Specific T cell responses to Tumor-associated Antigens from Melanoma Patients Undergoing Immune Checkpoint Blockade Therapy

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Introduction

- The importance of T cell responses in effective anti-tumor responses has been exemplified by the success of Immune Checkpoint Blockade (ICB) immunotherapies. However, ICB is effective in only a small subset of patients and may be accompanied by significant toxicities
- Companion diagnostics, such as PD-L1 immunohistochemistry, have improved outcomes after stratification, however evaluation is inconsistent and tumor samples are often inaccessible
- The development of a non-invasive, blood-based assay to predict responsiveness to ICB can enable more successful immunotherapy
- ATLAS™ is a T cell antigen discovery platform with which any patient's CD4⁺ or CD8⁺ T cell responses can be measured for dozens to hundreds of tumor-associated antigens (TAA)
- Profiling T cell responses to a broad TAA library suggests that a blood-based assay that is predictive of effective ICB is plausible

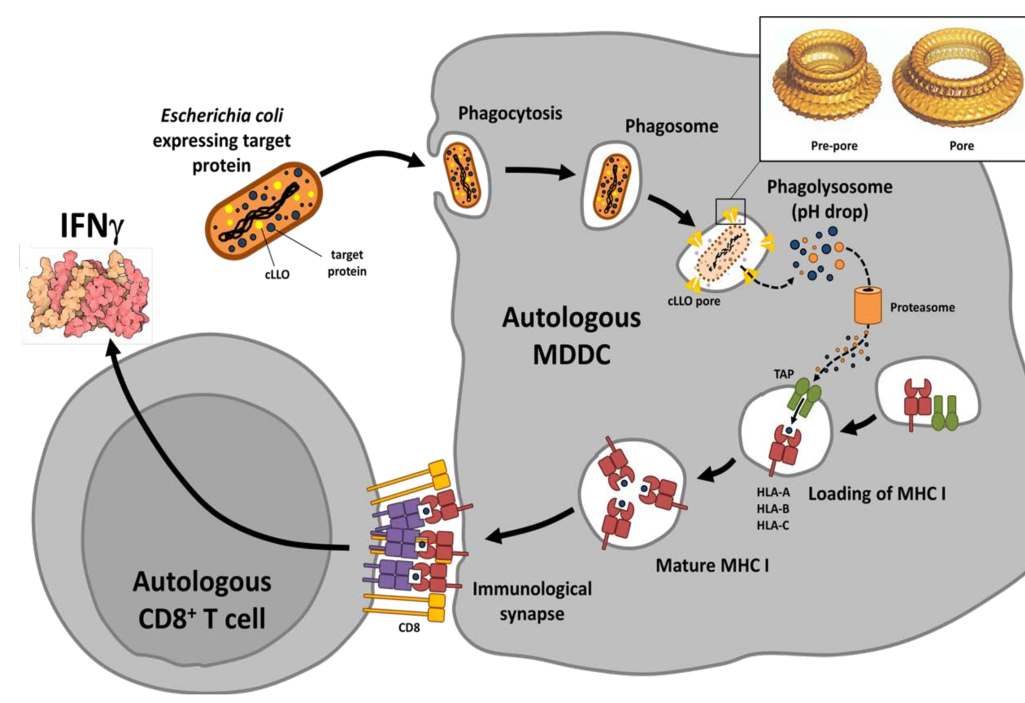
Sample Information

Patient ID	Cohort	CD4	CD8
CAL033	Responder	no	yes
CAL001	Responder	yes	yes
CAL003	Responder	no	yes
CAL006	Responder	yes	yes
CAL008	Responder	yes	yes
CAL009	Responder	yes	yes
CAL011	Responder	yes	yes
CAL013	Responder	yes	yes
CAL016	Responder	yes	yes
CAL017	Responder	yes	no
CAL018	Responder	yes	yes
CAL019	Responder	yes	yes
CAL020*	Responder	no	yes
CAL021	Responder	yes	yes
CAL022	Responder	yes	yes
CAL023	Responder	yes	yes
CAL024	Responder	yes	yes
CAL025	Responder	yes	yes
CAL026	Responder	yes	yes
CAL027	Responder	yes	yes
CAL029	Responder	no	yes
CAL030	Responder	yes	yes
CAL004	Responder	yes	yes
CAL010	Responder	yes	yes
CAL012	Responder	yes	yes
CAL015*	Responder	yes	yes
CAL002	Non-Responder	yes	yes
CAL007	Non-Responder	yes	yes
CAL014	Non-Responder	yes	yes
CAL028	Non-Responder	no	yes

*CAL020 is a repeat sample (new collection) from CAL015
Cohort determination based upon imaging scans
The majority of, but not all, subjects received pembrolizumab
Responder= no progression, Non-Responder = disease progression, Stable Disease considered Responder

Methods: The ATLAS™ Platform

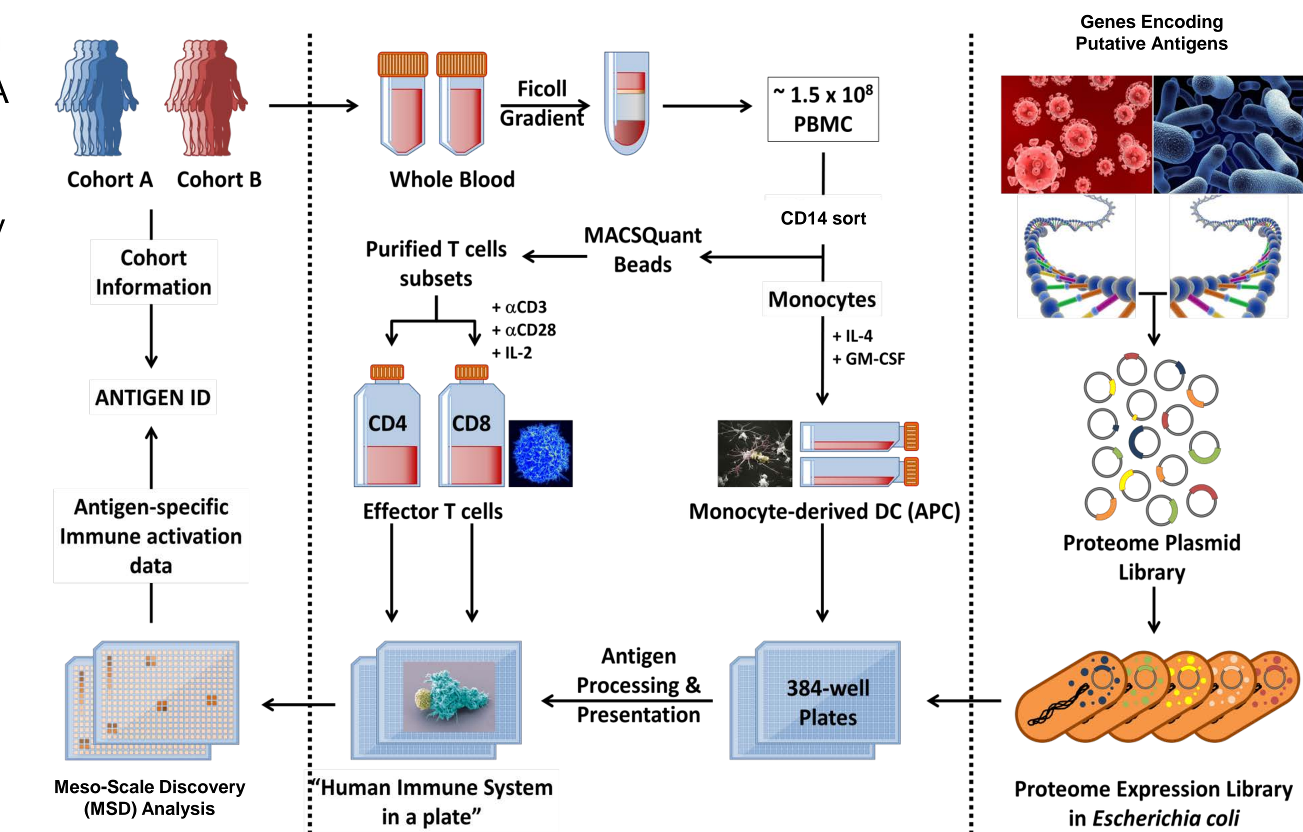
Figure 1. Lysteriolysin O (cLLO) Facilitates MHC class I presentation* by MDDC



*MHC cII presentation to CD4⁺ T cells facilitated through conventional endocytic route of processing of *E. coli* not co-expressing cLLO

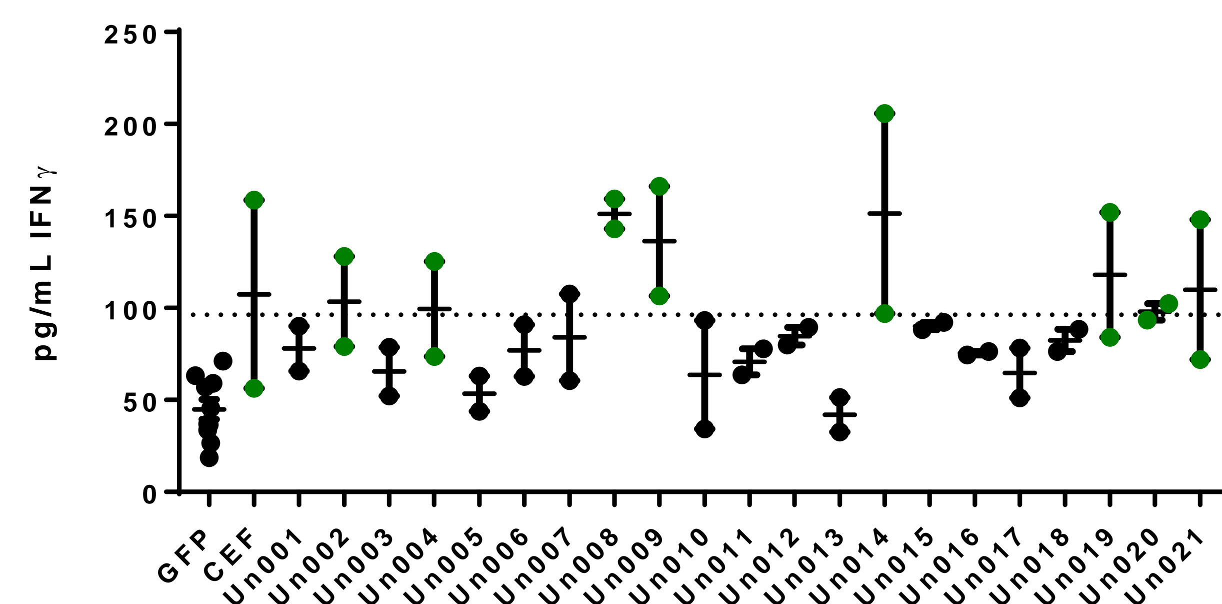
- 23 full-length TAA genes (labelled as Un001-023) were obtained from the DNA Resource Core at Harvard Medical School, recloned into the ATLAS™ expression vector, and sequence verified. Each TAA was recombinantly expressed in *E. coli*.
- Blood samples were collected from 32 consented patients who had previously undergone ICB therapy (one subject had two separate collections)
- Peripheral blood mononuclear cells (PBMC) were enriched by density gradient centrifugation. CD4⁺ and CD8⁺ T cells were sorted and non-specifically expanded, and CD14⁺ monocytes were differentiated into dendritic cells (MDDC).
- Library clones were screened in duplicate using 5,000 MDDC and 80,000 T cells, at an *E. coli*:MDDC ratio of 100:1; ten replicates of *E. coli* expressing GFP were included as negative controls
- Assay supernatants were harvested at 24hr and stored at -80°C.
- Supernatant cytokines were analyzed using Meso Scale Discovery V-PLEX Proinflammatory Panel 1 (human) Kit.

Figure 2. ATLAS™ Technology WorkFlow



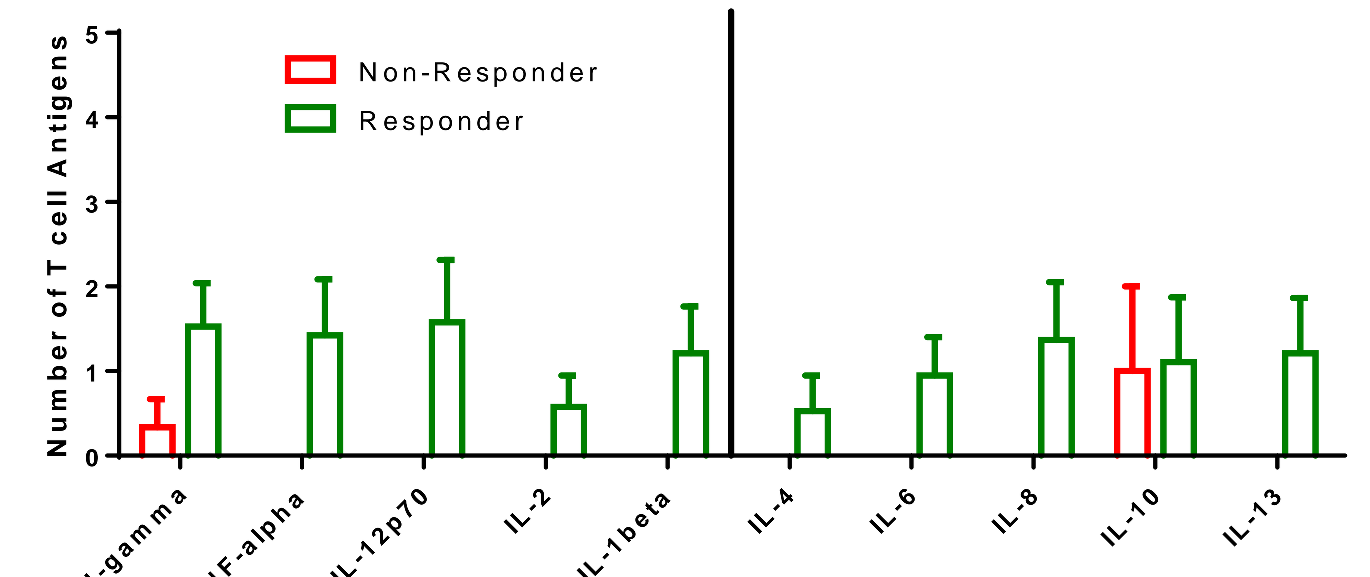
Results

Figure 3. Multiple TAA are Recognized by T cells from Patients Who Received Immune Checkpoint Blockade Therapy



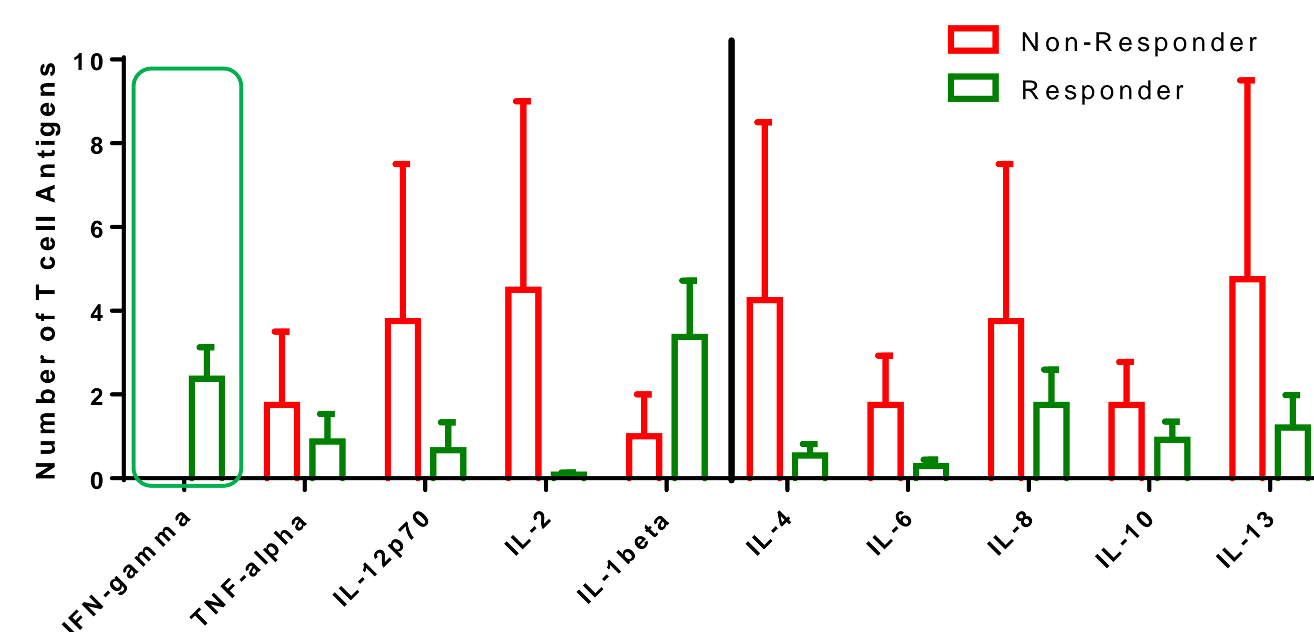
Exemplary data from one patient (CAL020). Non-specifically expanded CD8⁺ T cells were screened in duplicate against autologous APC individually pulsed with *E. coli* expressing each of 21 tumor-associated antigens. Clones that induced mean IFNγ responses that were statistically different from background (Wilcoxon Rank Sum, $p<0.05$) and exceeded 3 standard deviations (SD) of the mean of the negative control GFP clones (N=10) were considered antigens (indicated by horizontal dotted line). CEF = positive control peptide pool. GFP = green fluorescent protein. Each symbol represents an individual measurement, horizontal line = mean. Un022 & Un023 were not included in the CD8 library.

Figure 4. CD4⁺ T cell Responses may Predict Response to Immune Checkpoint Blockade



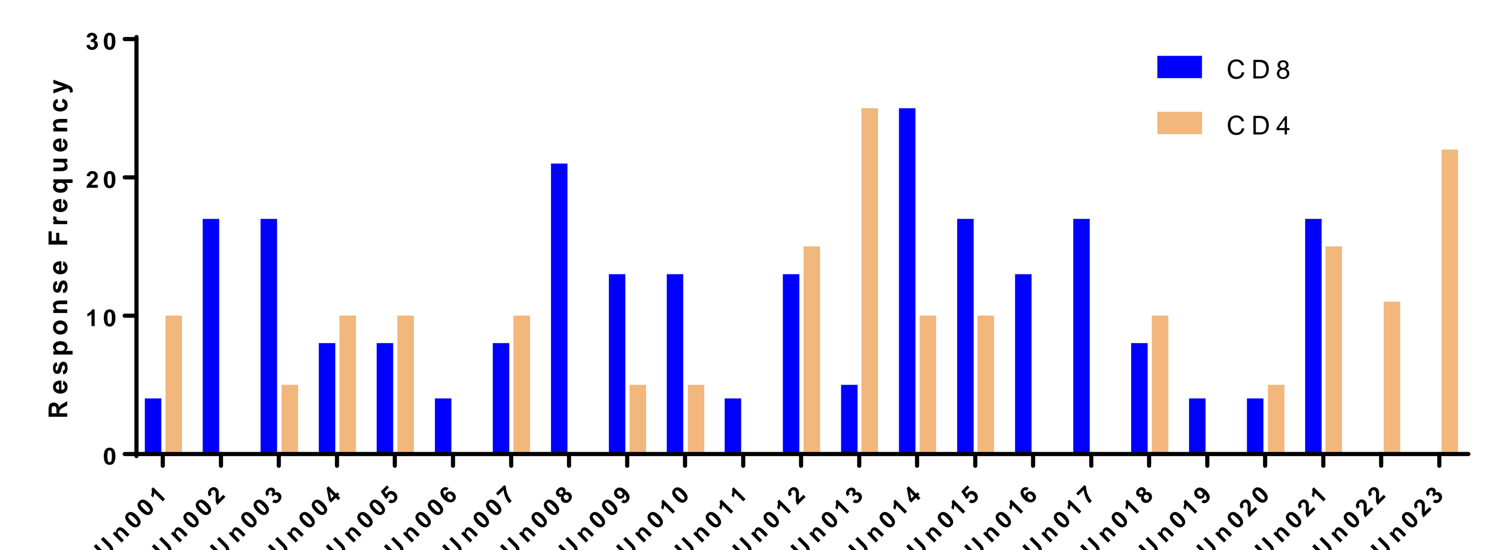
Subjects were cohorted into "Responder" (green bars) or "Non-Responder" (red bars) groups based on clinical evaluation of disease. Using a cutoff of 3SD above the mean of the negative control response per patient for each cytokine evaluated, the number of TAA to which each subject responded with their CD4⁺ T cell subset is represented. In contrast to the Responder cohort, the Non-Responder group had minimal discernable CD4⁺ T cell responses, measured by any cytokine, to any of the TAAs included in the library. Data are shown as the mean number (± SE) of TAA to which each cohort responded with each cytokine measured

Figure 5. IFN-γ-secreting CD8⁺ T cells may Predict Response to Immune Checkpoint Blockade



Subjects were cohorted into "Responder" (green bars) or "Non-Responder" (red bars) groups based on clinical evaluation of disease. Using a cutoff of 3SD above the mean of the negative control response per patient for each cytokine evaluated, the number of TAA to which each subject responded with their CD8⁺ T cell subset is represented. CD8⁺ T cells secreting IFNγ were undetectable in Non-Responders, but Responders had responses to a mean of ~ two TAA (boxed data). Data are shown as the mean number (± SE) of TAA to which each cohort responded with each cytokine measured.

Figure 6. No Dominant TAA Emerged in Subjects Considered Responders to Immune Checkpoint Blockade



The frequencies of subjects with TAA-specific (Un001-Un023) CD4⁺ (tan bars) and CD8⁺ (blue bars) T cell responses in the Responder cohort were evaluated to determine if any TAA was found to which the majority of subjects responded. As exemplary data, shown are the percent of subjects who were defined as a responder to each antigen by IFNγ secretion as described in Figure 3. No single TAA emerged as dominant after successful Checkpoint Blockade therapy. Un022 and Un023 were not in the CD8 library. The number of samples included in the analysis is as shown in the table.

Conclusions

- ATLAS can detect TAA-specific immune responses in ICB-treated melanoma patients
- The potential for a blood-based assay to predict efficacy of ICB exists by:
  - The presence of TAA-specific CD4⁺ T cell responses
  - IFN-gamma secreting CD8⁺ T cells to > N antigens
- No dominant TAA responses were identified that associated with successful outcome after ICB treatment, suggesting a "common" vaccine may be untenable
- Prospective studies are planned to determine if pre-existing T cell responses predict outcomes after treatment

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