Viral Biomarker Discovery using CyTOF

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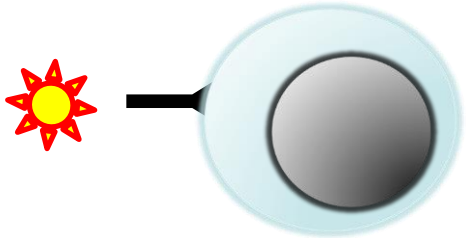
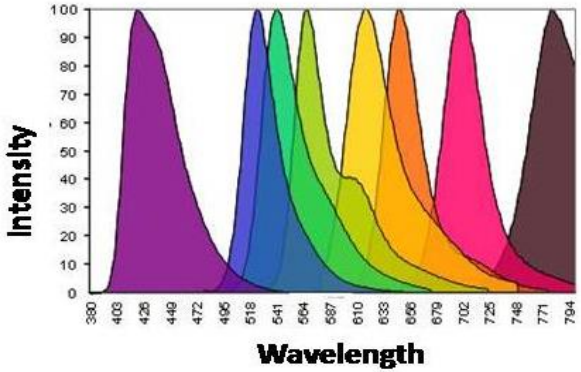
What makes a protective cellular response?

- Cytotoxic CD8+ T cells
- Multifunctional T cells (e.g., IL-2, TNF, IFNγ, MIP-1β, CD107)
- Central memory or effector memory T cells
- Lack of exhaustion markers (PD-1, LAG-3, etc.)

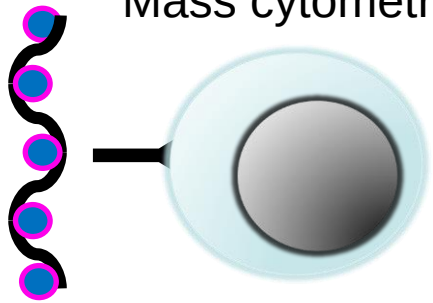
Mass Cytometry (CyTOF) Rationale

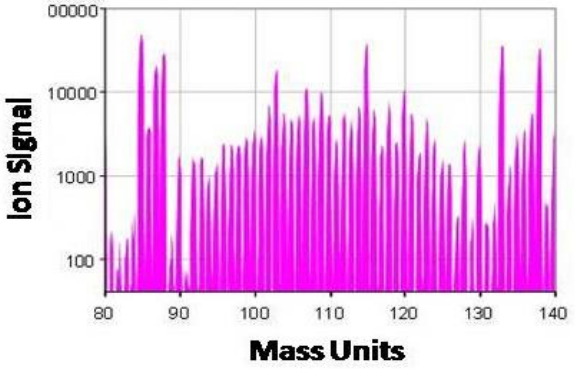
Fluorescence cytometry





Mass cytometry



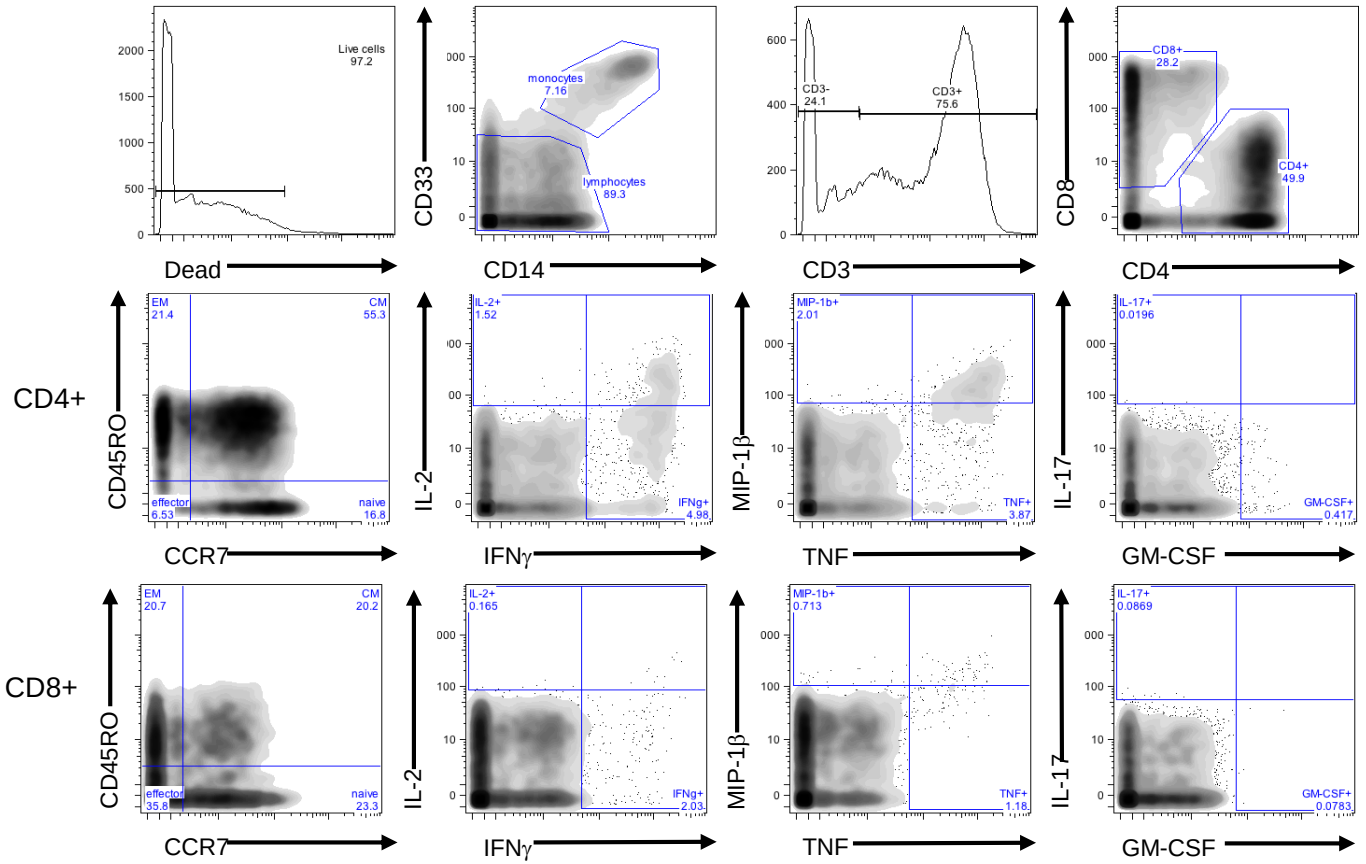


- Many more labels (antibodies)
- Little or no spillover

Difficult scenarios for CyTOF

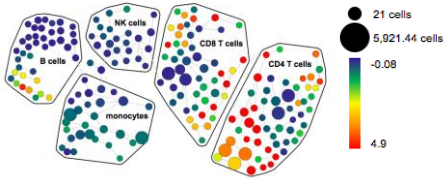
- Very rare cell populations
  - Time to acquire
  - Cell loss
- Very large studies
  - Time to acquire
  - Expense

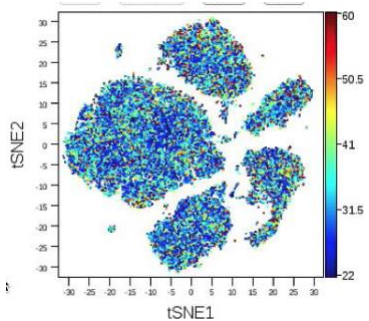
CyTOF ICS – CMV specific response

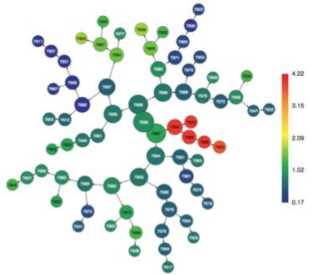


Analytical Approaches

- Manual gating and multivariate modeling of gated populations
- Automated clustering, visualization, and statistical comparison algorithms :
  - SPADE: clustering and 2-D representation of clusters
  - viSNE: dimension reduction and 2-D display of individual cells
  - Citrus: clustering and statistical comparison of groups by cluster







CMV in lung transplantation

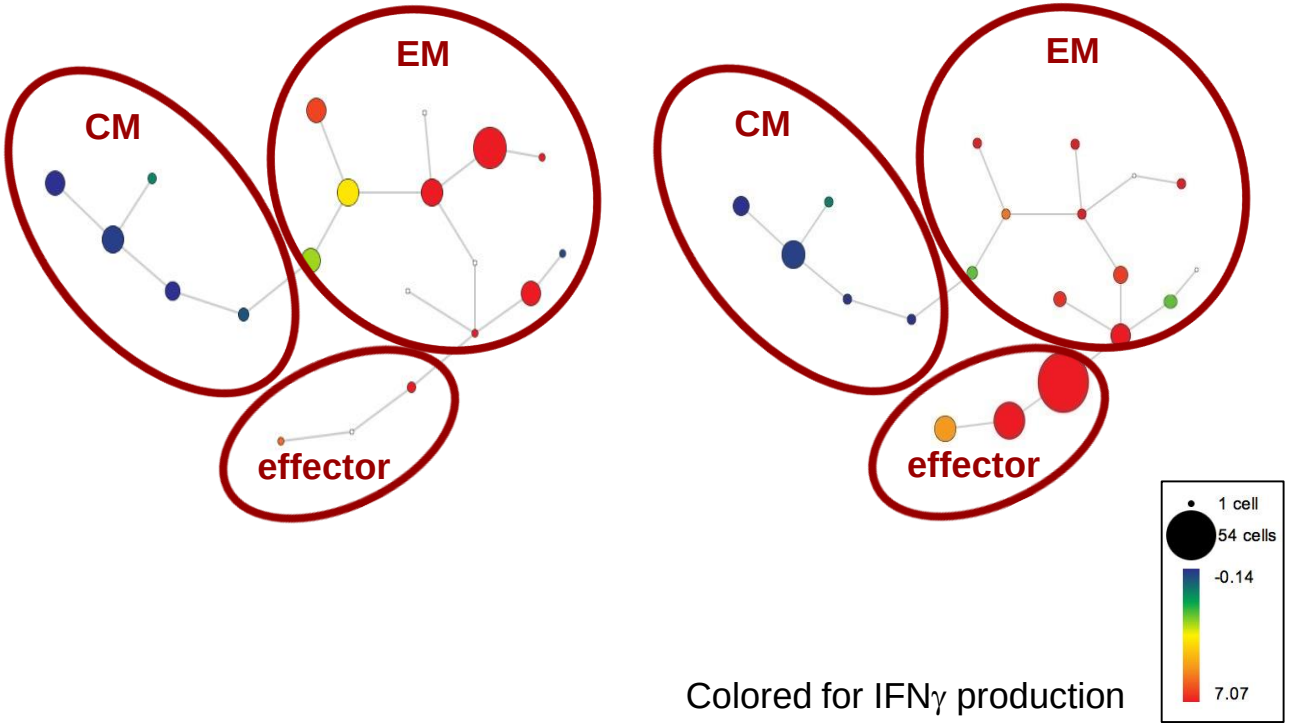
(Sheena Gupta, Sharon Chen, David Lewis, Tyson Holmes)

- Can the profile of CMV-specific T cells pre-transplant predict who is at risk of viremia/rejection post-transplant?
  - only for CMV+ recipients
  - may be a long shot
- 24 lung transplant patients with PBMC samples pre-transplant and median clinical follow up for 1.4 years post-transplant
- 18 healthy elderly controls from Jorg Goronzy HIPC cohort

Identification of antigen-specific T cells

- Separately for CD4+ and CD8+ T cells:
  - Gate on positive cells for each individual cytokine
  - Create a Boolean “or” gate for all cytokines
  - Export new FCS file of only cytokine-positive cells (positive for one or more cytokines)

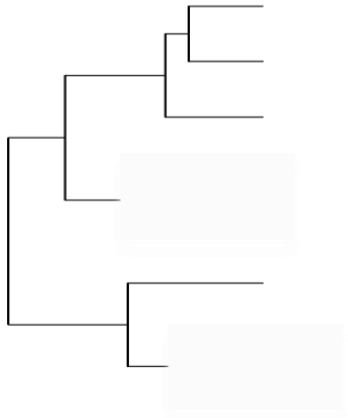
SPADE clustering of CMV-specific T cells



Colored for IFNγ production

Denoised Ragged Pruning (Tyson Holmes)

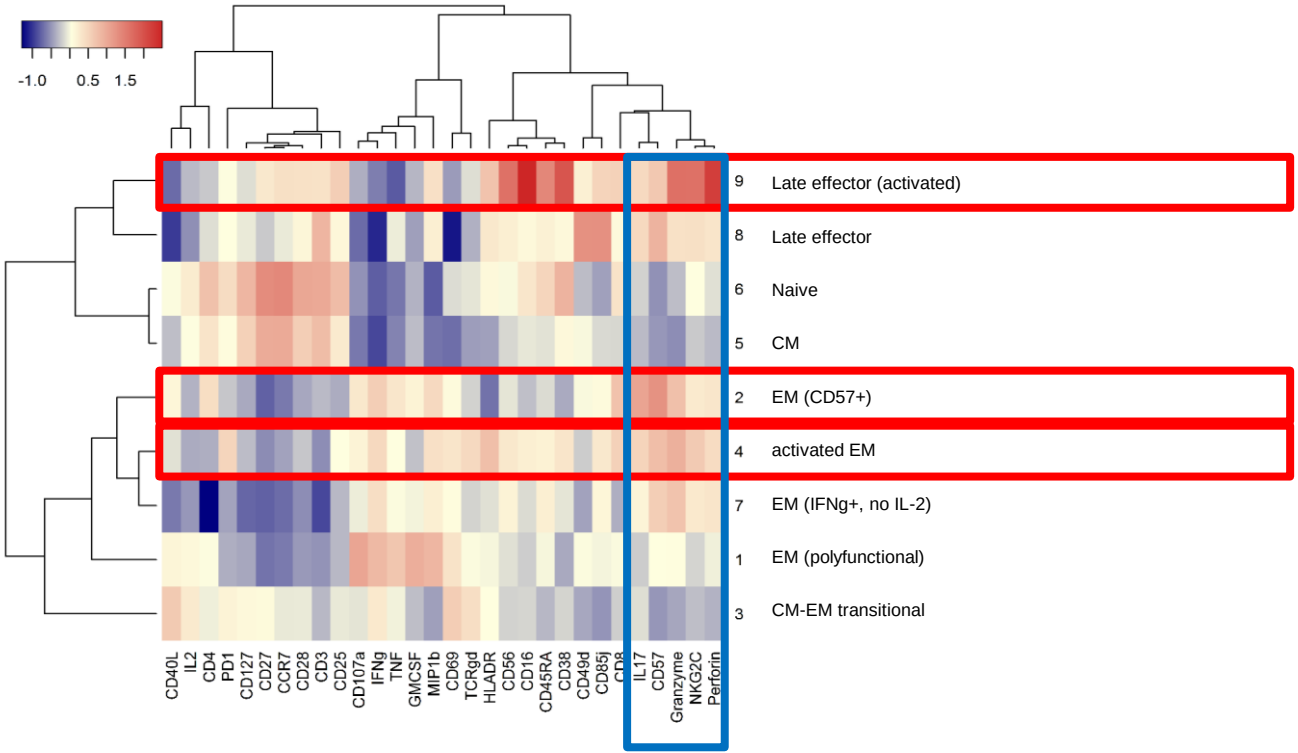
- Denoising via Eigendecomposition to de-emphasize parameters with minimal structure
- Hierarchical clustering using principal components
- Ragged pruning to obtain an optimal cluster solution



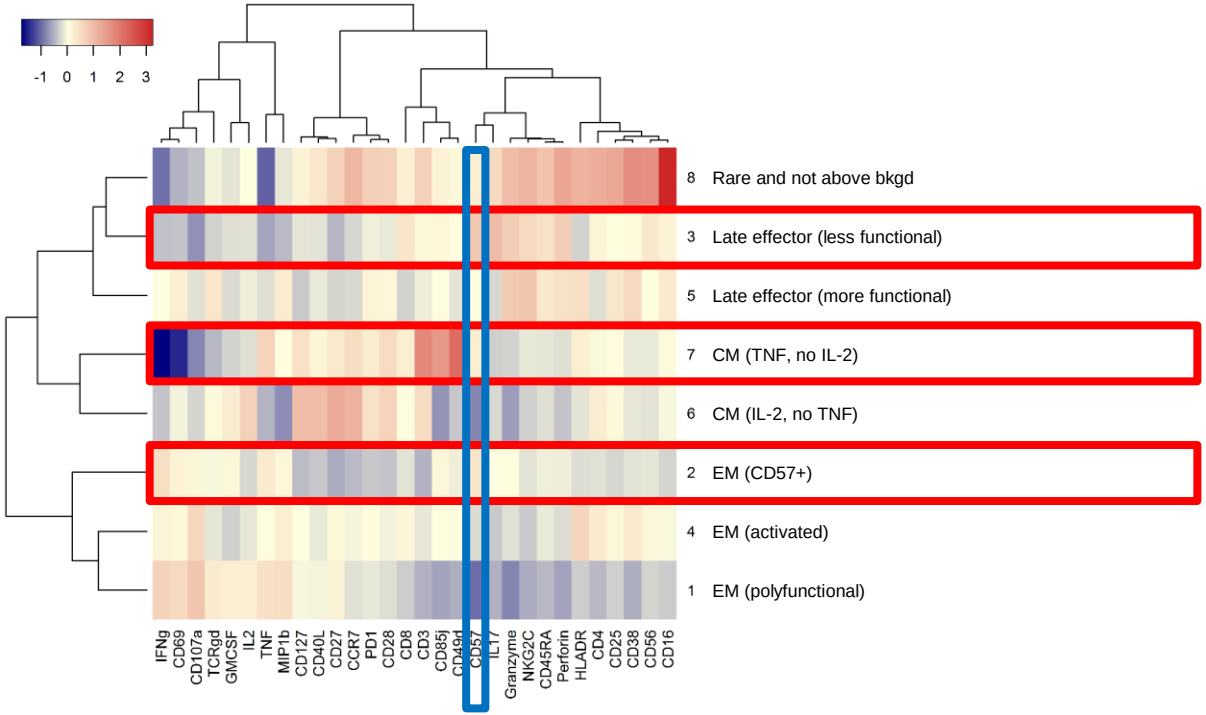
CMV-specific peptide pool stimulations

- Immediate early antigen pool:
  - IE-1
  - IE-2
  - US-3
  - UL-36
- Late antigen pool:
  - Pp65
  - UL-32
  - UL-48AB
  - UL-55 (gB)

CMV-specific CD4+ T cell clusters

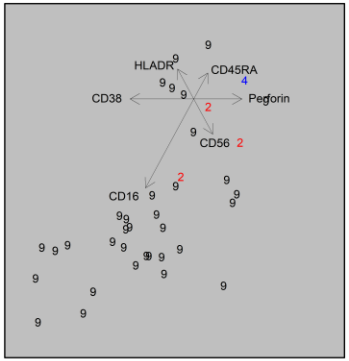


CMV-specific CD8+ T cell clusters

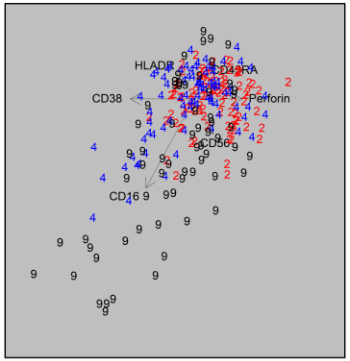


Healthy vs. transplant CMV response: CD4

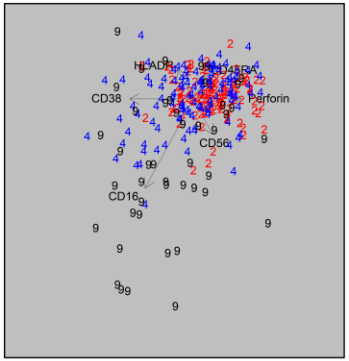
Healthy Unstimulated



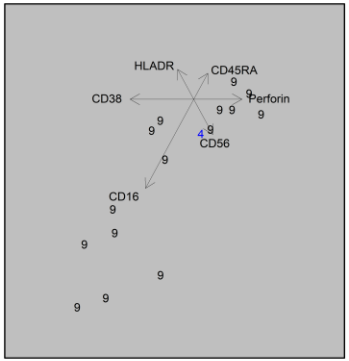
Healthy Early Stimulation



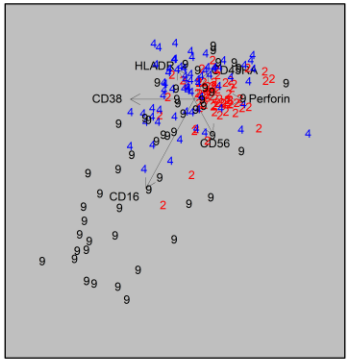
Healthy Late Stimulation



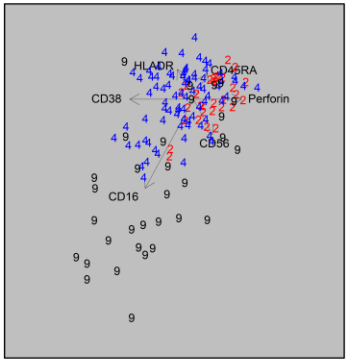
Transplant Unstimulated



Transplant Early Stimulation

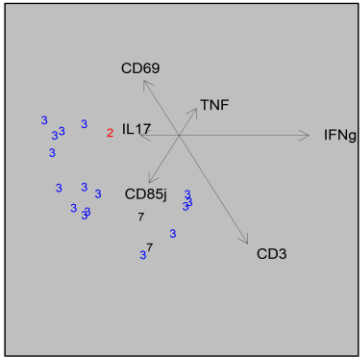


Transplant Late Stimulation

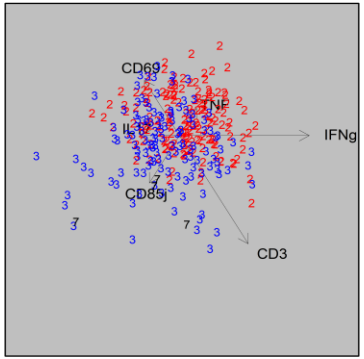


Healthy vs. transplant CMV response: CD8

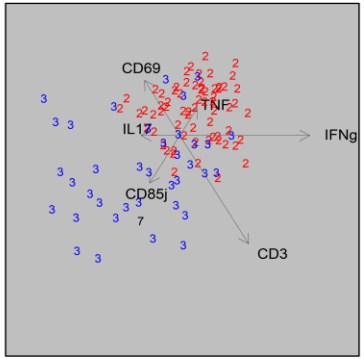
Healthy Unstimulated



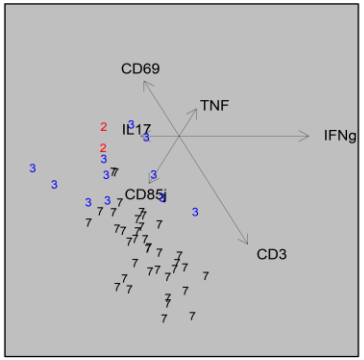
Healthy Early Stimulation



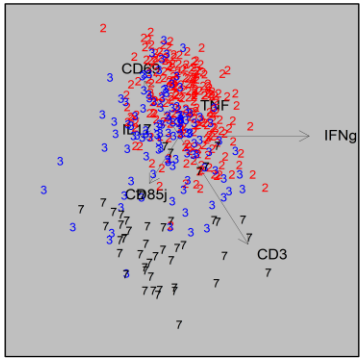
Healthy Late Stimulation



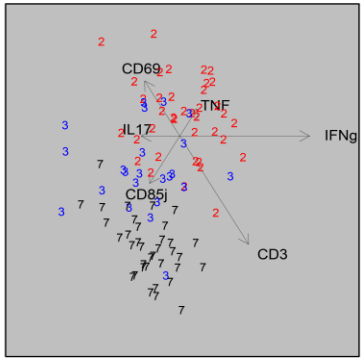
Transplant Unstimulated



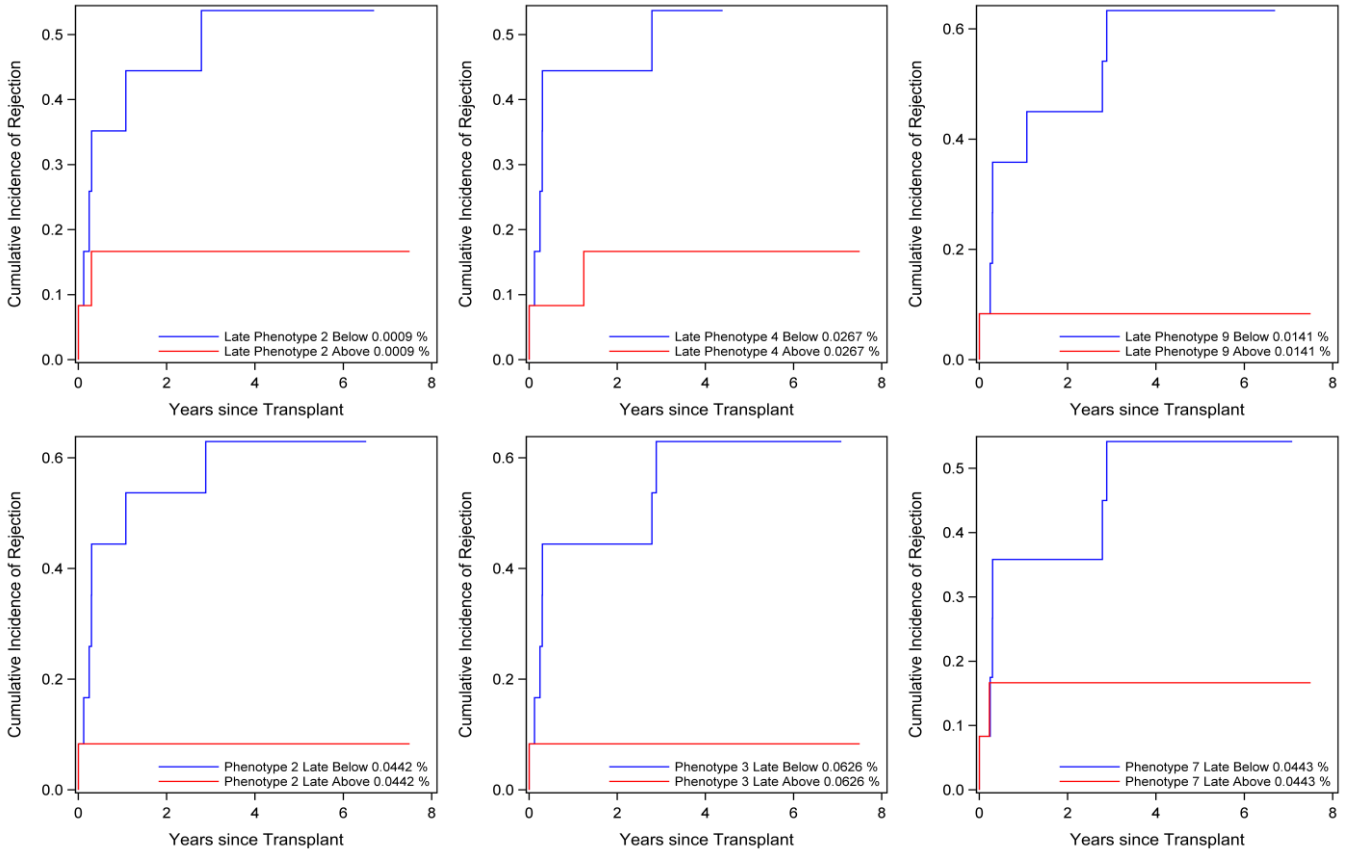
Transplant Early Stimulation



Transplant Late Stimulation



Cumulative risk of rejection for significant clusters



Predicting RSV vaccine response

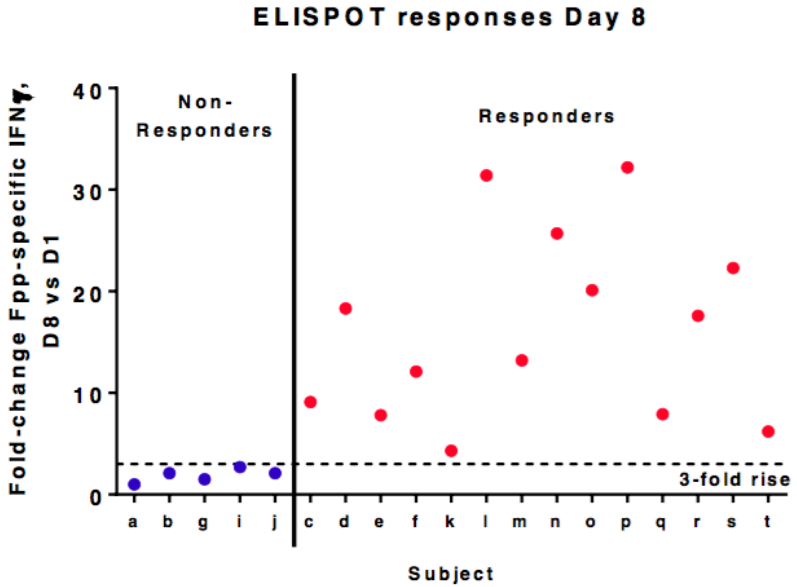
- MedImmune trial of experimental RSV vaccine in elderly
- 5 non-responders,

14 responders by ELISPOT

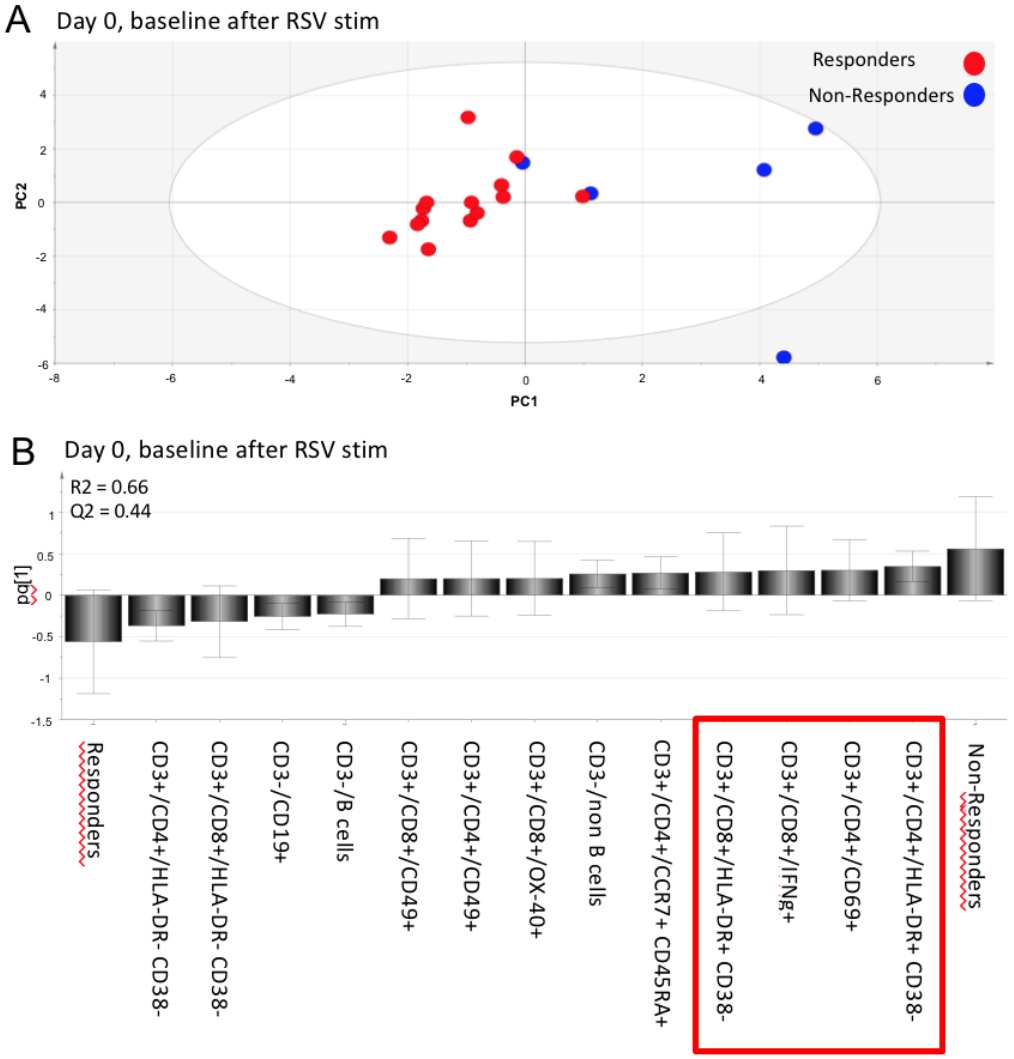
- PCA of all cells

after RSV stimulation

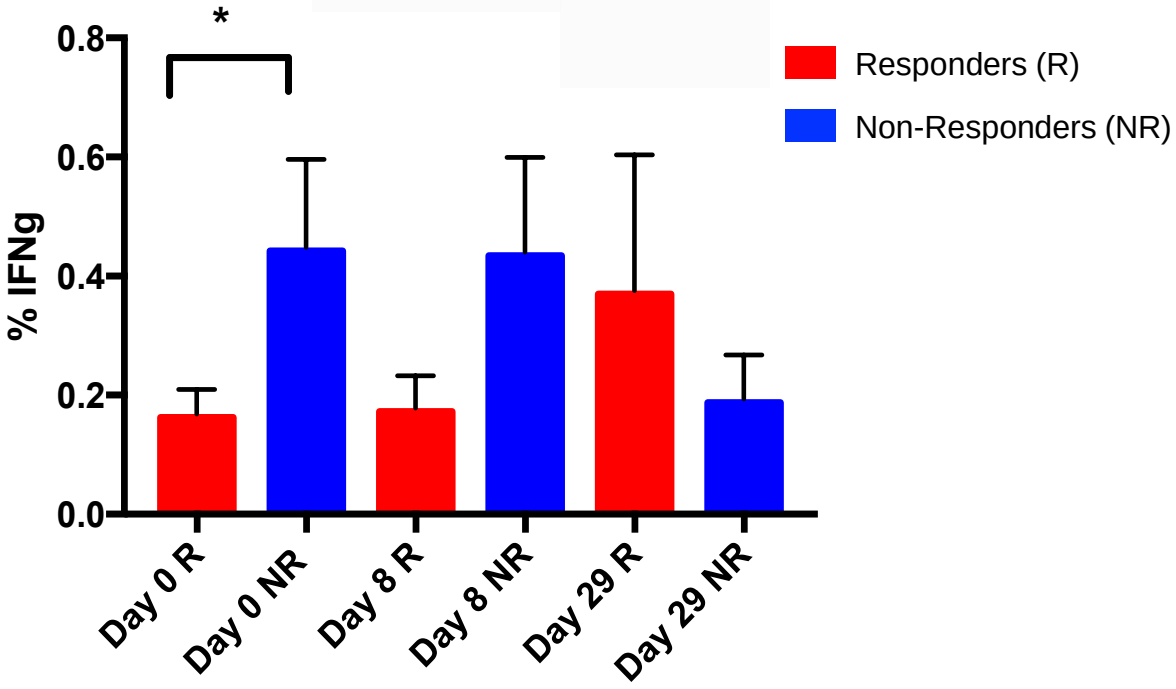
- Frequency of RSV-specific cells



PCA of reponders and non-responders



Responders have lower baseline IFNγ+CD8+ T cells



Conclusions

- CyTOF ICS assays can be used to monitor detailed phenotypes and functions of antigen-specific T cells
- The quality of a patient's T cell response to CMV prior to organ transplant may predict rejection post-transplant
  - CD57+ CD4 and CD8 T cells
- Response to an RSV vaccine may be associated with baseline immune parameters
  - Activated T cells, anti-RSV CD8 T cells as *negative* predictors

Acknowledgements

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