Tumoricidal potential of T cells have been demonstrated using infusion of ex vivo expanded tumor-infiltrating lymphocytes and most recently using trials of T cell checkpoint molecule blocking antibodies. These advances in tumor immunotherapy has shown that the functional targets of these therapies are tumor-specific, mutation-derived novel protein sequences or neoantigens. Despite substantial promise, most treated patients fail to respond to checkpoint therapies, therefore, application of neoantigens may be important for the development of next-generation of cancer immunotherapies. Current approaches to rank patient-specific neoantigens, as therapies or therapeutic targets, rely on in silico epitope prediction algorithms or proteomics. These methods fail to provide functional evidence of T cell reactivity and specificity. We used our proprietary ATLAS™ platform to screen T cell responses to the landscape of detected neoantigens in a non-small cell lung cancer (NSCLC) patient successfully treated with anti PD-L1 therapy pembrolizumab.

**Construction of Personalized Neoantigen Expression Library**

- Whole exome sequences were obtained from patient DNA isolated from PBMCs and tumor biopsy samples, and led to the identification of 201 tumor-specific somatic mutations.
- Individual DNA sequences spanning each mutation site (coding 133 amino acids) were synthesized and cloned into the ATLAS™ bacterial expression construct.
- Each neoantigen was co-expressed with listeriolysin O to facilitate MHC class I presentation.
- The expression level of each neoantigen was detected using a surrogate T cell assay that identifies C-terminal fusion tag SIINFEKL (OVA257-264 class I epitope).

**Personalized ATLAS™: A Platform to Rank Patient-Specific Neoantigens**

- Peripheral blood mononuclear cells (PBMC) that were collected pre- and post-pembrolizumab therapy were thawed. CD14+ monocytes were enriched and derived into dendritic cells (APC) and CD8+ T cells were sorted and expanded non-specifically using microbeads.
- *E. coli* expressing individual neoantigens were pre-arrayed in 384 well plates. The arrayed library was added to the screening plates containing APCs, then incubated with expanded CD8+ T cells overnight.
- The supernatant from each individual well was collected and the levels of IFN-γ and TNF-α cytokines were measured using Mesol Scale Discovery (MSD).
- *E. coli* expressing Neon Green were used as negative control. Ten percent of the wells of the screening plate contained negative controls to define true T cell responses.
- Only 103 neoantigens in the library were assayed due to the limitations in the yield of dendritic cells. Further work is in progress to screen the rest of the expressed clones in the library.

**References**


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