Lineage-specific immune recognition in checkpoint blockade therapy-treated melanoma patients

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Introduction

Immune checkpoints (IC) are used to establish self-tolerance but are often hijacked by tumors for immune evasion. Immune checkpoint inhibitors (ICI) have seen recent success in the clinic, producing durable responses in a subset of melanoma patients but clinicians have yet to fully characterize mechanisms of de novo and acquired responsiveness to therapy.

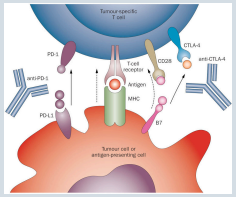


Figure 1: Examples of immune checkpoint inhibitors (ICI) include ipilimumab (anti-CTLA4), nivolumab (anti-PD1) and pembrolizumab (anti-PD1). These therapies block the interactions that cancer cells typically hijack to induce T-cell exhaustion.

We have noted a correlation between higher expression of lineage-specific proteins and immunotherapy response, suggesting a breakdown of tolerance to melanocyte lineage specific self-antigens. This parallels the clinical observation that vitiligo, a pigmentation disorder resulting from autoimmune destruction of melanocytes, is more prevalent in patients that respond well to immunotherapy. This effect is absent from most other cancers, implying that there may be epitope spread or elements of lineage-specific tissue recognition.

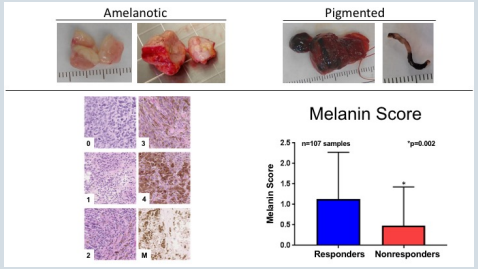


Figure 2: Melanin scoring of tissue from escape lesions of responders and non-responders reveals a correlation between higher pigmentation and response to ICI therapy

Methods

Blood Processing and CD8⁺ Isolation

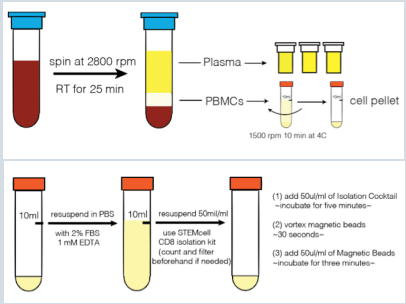


Figure 3: Longitudinal samples from checkpoint-blockade therapy treated melanoma patients are collected from the peripheral blood. The CD8 cells are isolated by magnetic negative selection from the patient PBMCs

Overnight Recovery of Frozen PBMCs

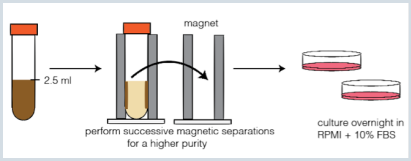


Figure 4: Frozen PBMCs collected from patients were cultured in RPMI + 10% FBS overnight to allow for recovery and expansion of cells prior to staining. Adding this step was found to improve the viability of the cells, as confirmed by DAPI staining.

Dextramer Staining Panel

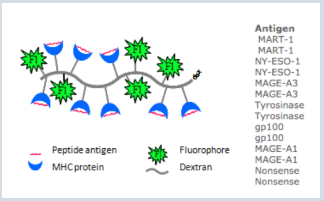


Figure 5: Dextramers are fluorescently labeled dextran molecules with bound MHC molecules. These reagents carry more MHC molecules and more fluorochromes than conventional multimers, thereby increasing their avidity to T-cells and enhancing the resulting signal. Each patient sample was tested against a panel of known melanoma-self antigen functionalized dextramers.

Results

Dextramer Staining showed MART-1 and Tyrosinase Positive Populations

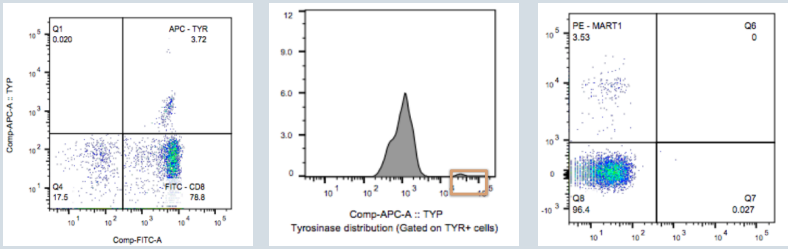


Figure 6: Using an MHC dextramer panel with nine melanocyte-specific antigens, we have identified a subpopulation of CD8+ T-cells that are reactive to Tyrosinase and MART1. This subpopulation of CD8+ T-cells increased in frequency following anti-PD1 therapy, suggesting that checkpoint blockade promotes the breakdown of tolerance toward melanocyte-lineage self-antigens.

Longitudinal analysis of patient samples show changes in MART-1/Tyr positive populations correlate to clinical events

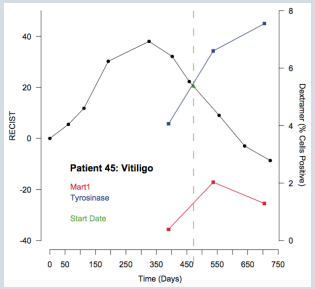
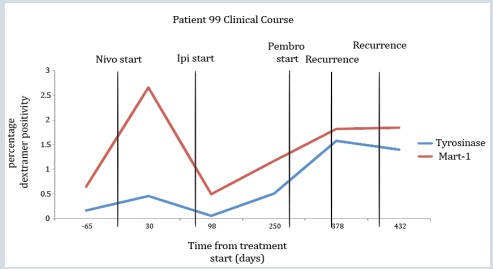
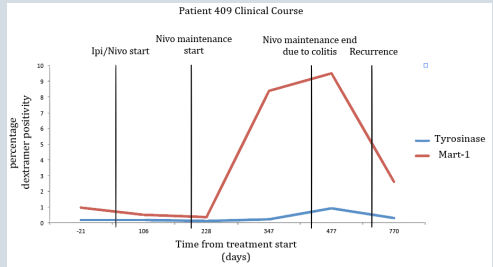


Figure 7: In several patients, an increase in lineage-specific antigen positive CD8+ T-cells correlated inversely with RECIST response and was more pronounced in patients with vitiligo.

Figure 8/9: A longitudinal analysis of MART-1 and Tyrosinase positive populations in patient samples overlaid with clinically relevant timepoints demonstrates that there is some correlation between checkpoint blockade therapy response and populations of peripheral CD8 T-cells that are reactive to melanoma self-antigens.





Conclusions and Future Work

- There is strong evidence for a lineage specific T-cell response generated as a result of checkpoint blockade therapy. We hypothesize that monitoring of peripheral blood-derived CD8⁺ T-cell subsets may provide a tool for analysis of lineage-specific cellular recognition during ICI therapy.
- Moving forward, we wish to characterize circulating antigen-specific T-cell responses during ICI and undertake comparative analysis with paired tumor infiltrating lymphocytes (TIL) prior to and during ICI therapy

References:

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