Empiric profiling of neoantigen-specific T cell responses in lung cancer patients with ATLAS™ reveals unexpected neoantigen and inhibitory antigen profiles

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Introduction

Immunotherapy of non-small cell lung cancer (NSCLC) has resulted in unprecedented, but sometimes short-lived efficacy in first- and second-line settings. The importance of T cells recognizing patient-specific mutations, or neoantigens, in successful immune checkpoint blockade (ICB) treatment is well established. Given the role of neoantigens in successful ICB treatment, coupled with the aspiration to improve response rates to ICB in the absence of added toxicity, efforts are underway to develop neoantigen vaccines to enhance specific T cell responses. The challenge is identifying which mutation, out of the tens to thousands present in a patient’s tumor, to include in their vaccine. Here we comprehensively identify neoantigen-specific T cell responses in the peripheral blood of lung cancer patients to identify their characteristics.

Methods: The ATLAS™ Platform

Figure 1: The ATLAS screening workflow. 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. All variants were 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. PBMCs were isolated by density gradient centrifugation from which CD14+ monocytes and T cells were isolated via immunomagnetic separation. 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 Multi-Scale Discovery. Stimulatory or inhibitory antigens are defined as those that elicit a mean cytokine response of either two standard deviations or two median absolute deviations from the mean or median of the background control responses, respectively.

Figure 2: Listeriolysin O (cLLO) facilitates MHC class I presentation by monocyte-derived dendritic cells (MDDC). Co-delivery of a putative antigen and cLLO by E. coli leads to pore formation in the phagolysosome upon acidification. The putative antigen is released into the cytoplasm, proteasomally processed and loaded onto the subject’s MHC class I molecules for recognition by autologous CD8+ T cells. MHC class II presentation to CD4+ T cells is achieved with a separate cLLO-negative E. coli library and conventional endocytic processing of extracellular antigens (not shown).

Figure 3: Normalized cytokine responses for IFNγ and TNFs across 8 lung cancer subjects. Each point represents the average observed cytokine response for a given analyte for each mutation profiled. Horizontal lines indicate statistical cutoffs: Blue = stimulatory neoantigens, maroon = inhibitory neoantigens.

Figure 4: Normalized cytokine responses for IFNγ in CD4+ and CD8+ T cells shown for two lung cancers. Each point represents the average observed response for each mutation profiled. Horizontal lines indicate statistical cutoffs: Blue = stimulatory neoantigens, maroon = inhibitory neoantigens. Green dots represent the background control antigen, Neon Green.

Figure 5. Venn Diagrams: Comparison of ATLAS-identified T cell responses with NetMHCPan II 4.0 or NetMHCPan II 4.0 predictions using a <500 nM affinity cutoff.

Patient Demographics and Mutations

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<th>Pharmacy?</th>
<th>Current therapy</th>
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Across subjects there was a minimum of 84 and a maximum of 971 somatic variants identified, with a median of 700. In total 1,015 unique antigens were profiled: 934 variants for CD8+ T cell responses and 525 variants for CD4+ T cell responses.

ATLAS provides the ability to monitor changes in T cell responses upon treatment with checkpoint blockade

Figure 6. Top row figures: Comparison of differential agretopicity index (DAI) between wild-type and mutant peptide sequences and ATLAS-identified antigens. This analysis is restricted to single nucleotide variants and plots the maximum DAI calculated for each mutation.

Figure 7. Bottom row figures: For each mutation, the minimum predicted affinity is plotted against the maximum calculated DAI.

ATLAS provides the ability to monitor changes in T cell responses upon treatment with checkpoint blockade

• Three inhibitory antigens resolved to non-antigenic
• Five mutations emerged as neoantigens upon treatment
• Five inhibitory neoantigens emerged upon treatment

Conclusions

• The ex vivo ATLAS platform is a powerful tool with which to identify and characterize neoantigens in the peripheral blood of oncology patients; here more than 1,000 unique mutations were screened identifying both stimulatory and inhibitory CD4+ and CD8+ T cell responses to neoantigens.
• Data not shown indicated identified neoantigens are unexpected relative to common neoantigen prioritization criteria, such as mutation type (short variant vs. long), RNA expression level, mutant DNA allele frequency, and whether the mutation occurs in a known cancer gene. In addition, we did not identify common CD4+ and CD8+ T cell shared responses in this cohort.
• The proportion of inhibitory to stimulatory responses may be a useful tool for early prognosis of checkpoint blockade efficacy. A Phase 1/2a clinical trial of a targeted personalized cancer vaccine, GEN-009, using ATLAS-identified antigens, is ongoing.

Acknowledgements

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