Immunophenotyping of tumor-specific CD8 T cells using high-dimensional mass cytometry

Michael Fehlings[1], Brian Abel[2], Alessandra Nardin[1], Evan Newell[3], Mahesh Yadav[4]

[1]immunoSCAPE, Singapore, [2] immunoSCAPE, USA, [3] Fred Hutchinson Cancer Research Center, USA, [4] Genentech, Inc, USA

Background: There is strong evidence that immunotherapy-mediated tumor rejection can be driven by tumor-specific CD8+ T cells reinvigorated to recognize neoantigens derived from tumor somatic mutations. Thus, the frequencies or characteristics of tumor-reactive, mutation-specific CD8+ T cells could be used as biomarkers of an anti-tumor response. However, such neoantigen-specific T cells are difficult to reliably identify due to their low frequency in peripheral blood and wide range of potential epitope specificities.

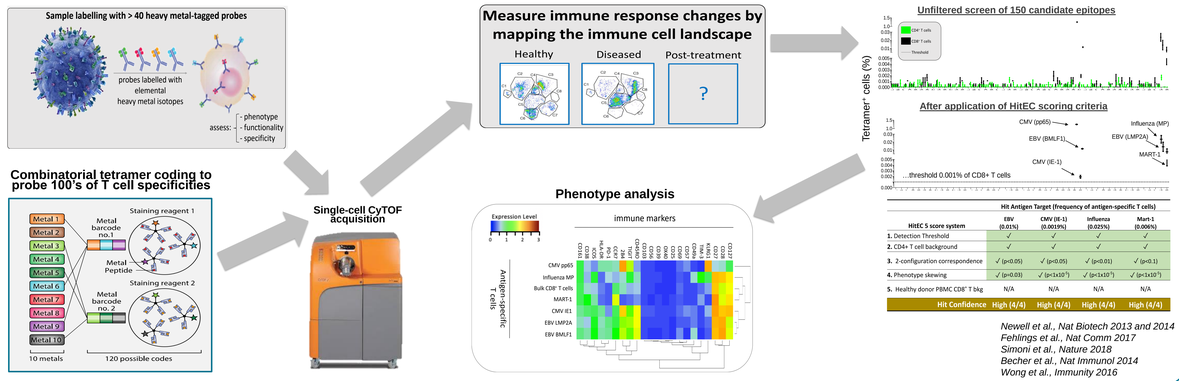
immunoSCAPE leverages the high-dimensional immune profiling capabilities of mass cytometry combined with a unique technology for the identification of antigen-specific T cells to address this challenge. By applying CyTOF in conjunction with combinatorial peptide-MHC tetramer staining and high-performance dimensional analysis tools, we can screen and identify rare neoantigen-specific T cells while concurrently performing deep phenotypic characterization.

Methods: Peripheral blood mononuclear cells (PBMC) from 14 non-small cell lung cancer (NSCLC) patients were collected pre- and post-treatment with the anti-PD-L1 antibody, atezolizumab, for characterization. Using whole exome and RNA sequencing we identified tumor neoantigens that are predicted to bind to major histocompatibility complex class-I (MHC-I) and utilized mass cytometry, together with cellular 'barcoding', to profile immune cells from patients with objective response to therapy (PR, n=8) and those with progressive disease (PD, n=6). In parallel, a highly-multiplexed combinatorial tetramer staining was used to screen antigen-specific CD8+ T cells in peripheral blood for 782 candidate tumor neoantigens and 71 known viral-derived control peptide epitopes across all patient samples.

Patient Response	PR	PD
Patients	8	6
Total NeoAgs predicted	487	302
Mean number of NeoAgs/patient	61	50

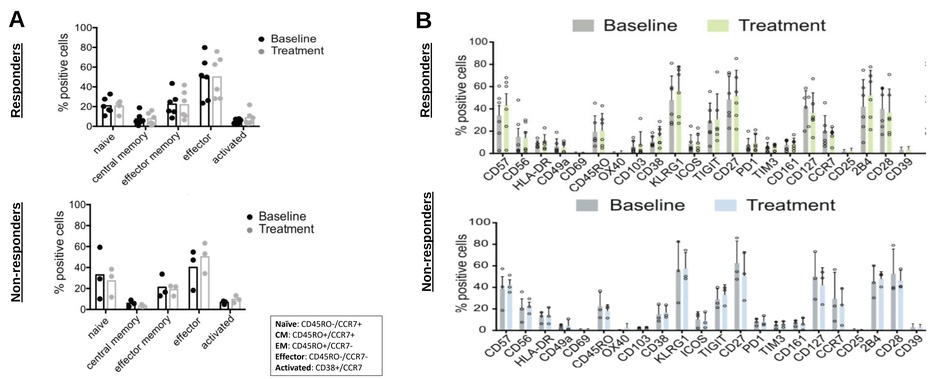
Summary of patient samples and characteristics (Fehlings *et al.*, JITC. 2019)

targetSCAPE: Large-scale epitope screening (100's) and deep antigen-specific T cell profiling *ex vivo*



Newell et al., Nat Biotech 2013 and 2014
Fehlings et al., Nat Comm 2017
Simoni et al., Nature 2018
Becher et al., Nat Immunol 2014
Wong et al., Immunity 2016

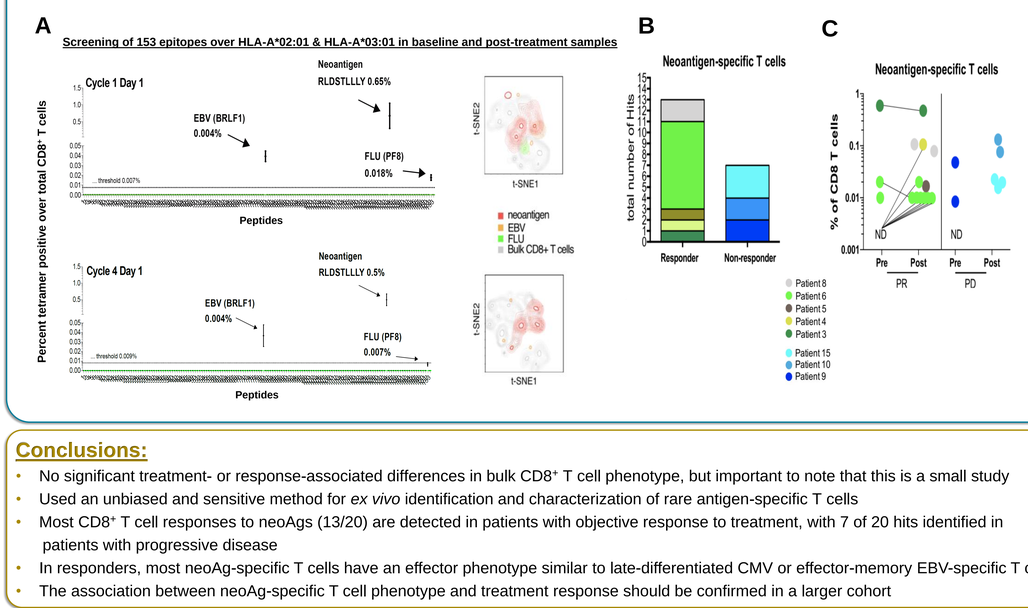
No difference in bulk CD8+ T cells phenotype at baseline or following treatment between responders and non-responders



Comparative phenotypic analysis of bulk CD8+ T cells before and after atezolizumab treatment.

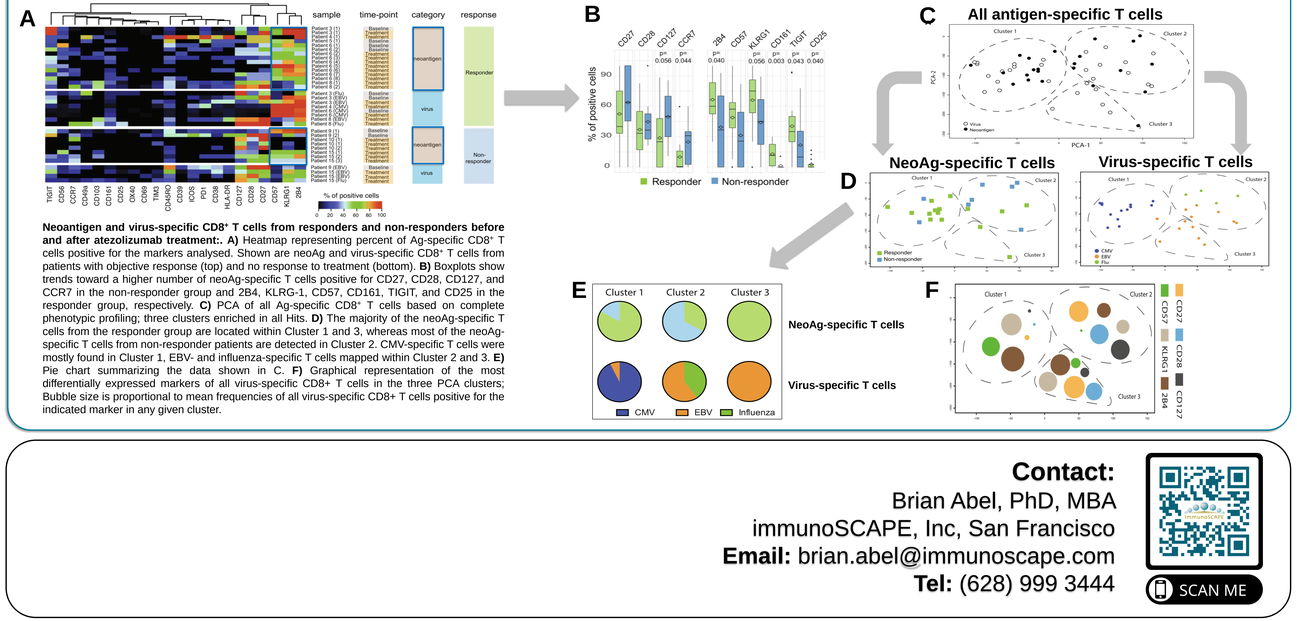
A) Frequencies of major CD8+ T cell subsets (naïve, central memory, effector memory, effector, and activated cells) at baseline and on atezolizumab treatment in responders (n=6, top) and non-responders (n=3, bottom). **B)** Frequencies of CD8+ T cells positive for all the analysed markers at baseline versus atezolizumab treatment within responders (n=6, top) and non-responders (n=3, bottom).

Neoantigen-specific T cells are enriched in patients responding to atezolizumab treatment



Screening of CD8+ T cells for neoantigens and virus control antigens using a multiplex pMHC tetramer staining approach by CyTOF. A) Shown is one screen (153 antigen candidates) from CD8+ T cell in the same patient before and post treatment. t-SNE plots are based on the expression of all phenotypic markers. **B)** Total number of unique neoantigen-specific CD8+ T cell hits detected in a total of 782 candidates within the responders (n=8) and non-responders (n=6). **C)** Frequencies of all neoantigen-specific CD8+ T cells detected within the responder (13 neoantigens) and non-responder (7 neoantigens) groups pre- and post-treatment. Abbreviations: ND= not detected; PR=responders; PD= non-responders

Neoantigen-specific T cells in responders have an effector phenotype similar to late-differentiated CMV-specific T cells



Neoantigen and virus-specific CD8+ T cells from responders and non-responders before and after atezolizumab treatment:. A) Heatmap representing percent of Ag-specific CD8+ T cells positive for the markers analysed. Shown are neoAg and virus-specific CD8+ T cells from patients with objective response (top) and no response to treatment (bottom). **B)** Boxplots show trends toward a higher number of neoAg-specific T cells positive for CD27, CD28, CD127, and CCR7 in the non-responder group and 2B4, KLRG-1, CD57, CD161, TIGIT, and CD25 in the responder group, respectively. **C)** PCA of all Ag-specific CD8+ T cells based on complete phenotypic profiling; three clusters enriched in all Hits. **D)** The majority of the neoAg-specific T cells from the responder group are located within Cluster 1 and 3, whereas most of the neoAg-specific T cells from non-responder patients are detected in Cluster 2. CMV-specific T cells were mostly found in Cluster 1, EBV- and influenza-specific T cells mapped within Cluster 2 and 3. **E)** Pie chart summarizing the data shown in C. **F)** Graphical representation of the most differentially expressed markers of all virus-specific CD8+ T cells in the three PCA clusters; Bubble size is proportional to mean frequencies of all virus-specific CD8+ T cells positive for the indicated marker in any given cluster.

Conclusions:

- No significant treatment- or response-associated differences in bulk CD8+ T cell phenotype, but important to note that this is a small study
- Used an unbiased and sensitive method for *ex vivo* identification and characterization of rare antigen-specific T cells
- Most CD8+ T cell responses to neoAgs (13/20) are detected in patients with objective response to treatment, with 7 of 20 hits identified in patients with progressive disease
- In responders, most neoAg-specific T cells have an effector phenotype similar to late-differentiated CMV or effector-memory EBV-specific T cells
- The association between neoAg-specific T cell phenotype and treatment response should be confirmed in a larger cohort

Contact:
Brian Abel, PhD, MBA
immunoSCAPE, Inc, San Francisco
Email: brian.abel@immunoscape.com
Tel: (628) 999 3444

SCAN ME