**Background**

Neoantigens arise from tumor-specific, somatic mutations and have the potential to be recognized by T cells that are associated with anti-tumor immune responses. Since neoantigens are non-self, they are hypothesized to provide an attractive therapeutic modality because T cells that can respond to those sequences have not undergone thymic selection. The ATLAS platform enables identification of biologically relevant CD4+ and CD8+ T cell neoantigens in any subject in an unbiased manner, by using subjects’ own antigen presenting cells (APCs) and T cells rather than predictive algorithms.

**Materials and Methods**

- Eleven patients with solid tumors were analyzed.
- Whole exome and RNA sequencing was performed on tumor biopsies and matched normal genomic DNA from which single nucleotide variants and insertion/deletions were identified, cloned into expression vectors and expressed in E. coli with and without co-expression of listeriolysin O to enable presentation via MHC class I or class II, respectively.
- CD14+ monocytes and T cell subsets were isolated from patient peripheral blood mononuclear cells. Monocytes were differentiated into dendritic cells (MDDCs), and T cells were non-specifically expanded.
- For each patient, their unique clones were co-cultured with autologous MDDCs in an ordered array, then their CD4+ or CD8+ T cells were added and incubated overnight.
- T cell activation was determined by measurement of TNF-α and IFN-γ in the supernatants by Meso-Scale Discovery.
- Cytokine concentrations were normalized against responses to negative control protein (neon green (NG)), and neoantigens were defined as clones that elicited responses >2 median absolute deviations (MADs) above the median of the negative control replicates, unless otherwise indicated. Inhibitory antigens were defined as those that reduced responses >2 MADs below the median of negative controls.

**Tumor Types, Number of Mutations Profiled, and HLA Haplotypes**

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<tr>
<th>Tumor Type</th>
<th>Number of Mutations Profiled</th>
<th>HLA A Profile</th>
<th>HLA B Profile</th>
<th>HLA C Profile</th>
<th>HLA A-DP1 Profile</th>
<th>HLA B-DPB1 Profile</th>
<th>HLA A-DQA1 Profile</th>
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*ND* = Not Determined

**Results and Conclusions**

- **ATLAS empirically defines which somatic neoantigens eliciting immune responses in individuals independently of a patient’s HLA type.**
- **Neoantigen screening was performed (or is in process) for 22 individuals across eight tumor types with mutational burden ranging from 12 to 276.**
  - Different HLA alleles represented: HLA-A=17, B=24, C=18, DQ=15, DR=9, DP=7
  - Both stimulatory and inhibitory neoantigens for CD8+ T cells, as well as for CD4+ T cells for which the algorithms do not perform nearly as well (data not shown), are identified, identifying the opportunity to identify better targets to include in a vaccine.
  - Frequency of stimulatory responses
    - CD4: 7% of mutations
    - CD8: 5% of mutations
  - Frequency of inhibitory responses
    - CD4: 14% of mutations
    - CD8: 27% of mutations
  - CD4+ and CD8+ T cell antigens are different; only 12% of neoantigens are shared between T cell subsets.
  - To date, none of mutant allele frequency, gene expression, mutation type, nor predicted epitope binding has created insight into which mutations elicit T cell responses.
  - Inhibitory antigens identified by ATLAS cannot be distinguished by *in silico* approaches. The therapeutic impact of these types of antigens is being investigated pre-clinically.
  - ATLAS-selected antigens will be used in GEN-009 clinical study (IND expected Q1 2018).

**CD8+ T cell responses identified by ATLAS are not enriched for any mutation type**

Frequency of CD8+ responses to candidate neoantigens by type of somatic mutation and response. There was no significant enrichment of response type by mutation type. Similar results seen with CD4+ T cell responses (not shown).

**DNA mutant allele frequency is not associated with CD8+ T cell response**

Mutant DNA allele frequency was derived from whole exome sequencing and compared to response type observed. An equivalent comparison for CD4+ T cells was made and was similar (data not shown).

**Detection of a mutation in RNA does not correlate with gene expression**

For 8 patients, RNA-seq was performed on the tumor sample. Somatic mutations were identified via whole exome sequencing, and the RNA-seq data were interrogated for the presence or absence of mutations identified in DNA. True = mutation identified by RNAseq, False = mutation not identified by RNAseq.

**Acknowledgements**

We would like to thank the patients who consented to participate in this study and their families.