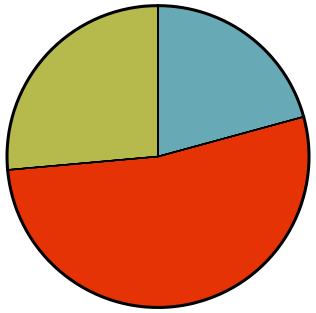
Sepsis severity



20.00% 3 Mild
66.67% 10 Severe
13.33% 2 Critical

Total=15

COVID-19 severity

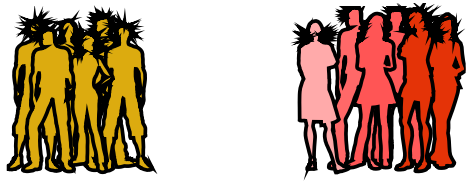


20.75% 11 Mild
52.83% 28 Severe
26.42% 14 Critical

Total=53

Feature selection process

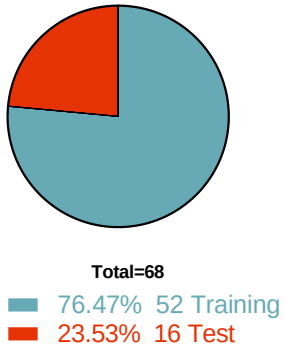
Initial dataset
n=68

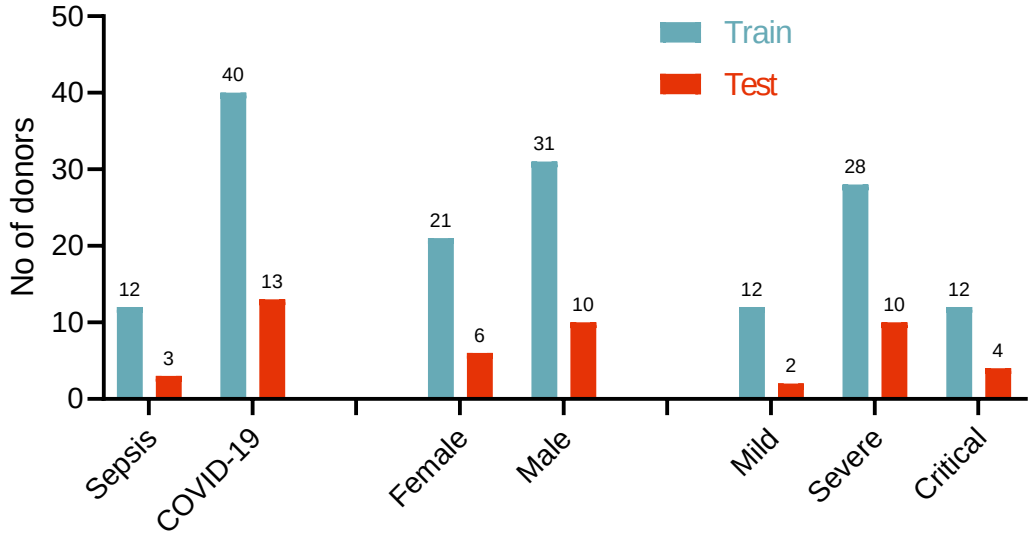


Sepsis
n= 15

COVID-19
n= 53

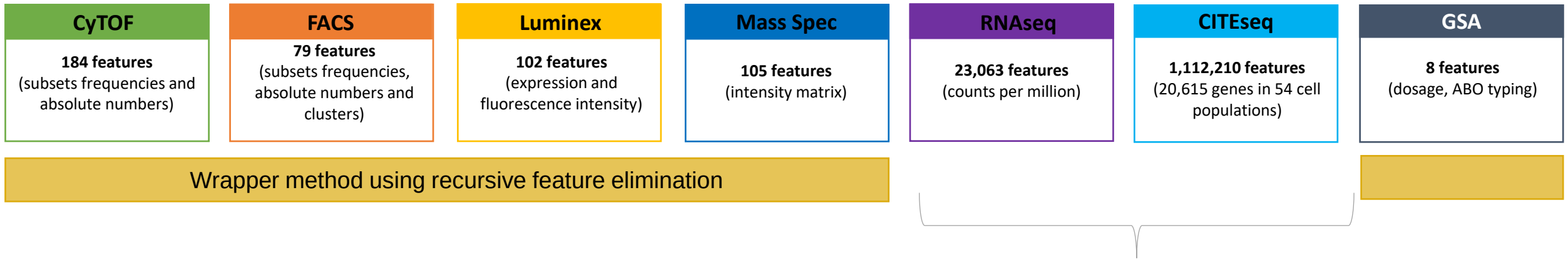
Dataset partitioning
3/4





1. Dataset partitioning
2. Feature selection on training set within each assay
3. Merging selected features
4. Machine learning using SIMON
5. Model performance on test set

Feature selection process



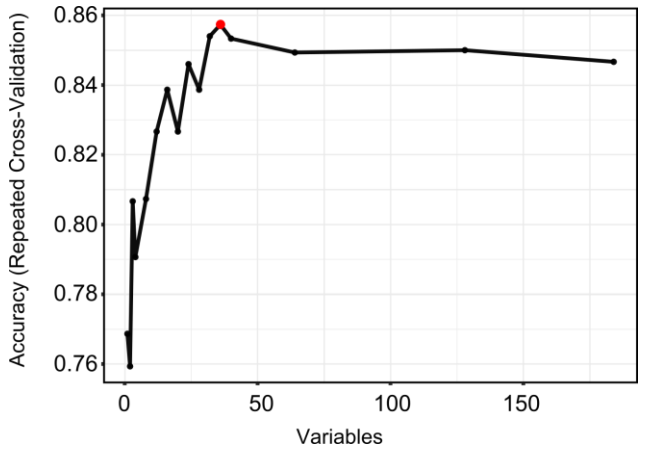
1. Dataset partitioning
2. Feature selection on training set within each assay
3. Merging selected features
4. Machine learning using SIMON
5. Model performance on test set

CyTOF

184 features
(subsets frequencies and absolute numbers)

36

36 features from CyTOF dataset selected by the top performing model

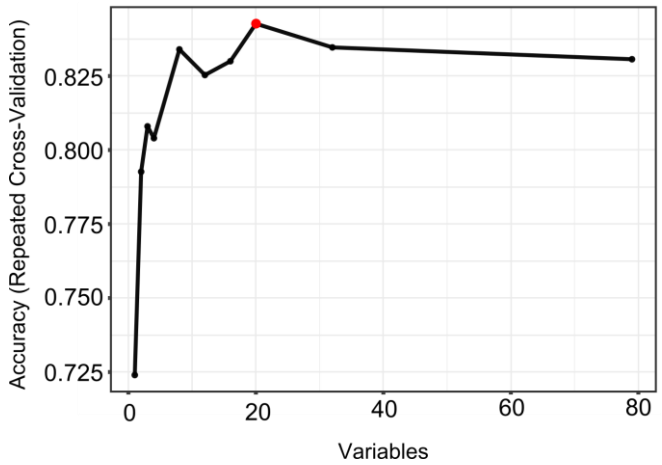


FACS

79 features
(subsets frequencies, absolute numbers and clusters)

20

20 features from FACS dataset selected by the top performing model

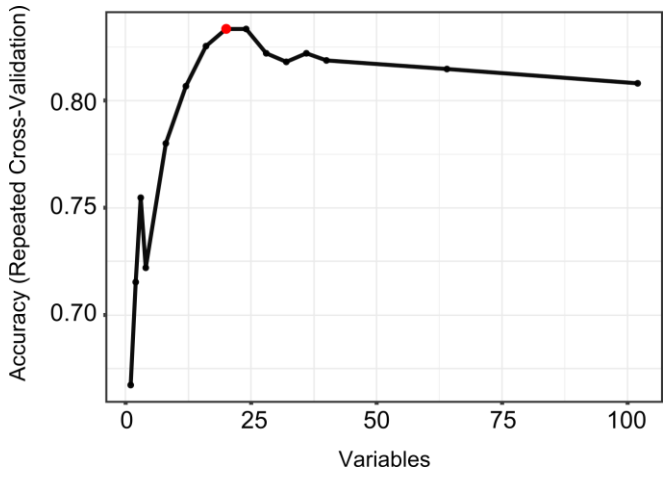


Luminex

102 features
(expression and fluorescence intensity)

20

20 features from Luminex dataset selected by the top performing model

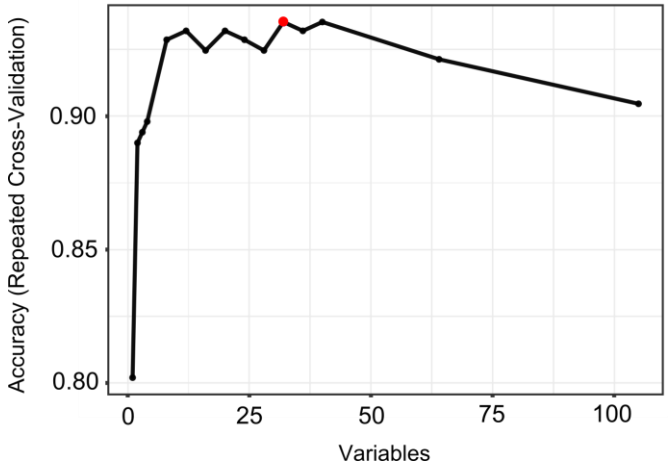


Mass Spec

105 features
(intensity matrix)

32

32 features from Mass Spec dataset selected by the top performing model



RNAseq

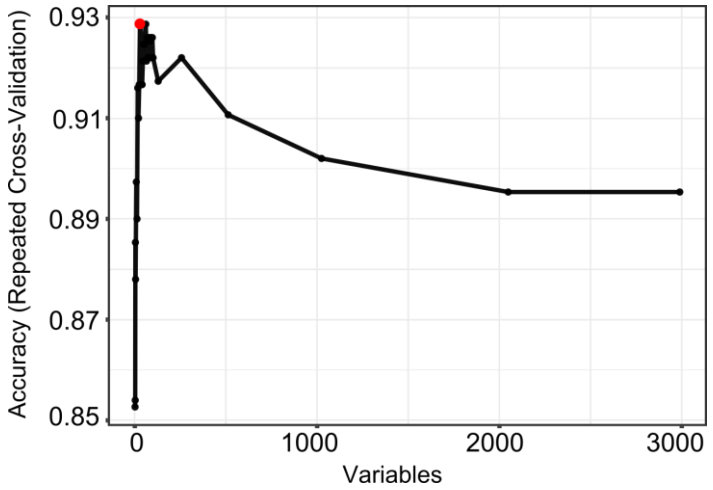
23,063 features
(counts per million)

↓

2,989 features
(DEGs, FDR<0.05 and FC>1.5)

28

28 features from RNAseq dataset selected by the top performing model

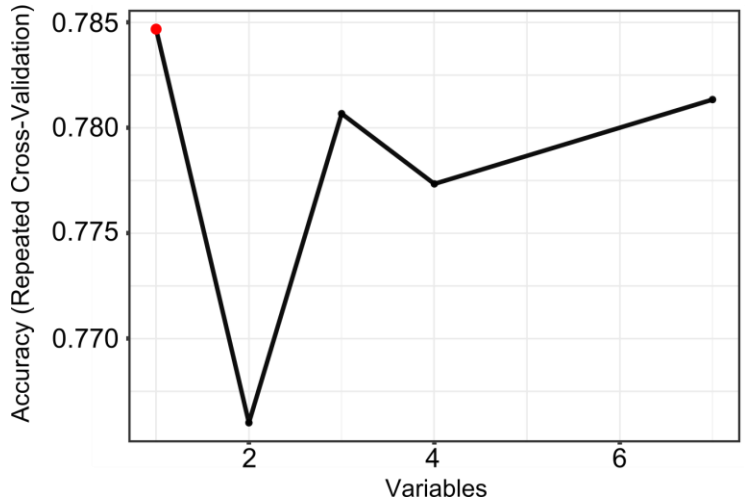


GSA

8 features
(dosage, ABO typing)

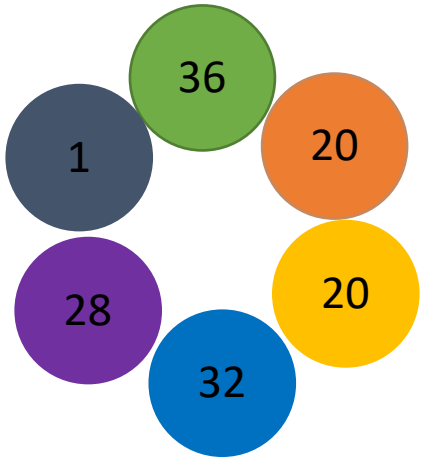
1

1 feature (SE genotype) from GSA dataset selected by the top performing model



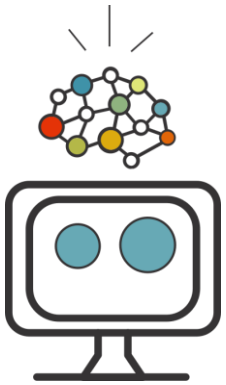
Feature selection process – merging selected features

Merged dataset



137 features and 52 donors

SIMON



4 ML algorithms

Naïve Bayes

Shrinkage discriminant analysis

Support vector machine

C5.0 – decision tree

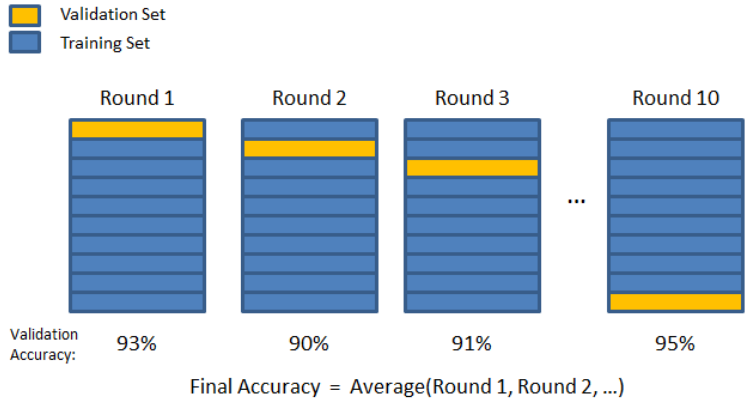
Model training

10-fold cross-validation

repeated 10 times

MODEL evaluation

10-fold cross-validation

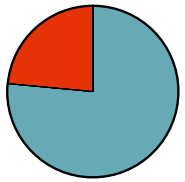


Confusion matrix

n=52	Predicted: Sepsis	Predicted: COVID-19	Accuracy
Actual: Sepsis	0 True positive	12 False positive	
Actual: COVID-19	0 False negative	40 True negative	
Overall accuracy	**Sensitivity** TP / (TP + FN) = 0	**Specificity** TN / (TN + FP) = 0.77	**ACCURACY** (0+40) / 52 = 0.77

1. Dataset partitioning | 2. Feature selection on training set within each assay | 3. Merging selected features | 4. Machine learning using SIMON | 5. Model performance on test set

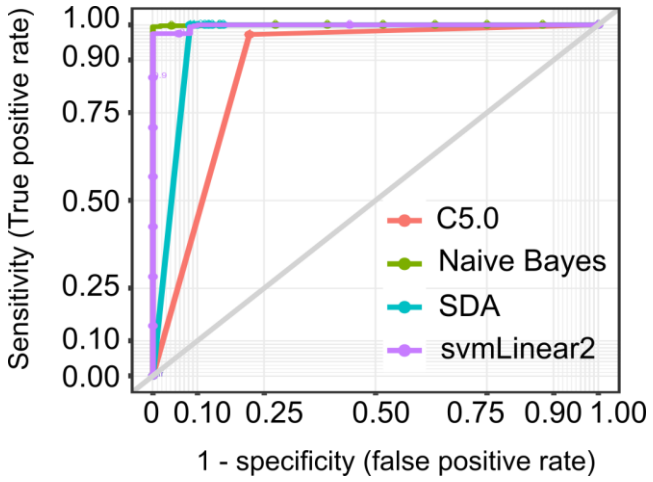
Feature selection process – final model

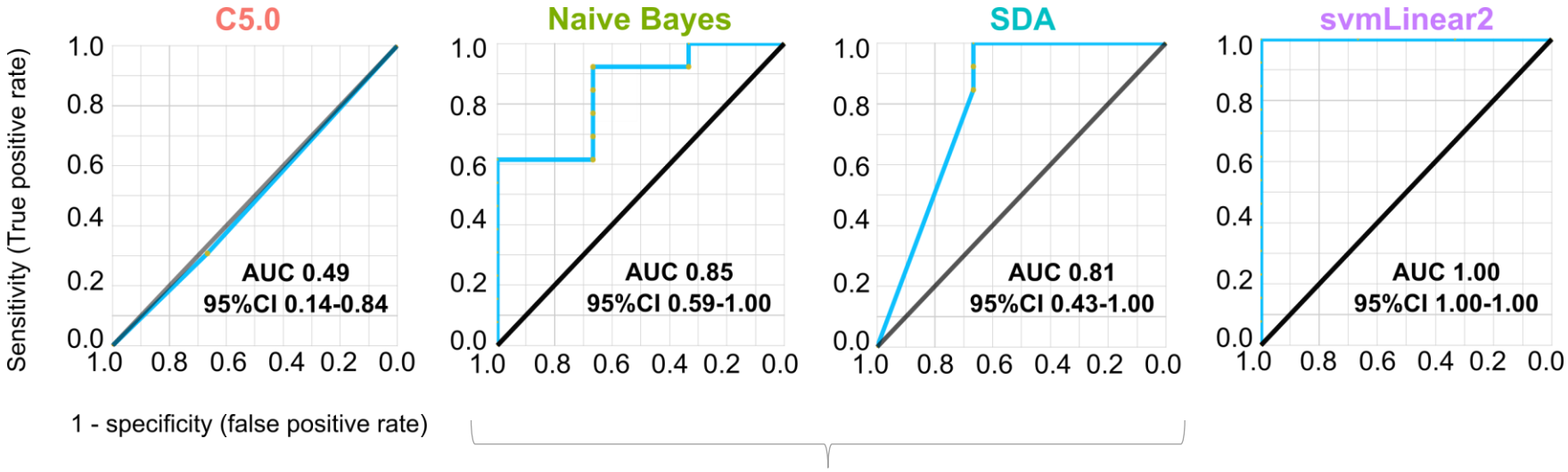


76.47% 52 Training
23.53% 16 Test

'unseen' test set – ROC plots across models

Training set – ROC plots across models





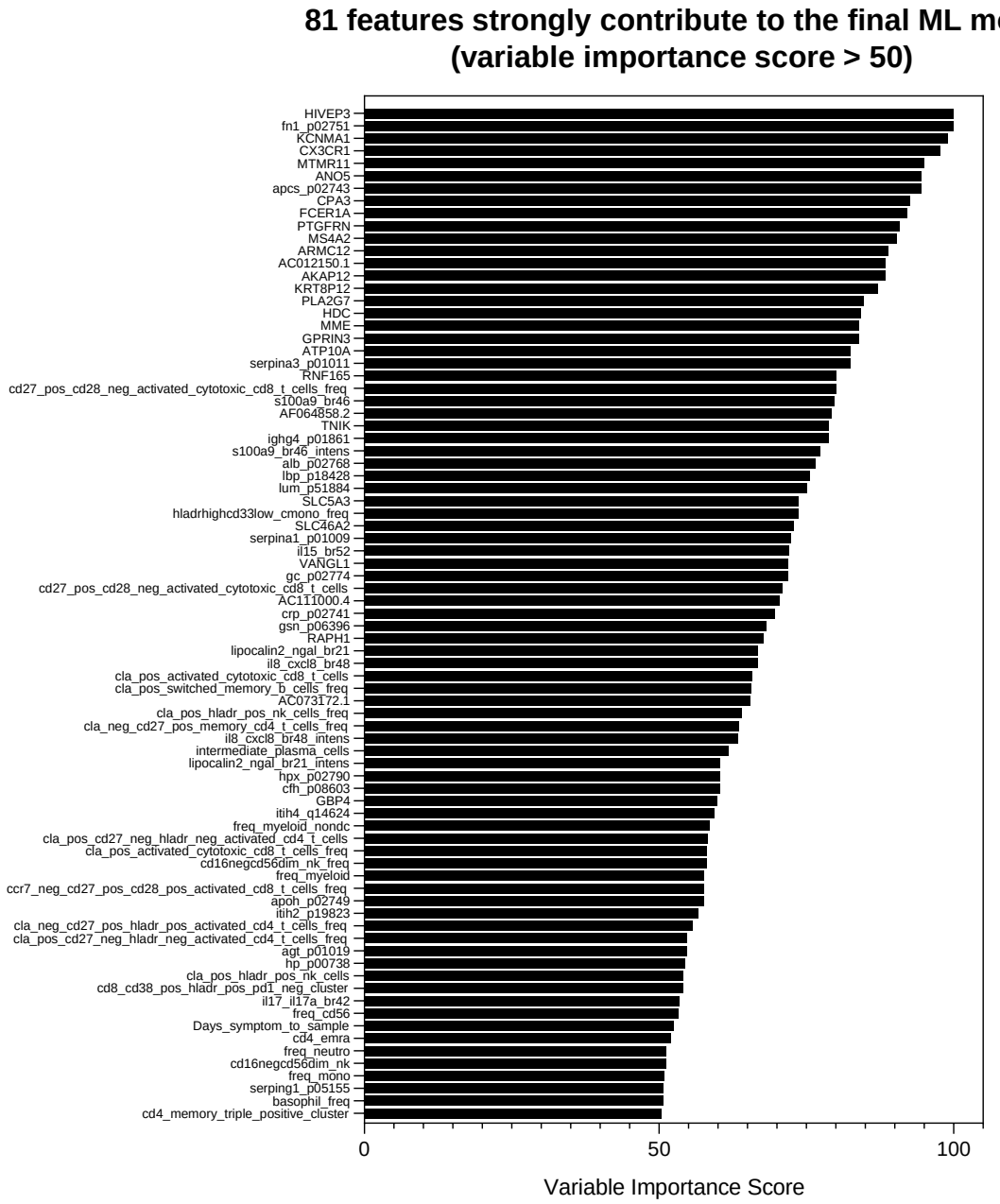
RANDOM

GOOD DISCRIMINATIVE ABILITY

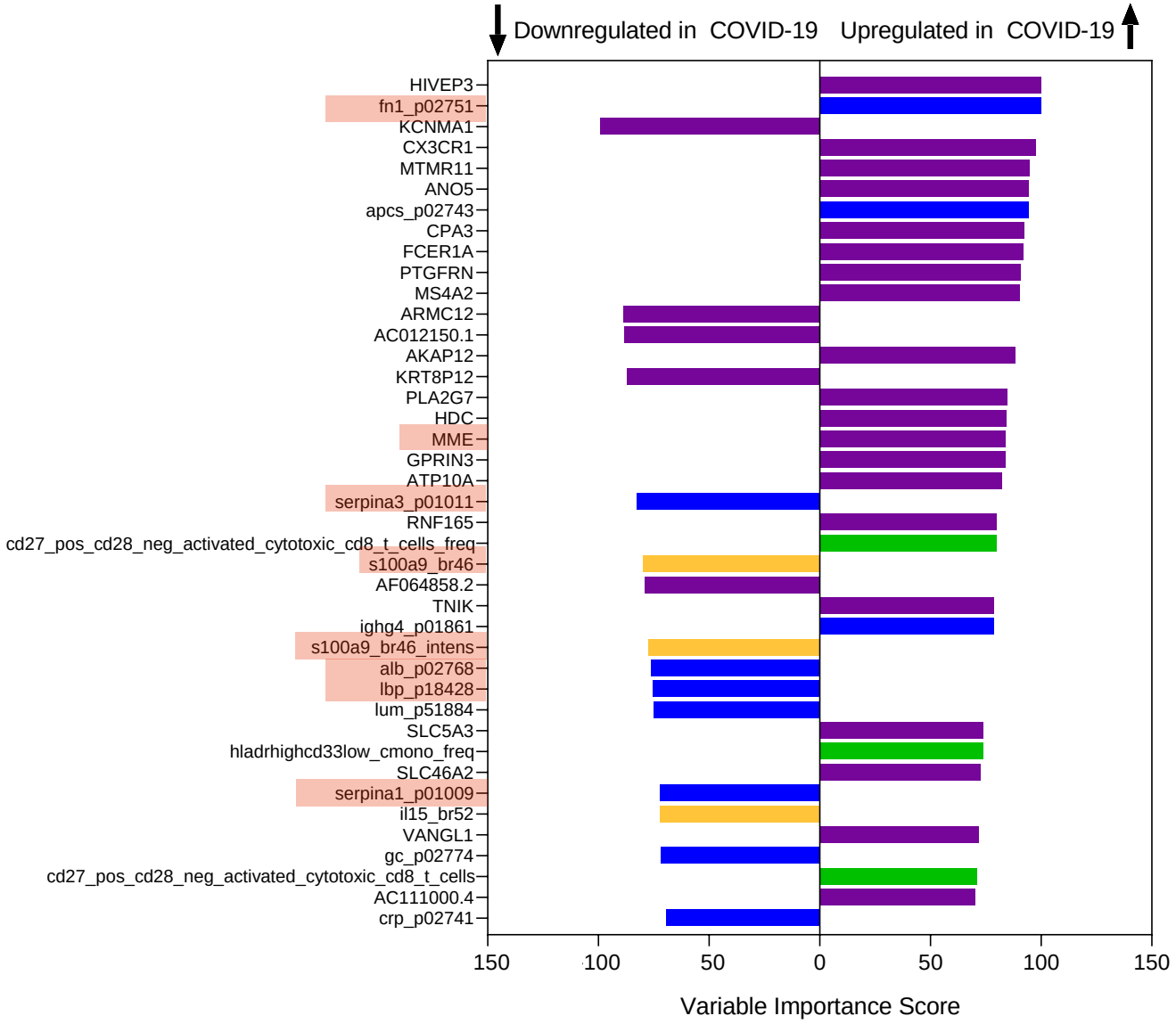
OVERFITTED
Specificity = 0
Sensitivity = 1
**model can only discriminate COVID-19 patients and always makes mistake on sepsis patients*

1. Dataset partitioning
2. Feature selection on training set within each assay
3. Merging selected features
4. Machine learning using SIMON
5. Model performance on test set

Pattern recognition in COMBAT using SIMON



COVID-19: Knowns and unknowns



FN1, fibronectin → lung fibrosis in COVID-19 patients
Xu et al, 2020. doi: 10.1186/s12931-020-01445-6.

MME - neprilysin → part of the ACE2 complex
Emameh et al, 2020. doi: 10.1186/s12575-020-00124-6.

Alpha-1-antichymotrypsin (Serpina 3) → up-regulated in 2006 SARS
Wan et al, 2006. doi: 10.1002/pmic.200500638.

Calprotectin (S100A9) → increased in severe COVID-19 patients
Silvin et al, Cell 2020. doi: 10.1016/j.cell.2020.08.002.

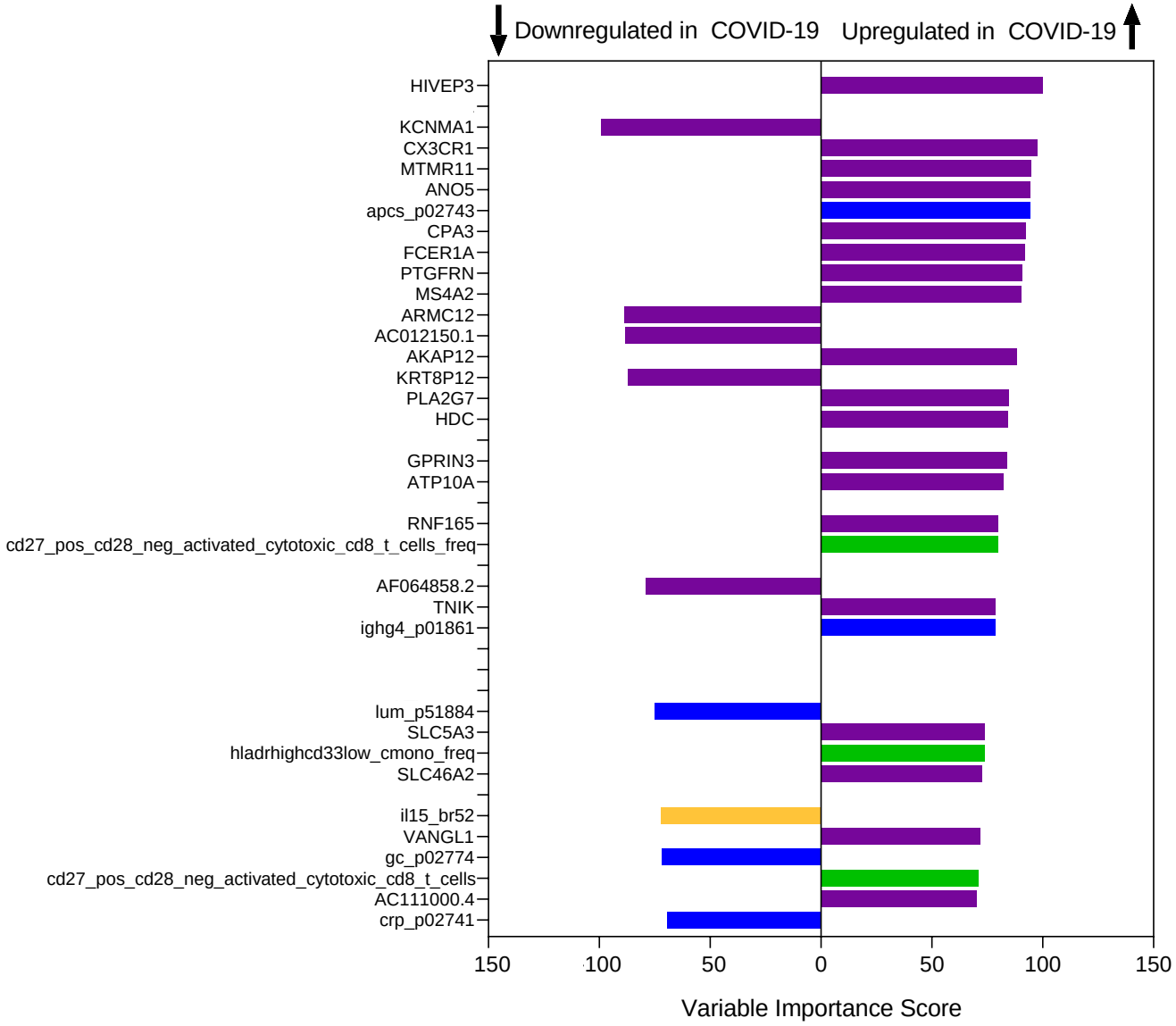
Albumin (ALB) → hypoalbuminemia in COVID-19 patients

LBP, LPS binding protein → increased in COVID-19 patients
Hoel et al, J Intern Med 2020. doi: 10.1111/joim.13178.

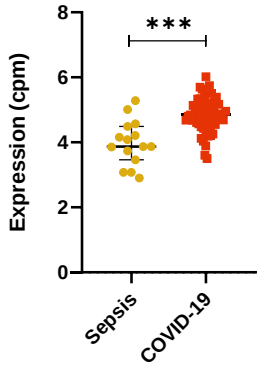
Alpha-1-antitrypsin (Serpina 1) → inhibits SARS CoV2 infection
de Loyola et al, 2020. doi: 10.1002/rmv.2157

41 features with score above 69
RNAseq – purple, Mass Spec – blue, CyTOF – green, Luminex - yellow

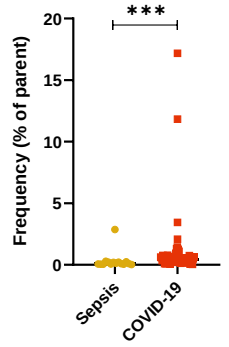
Pattern recognition in COMBAT using SIMON



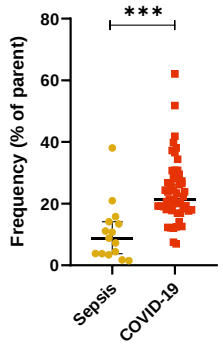
Hivep3



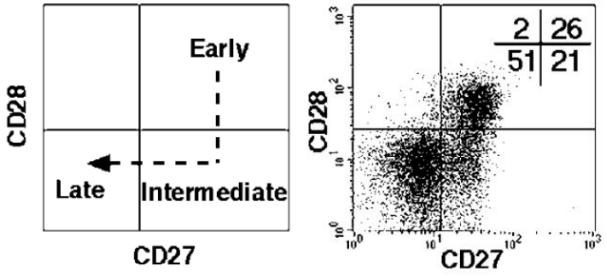
CD27+ CD28- Activated CD8+ T cells



HLA-DR high CD33 low cMono



Stages of differentiation

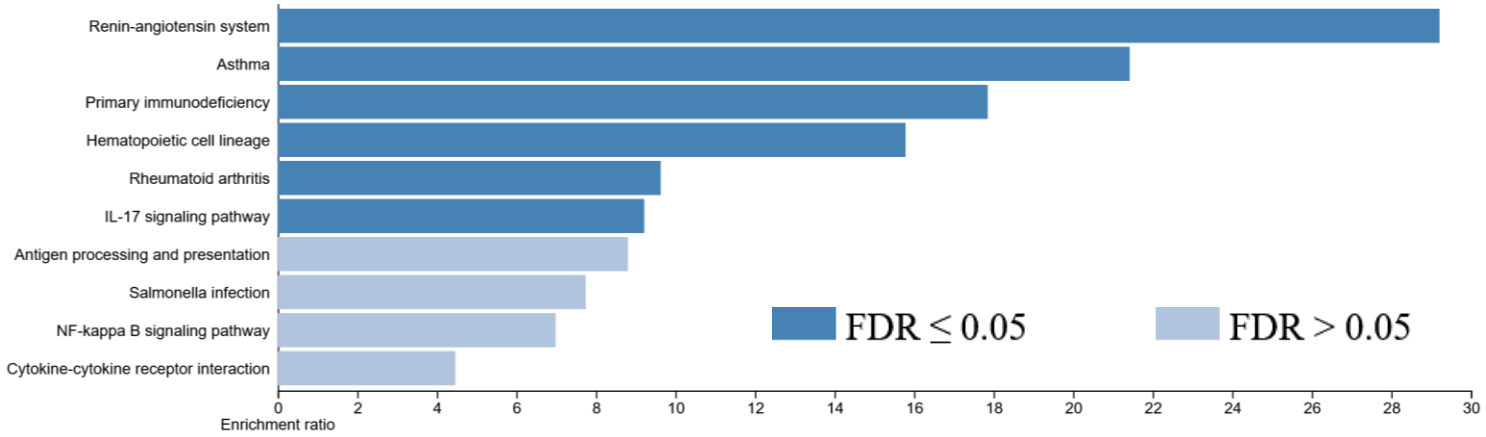


41 features with score above 69

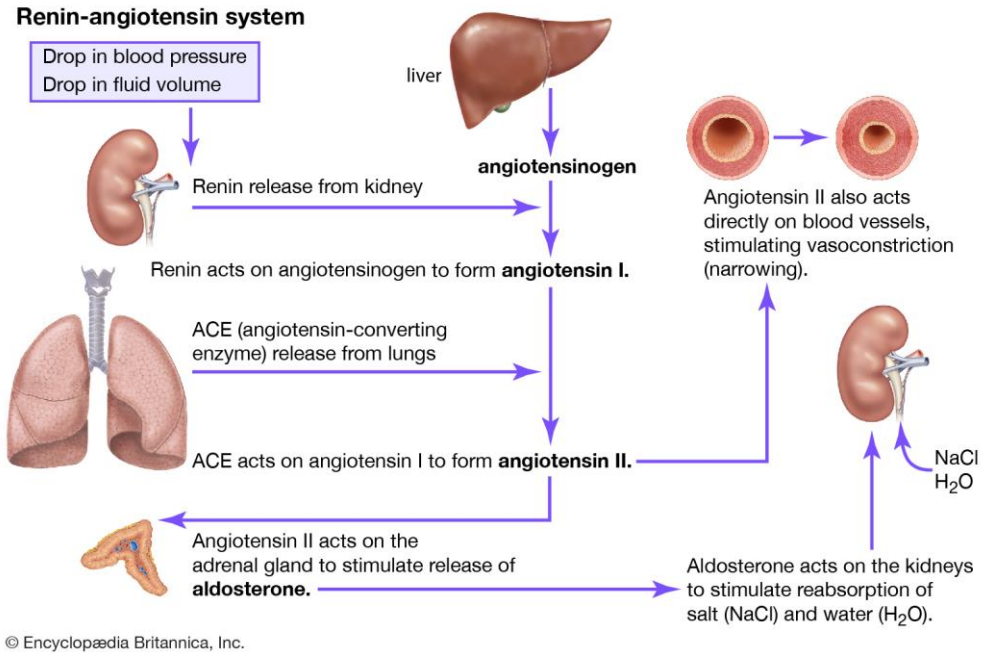
RNAseq – purple, Mass Spec – blue, CyTOF – green, Luminex - yellow

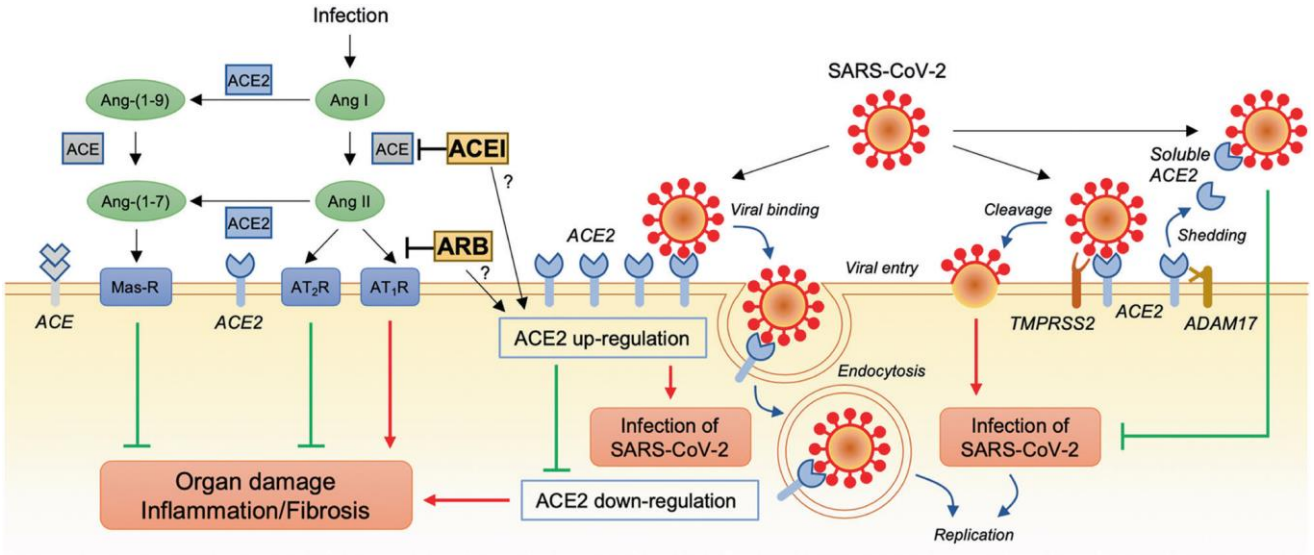
Apply et al, Nat Med, 2002

COVID-19 vs sepsis patients – KEGG pathway analysis



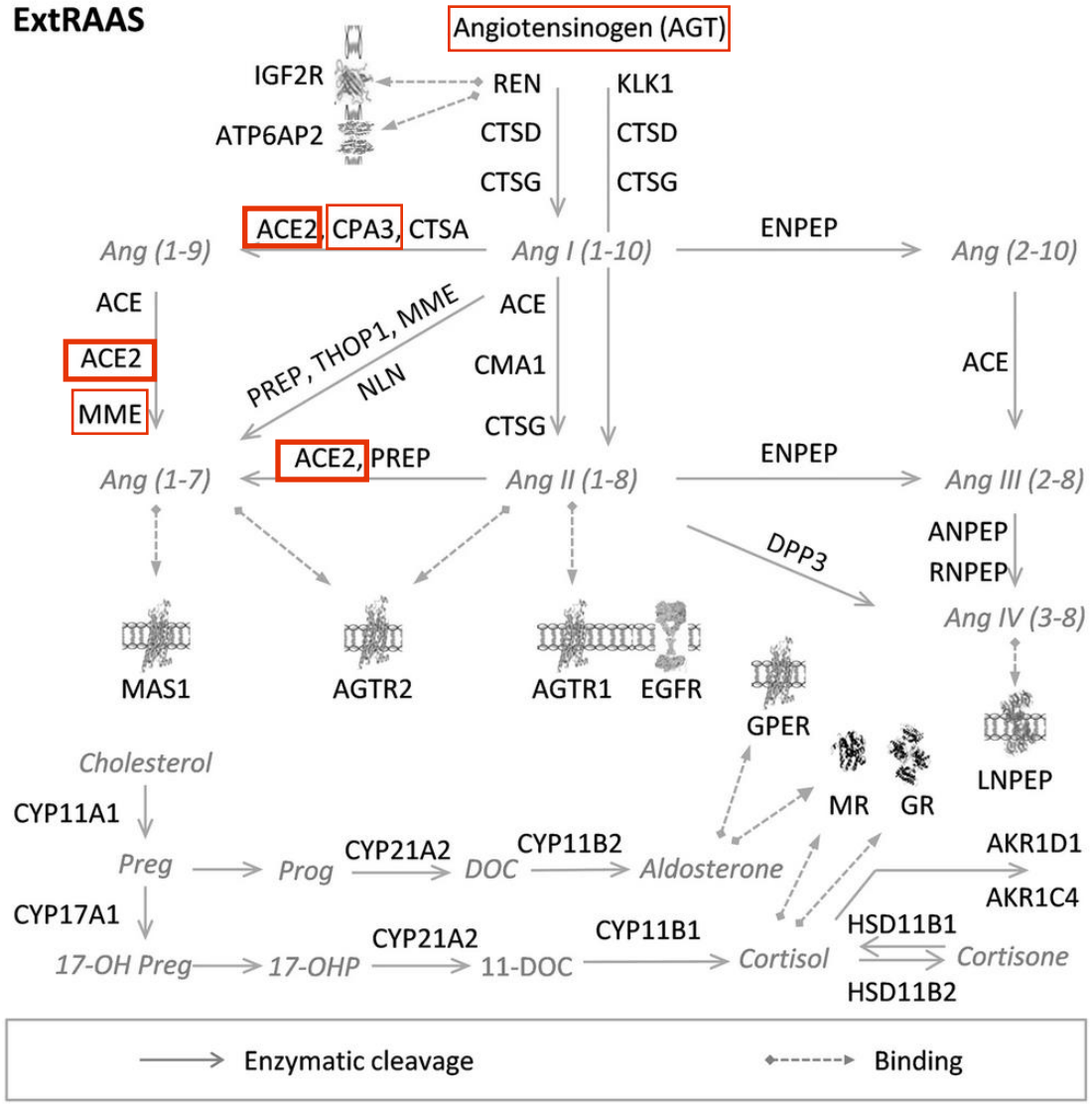
Possible associations of the renin-angiotensin system with SARS-CoV-2 infection and lung injury caused by SARS-CoV-2



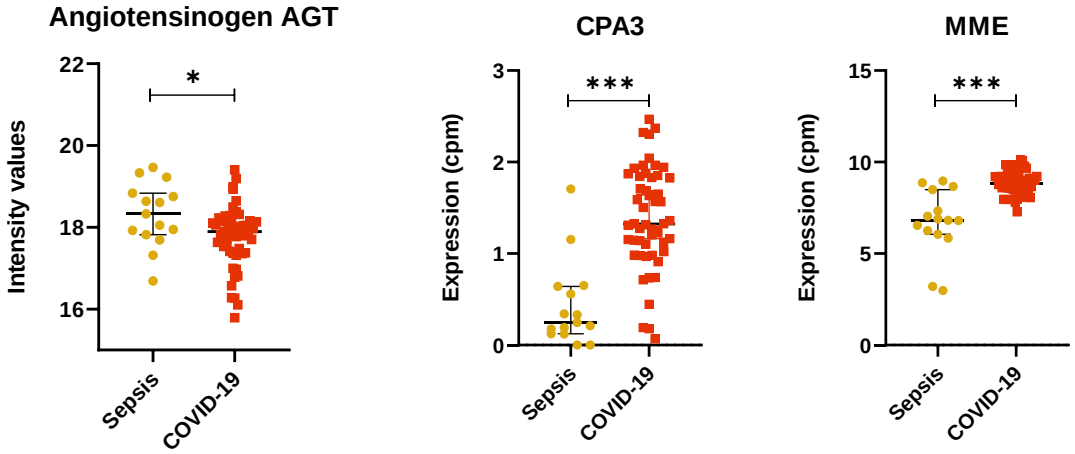


Furuhashi et al, Hypertension Research, 2020, doi: 10.1038/s41440-020-0478-1

Renin-angiotensin system in COVID-19

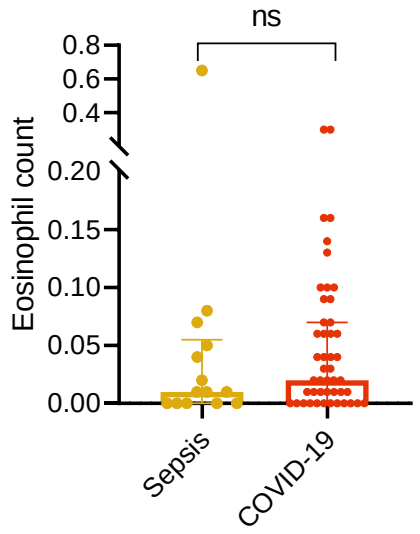


Nehme et al, Sci Rep, 2015, doi: 10.1038/srep10035



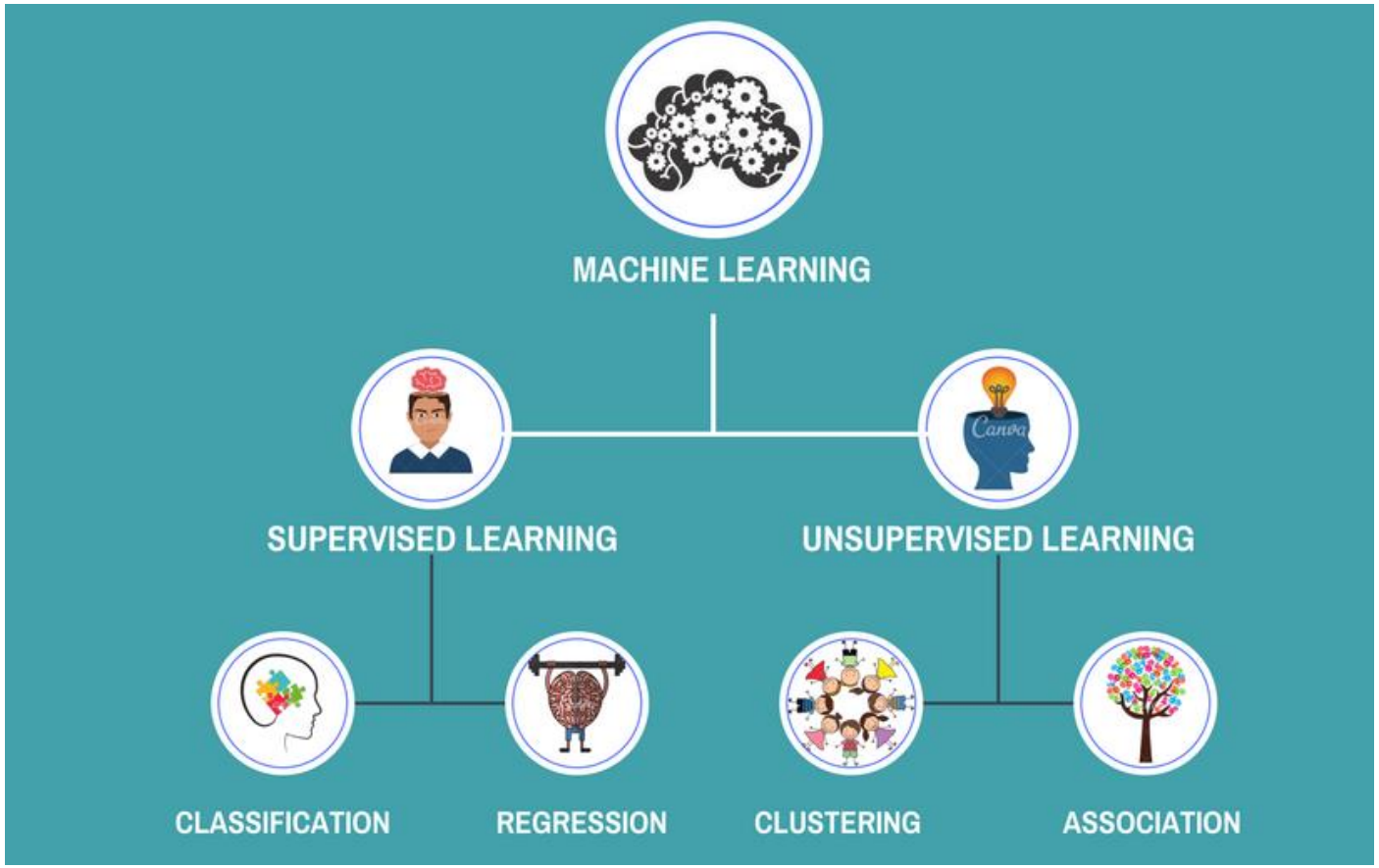
carboxypeptidase 3 (CPA3)
- Correlated with poorly controlled asthma
- Correlating with increased numbers of eosinophils
- Associated with **eosinophilic esophagitis**

Fricker et al, J Allergy Clin Immunol . 2019. doi: 10.1016/j.jaci.2018.12.1020.




KUKA
KNEXT
KNEXT barista robot
Perfect cofee every time

Part II – Let the algorithms run loose: the power of unsupervised ML



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

Susanna Dunachie Paul Klenerman
Chief Investigators, University of Oxford

Extension of the UK SIREN Study

Dept of Health & Social Care Funded

- Prospective longitudinal cohort study in 5 sites
  - Oxford (Eleanor 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)

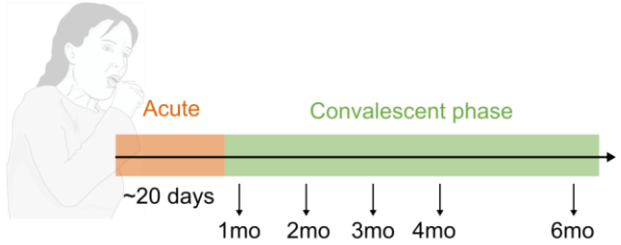
PHE (Susan Hopkins, Meera Chand, Victoria Hall, Natalie Gleeson)

1750 Healthcare workers recruited to date

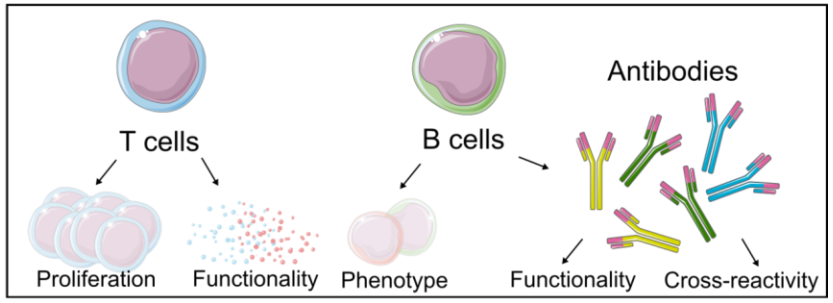
Department of Health & Social Care

PITCH
PROTECTIVE IMMUNITY from T CELLS in HEALTHCARE WORKERS

Identifying immunological signature of long-term protective immunity to SARS-CoV-2



T cells						
ELISpot		●		●		●
Proliferation		●				●
ICS		●				●
Antibodies						
Nucleocapsid IgG	●	●	●	●	●	●
Spike IgG	●	●	●	●	●	●
Pseudoneutralizing antibodies	●	●	●	●	●	●
MSD - 9 strains	●	●	●	●	●	●
ADCD		●				●
ADCD_immunology_cohort	●	●	●	●	●	●
ADMP	●	●	●	●	●	●
ADNP	●	●	●	●	●	●
ADNKA	●	●	●	●	●	●
Spike isotypes (IgA, IgG1-4, IgM)	●	●	●	●	●	●
B cells						
Spike ELISpot	●	●	●	●	●	●
ELISpot other coronaviruses	●	●	●	●	●	●
Memory ELISpot other coronaviruses	●	●	●	●	●	●



Total:

- **86 donors** (12 asymptomatic, 66 mild, 8 severe)
- **433 samples**

Tomic et al, Nat Comms, 2022

Research questions

Research question 1

What is driving the persistence of SARS-CoV-2-specific immune memory after infection?

Is there a difference between asymptomatic, mild and severe individuals?

Research question 2

Which immunological parameters at the baseline◊ are important for **durable response that can provide protection*** against SARS-CoV2 infection?

Durable response and protection

→Being seropositive 6 months pso (anti-N antibodies >=1.4)

ref. Lumley et al, NEJM, 2021. Antibody Status and Incidence of SARS-CoV-2 Infection in Health Care Workers

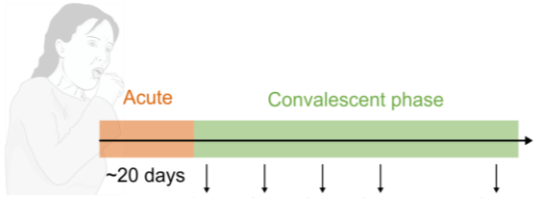
◊ *Day 28 PSO/PCR positivity*

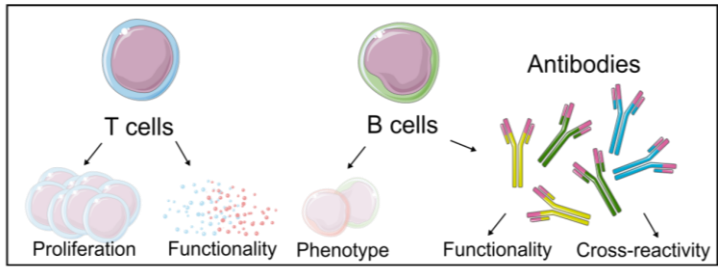
Research question 1

What is driving the persistence of SARS-CoV-2-specific immune memory after infection?

Is there a difference between asymptomatic, mild and severe individuals?

Integrative analysis of clinical and longitudinal immunological data of SARS-CoV2 infected individuals

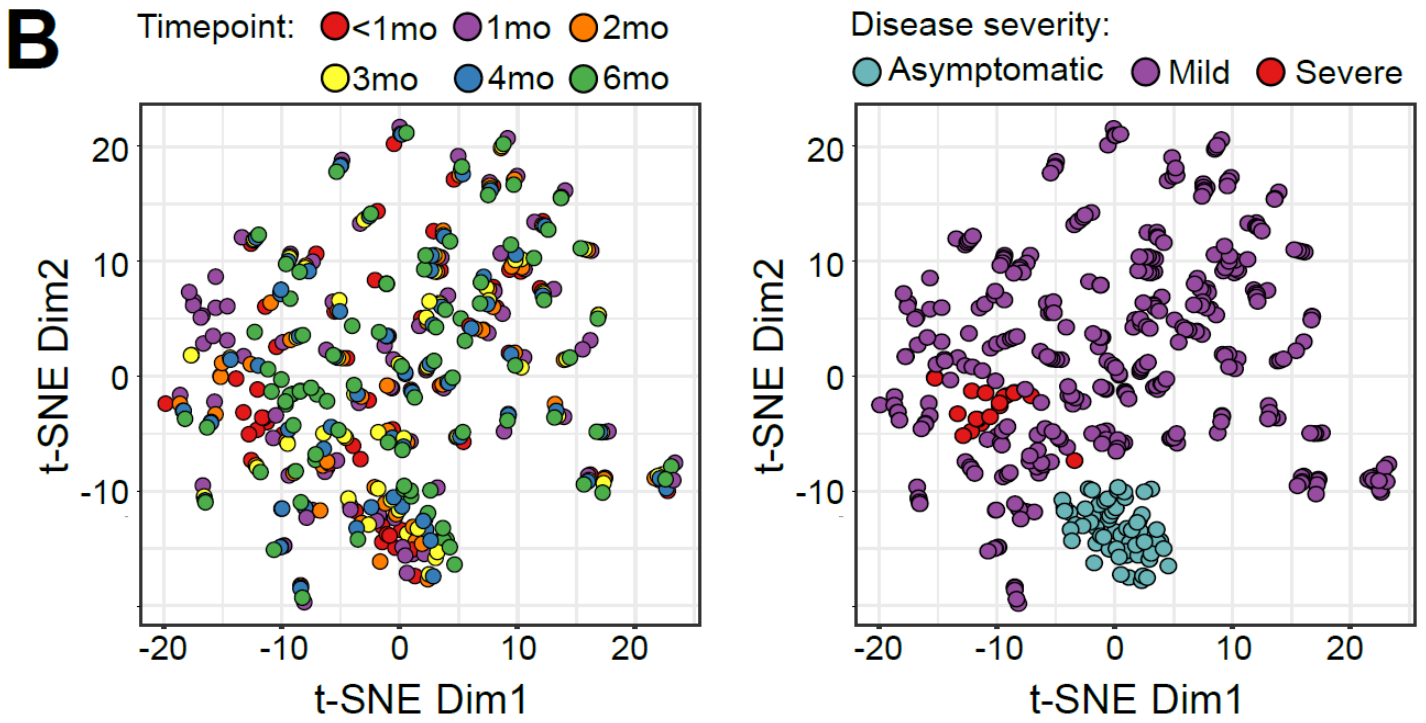




Clinical data: 20 features

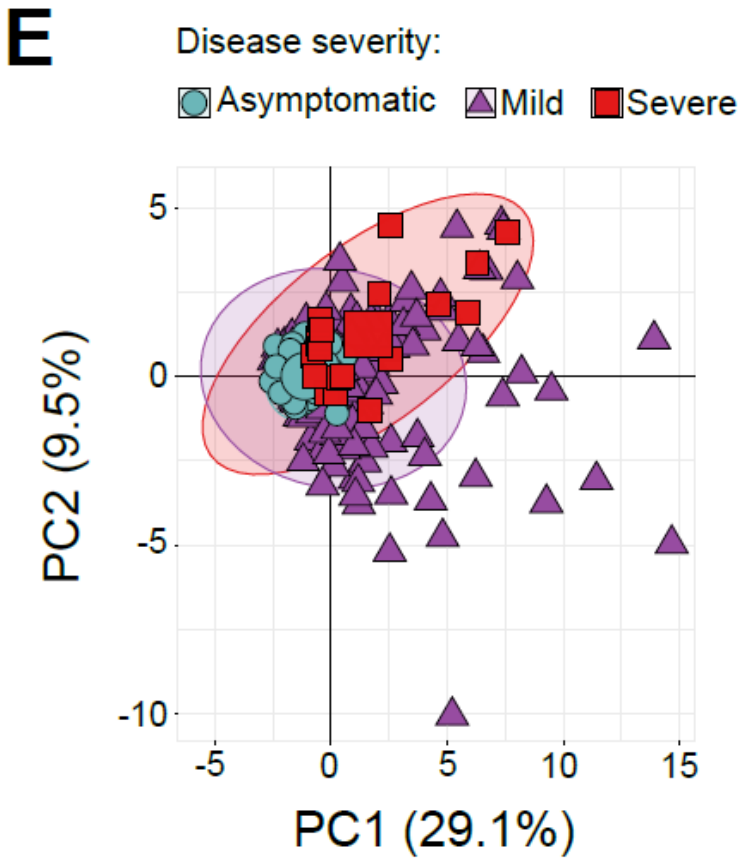
Immunological data: 51 parameters, 433 samples

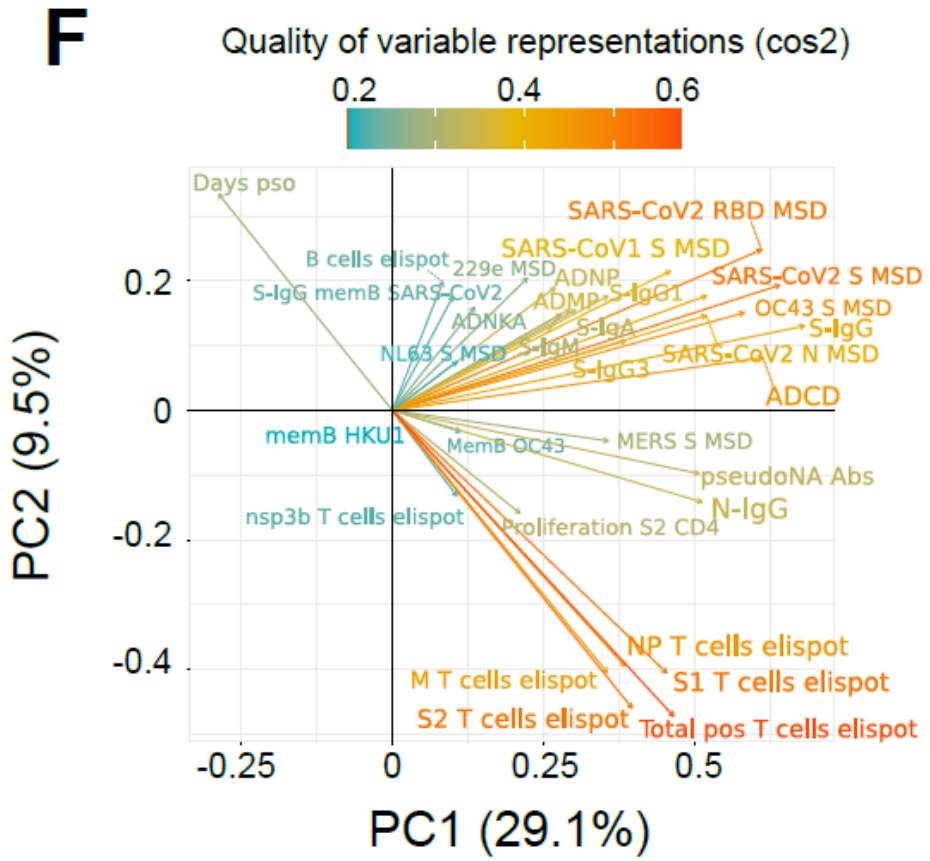
Integrated clinical and immunological data



Integrated analysis of longitudinal immune response of SARS-CoV2-infected individuals identifies **three immunophenotypic groups**

Mild individuals have heterogenous immune responses





38.6% of the variance is explained by all measured immunological data

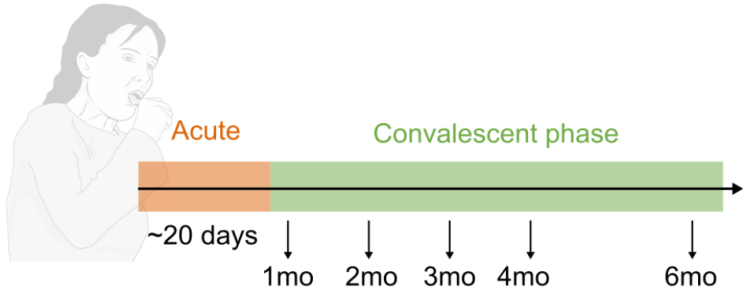
Conclusions I

SARS-CoV-2-infected individuals are separated in 3 distinct immunophenotypical groups (tSNE, PCA and hierarchical clustering):

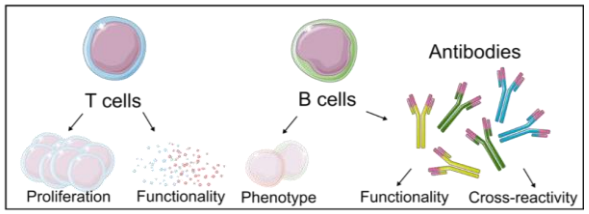
1. **Immunophenotype I**: Lower Ab and T cell responses (similar to asymptomatic)
2. **Immunophenotype II**: Higher Ab responses (similar to severe)
3. **Immunophenotype III**: Higher T cell responses

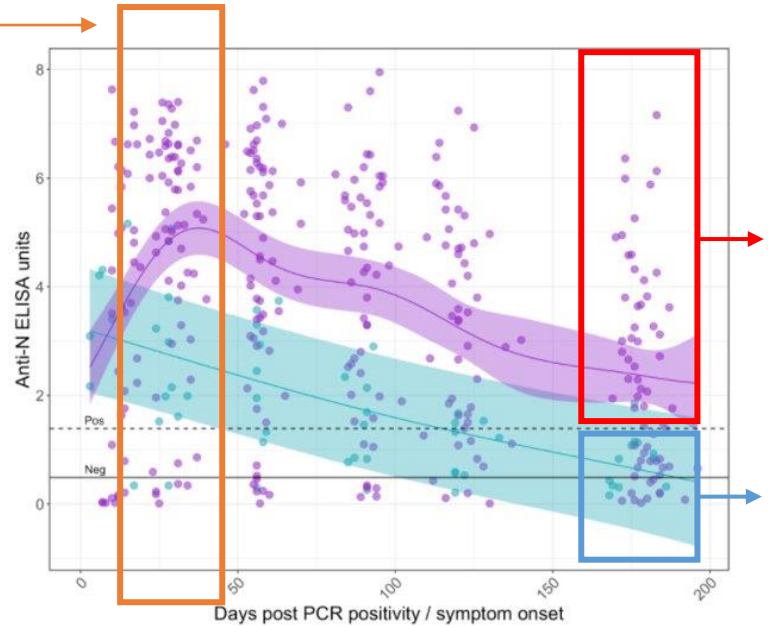
Research question 2

Which immunological parameters at the baseline are important for durable response that can provide protection against SARS-CoV2 infection?



Predictors (1mo pso)



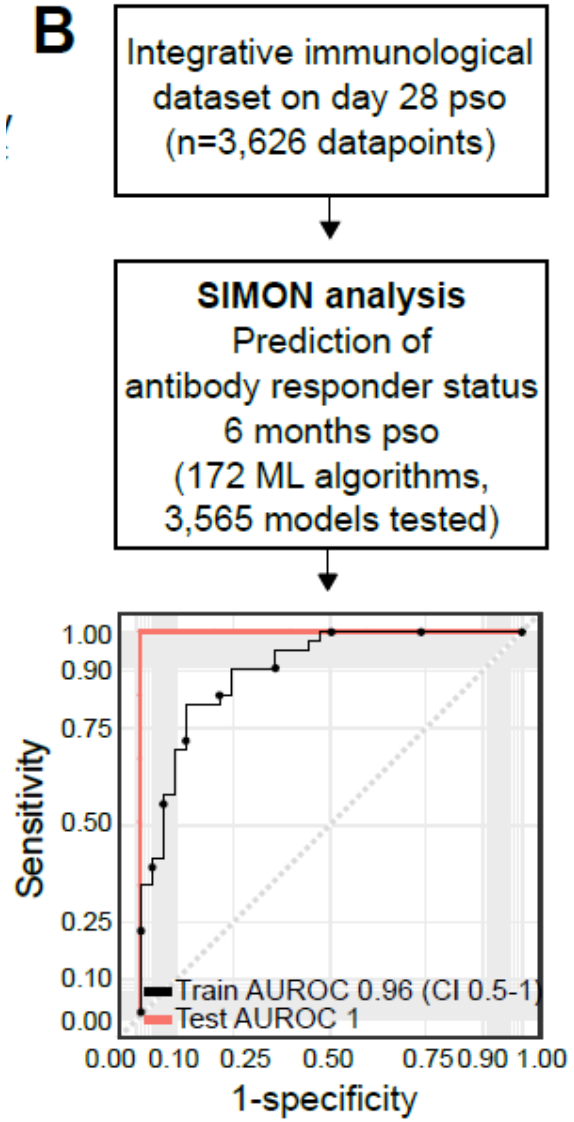


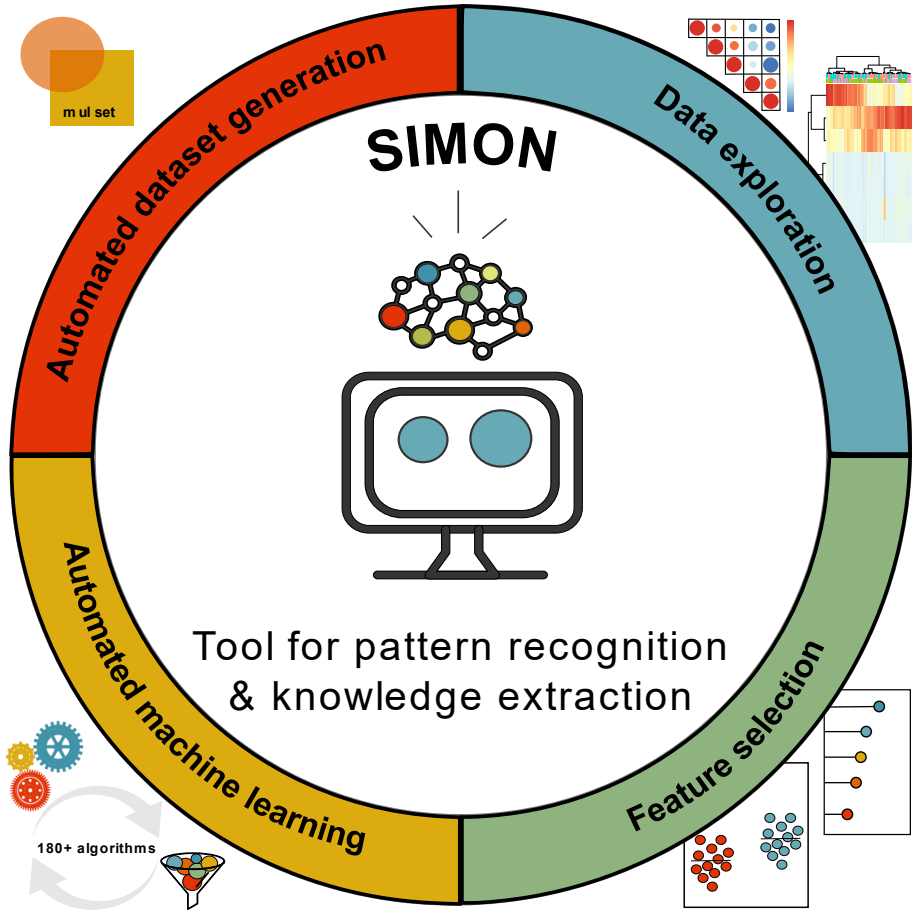
High responders (protected)

Lumley et al, NEJM, 2021. Antibody Status and Incidence of SARS-CoV-2 Infection in Health Care Workers

Low responders (unprotected)

Which immunological parameters at the baseline can predict durable and protective response against SARS-CoV-2 infection?

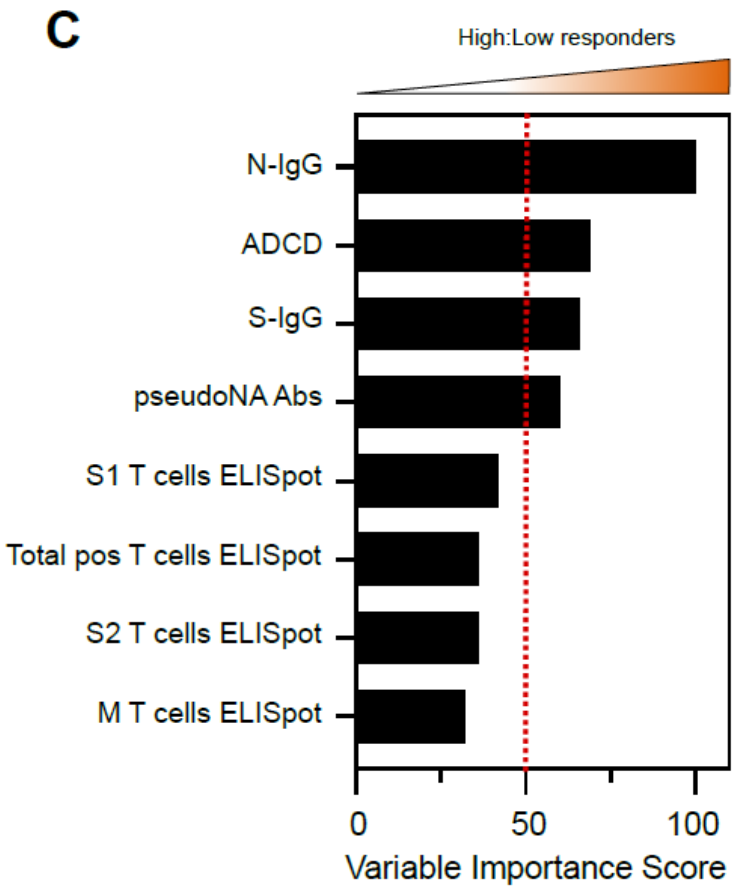




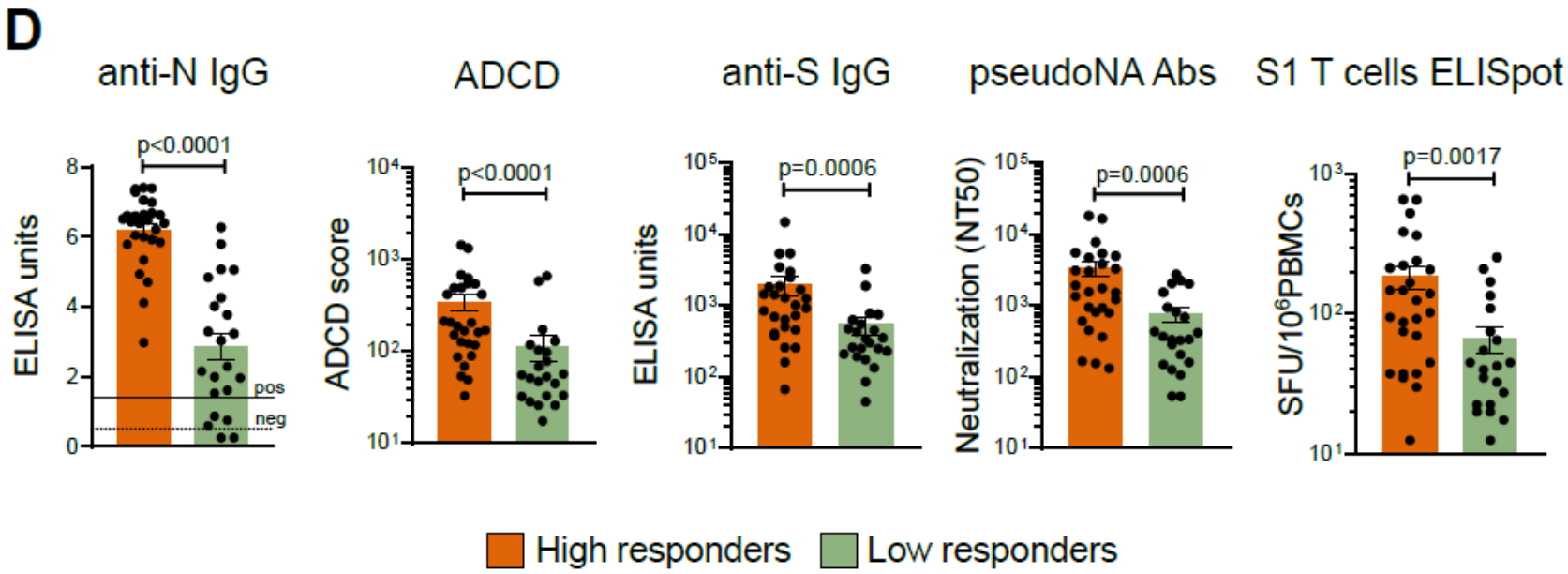
Tomic et al, JI, 2019 & Tomic et al, Patterns, 2021

SIMON identifies early signature of SARS-CoV-2 protective immunity

Baseline parameters that can predict individuals on a trajectory for long-term immunity



High responders have **higher antibody responses** (pseudoNA, anti-S, anti-N and ADCD) and **higher T cell responses** (total and S) than low responders 1 month pso



svmPoly algorithm

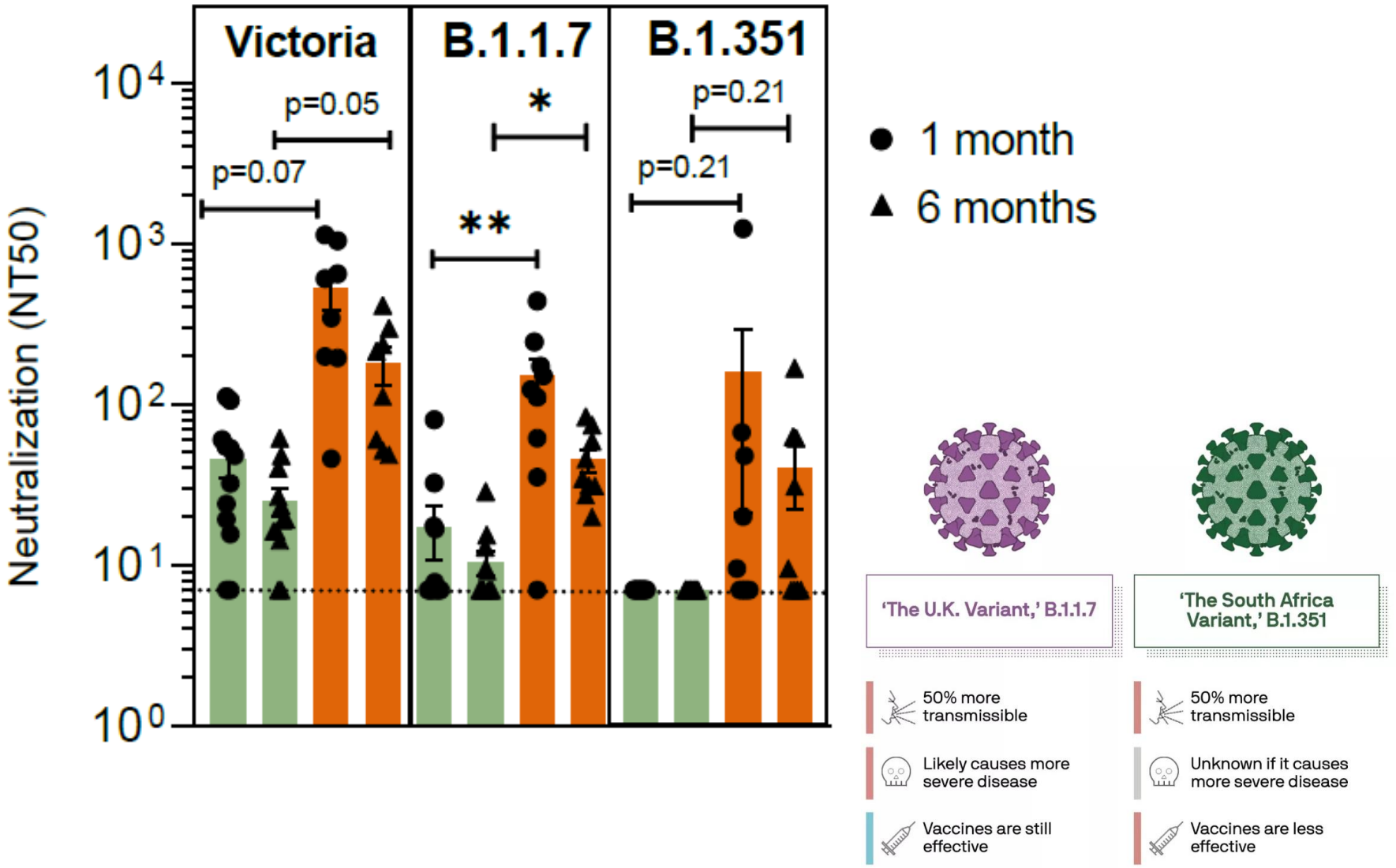
Conclusions II:

Immunological signature of long-term protective immunity to SARS-CoV2

1. Early generation of antibodies (S, N, pnAbs)
2. Early generation of T cell responses (S, M)

Could identified individuals with durable and protective SARS-CoV2 immunity be protected against novel variants?

SARS-CoV-2-infected individuals with long-term immunity have higher levels of neutralizing antibodies against variants of concern



Final conclusions

- persistence of SARS-CoV-2-specific immune memory depends on the magnitude of the immune response early after infection
- individuals with higher anti-S and anti-N antibodies and T cell responses generated early after infection mount protective SARS-CoV-2 immunity
- variable protection against novel variants

Medawar pathogen research

Paul Klenerman
Donal Skelly
Susanna Dunachie
Ane Ogbe
Carl-Philipp Hackstein
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MRC Medical Research Council

NIHR | Oxford Biomedical Research Centre

Department of Health & Social Care

wellcome

UKCIC
UK Coronavirus Immunology Consortium

UKRI UK Research and Innovation

NIHR | National Institute for Health Research

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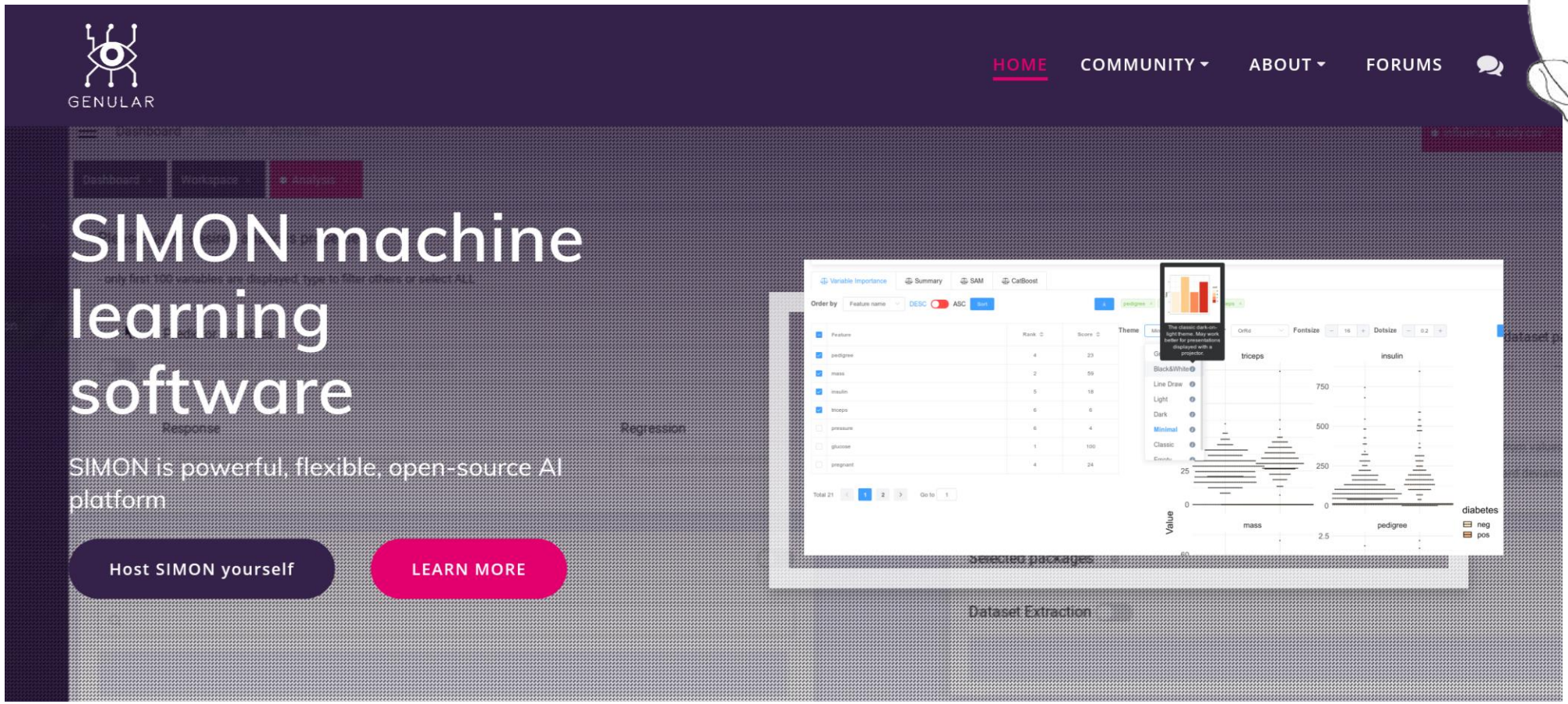

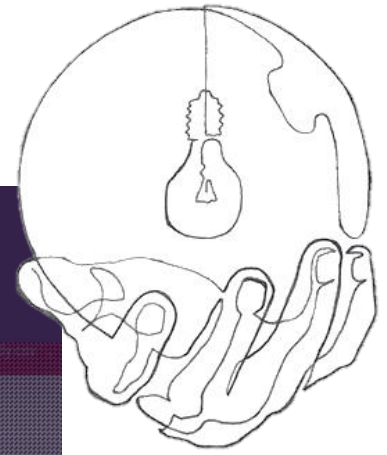

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Free as in Freedom

Check out SIMON at **genular.org**

Have a Question?

Search the documentation...

Search

Installation

- Installation Quickstart

Machine Learning

- How to perform SIMON analysis?
- Instruction videos

Star us on GitHub!!!

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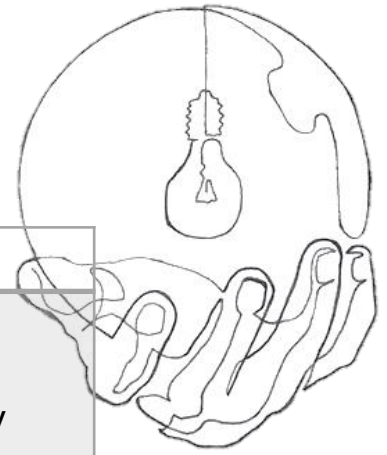

Project	How To Help	Next Step
Localization (English, German, French, Chinese, Arabic)	Help us translate SIMON into your language. If some translation is missing or incorrect you can easily help us by correcting it.	Join our Translation Community
Tutorials	Help others use and understand SIMON	Write a tutorial or record it, with usage examples
Organizing	Ask questions on recently opened GitHub issues to move the discussion forward	Go to GitHub Issues
Write article	Help other understand what is Machine Learning & how can they apply it, by publishing blog post	e-mail us

AGPL v3 Free Software
Free as in Freedom

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Thank you

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NIH National Institutes of Health
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