Mutation-Targeted T Cell Responses in Blood from Patients with Solid Tumors Prior to Treatment and which Evolve with Clinical Benefit from Anti-PD-1 Therapies

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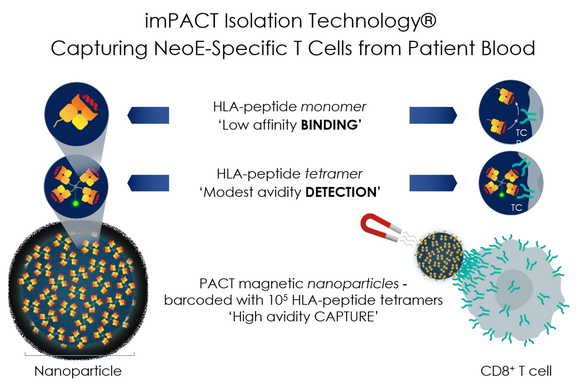
Abstract

T cells targeting tumor-exclusive neoepitopes (neoE) have been postulated to represent the primary mediators of clinical benefit for patients with solid tumors treated with immunotherapies. Identifying and tracking these T cells in patients can help to understand the mechanism for immune checkpoint inhibitor therapies, as well as provide new therapeutic candidates for personalized adoptive cell therapies. However, this has been hampered by the low frequency of neoE-specific T cells in peripheral blood. To this end, we demonstrate the use of the imPACT Isolation Technology®, an ultra-sensitive high-throughput technology, to capture neoE-specific CD8⁺ T (neoE-T) cells from peripheral blood. In addition, this technology can be utilized to quantify and monitor neoE-T cells longitudinally during therapy. We show here preliminary data applying the imPACT technology to clinical trial samples for the characterization of mutation-targeted T cell responses from patients associated with clinical benefit.

Methods



Peripheral blood mononuclear cells (PBMC) from patients with ovarian cancer treated with single agent or combinations containing an anti-PD-1 antibody (AB122) were analyzed. Briefly, tumor-exclusive neoE-HLA target candidates were predicted and barcoded snare libraries comprising personalized neoE-HLA reagents were produced for capture of neoE-specific CD8⁺ T cells from PBMCs. Longitudinal analysis of neoE-T cells responses throughout the duration of treatment was performed to obtain valuable information on neoTCR sequences and neoE-T cell quantification & phenotype.

Results

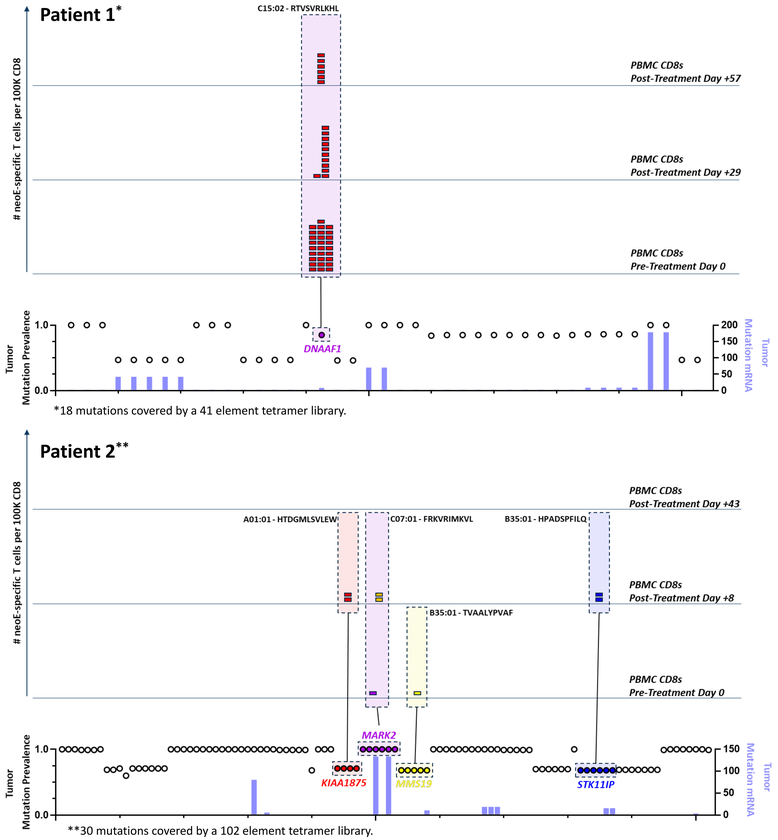


Figure 1. ***neoE-reactive T-cells from the blood of clinical trial patients.*** *PBMCs from Patient 1 and Patient 2 were isolated at different timepoints and analyzed by imPACT Isolation Technology®. Rectangles indicate neoE-reactive T-cells recovered per 100K CD8s analyzed. Different colors represent unique and novel TCRs.*

Patient	Cancer	Mutation Number	Treatment	Clinical Outcome
Patient 1	Ovarian	36	AB122	Stable
Patient 2	Ovarian	60	AB122, AB928	Stable

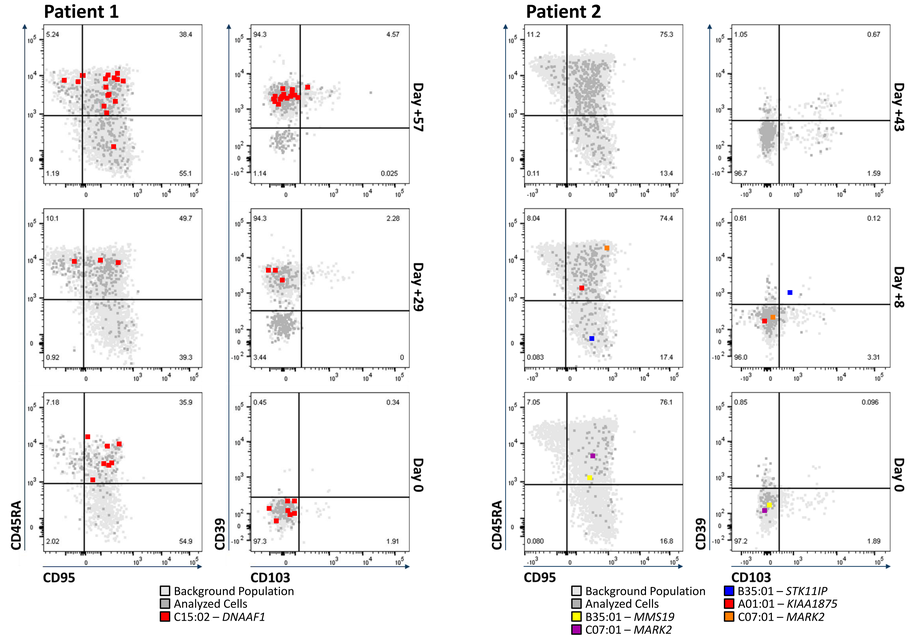


Figure 2. ***Phenotypic characterization of neoE-reactive cells from the blood of clinical trial patients.***

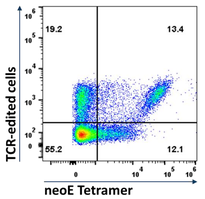


Figure 3. ***Functional characterization of neoE-reactive T-cell clones from cells recovered from Patient 1.*** *Gene-edited PBMCs (upper quadrant) with Patient 1 TCR bind their cognate neoE tetramer (upper right quadrant), verifying their reactivity against the* DNAAF1 *antigen.*

A baseline neoE-specific CD8⁺ T cell profile was identified in all patients prior to treatment. Among ovarian cancer patients exhibiting some clinical benefit in response to anti-PD1 therapy, some neoE-T cell clones identified at baseline persist in the blood and/or diversify in clonality over the course of treatment. In some circumstances, new neoE-T cell clones have emerged on treatment with anti-PD-1. Furthermore, phenotypic analysis suggested the neoE-T cells captured from blood have been activated, indicating previous encounter with their respective neoE-HLA targets.

Conclusions

Our data warrants the further testing of imPACT Isolation Technology® to assess the immune response in patients undergoing therapy:

- ❑ **imPACT Isolation Technology® identifies and tracks immune responses in oncology trial patients**, with information on phenotype and quantity of neoE-T cells in peripheral blood and TILs.
- ❑ The **neoE-T cell capture technology** shown here may prove to be **a powerful tool for mechanistically understanding the evolution of the immune responses associated with clinical benefit.**