**ATLAS™** identifies relevant neoantigens for therapeutic anti-tumor vaccination and may serve as a biomarker for efficacy of immunotherapy of solid tumors

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#P417

**Background**

Neoantigen cancer vaccines are promising next-generation cancer therapies. However, the success of vaccination is dependent on the ability to identify the right neoantigens for vaccine inclusion, which remains a critical challenge. Computationally-identified neoantigens do not necessarily generate immunogenic responses. Recently, we reported interim immunogenicity results from the ongoing GEN-009-101 Phase 1/2a personalized immunotherapy clinical trial (NCT03633310). For GEN-009, ATLAS™, an ex vivo cell-based assay, selects neoantigens for vaccine inclusion based on a patient's own pre-existing T cell responses. The interim results revealed that vaccination elicited T cell responses to over 98% of administered peptides. Here, we compare immunogenicity of ATLAS-identified and NetMHCpred-predicted neoantigens from a cohort of six patients enrolled in the GEN-009-101 Phase 1/2a clinical trial.

**The ATLAS Platform**

Vaccines generated using ATLAS™ identify both CD8+ and CD4+ neoantigens while detecting inhibitory neoantigens for vaccine exclusion regardless of cancer type or tumor mutational burden.

**Comparison of ATLAS and algorithms reveal that a subset of inhibitory and stimulatory neoantigens are misidentified by in silico approaches**

**Methods**

**Vaccine Development:** GEN-009 vaccine contains 4–20 stimulatory synthetic long peptides (SLPs) claded into 4 pools. Each pool consists of 1-5 SLPs with Poly-CLC as an adjuvant. Eligibility is based on histological confirmation of cutaneous melanoma, non-small cell lung cancer (NSCLC), squamous cell carcinoma of the head and neck (SCCHN), or urothelial cancer with no evidence of disease. Patients were dosed at 1, 22, and 43 days with booster vaccinations at 12 and 24 weeks. For more information please visit Poster P412.

**Fluorescent Assays:** Ex vivo and in vitro assays were conducted on endogenous MHC Class I and II predicted peptides using flow cytometry. MHC Class I and II responses to each SLP and pool were assessed using 96-well plates coated with human IFN-γ (ex vivo) or IFN-γ ELISPOTs (SVM) fluoroimmuno kits (Maltech). Peptides were applied using 96-well plates with peptide 30 days before sorting for CD4+ or CD8 T cells using magnetic bead isolation (Miltenyi) and then plating for the fluorescent assay. A response was indicated as positive if the mean SFC exceeded the LOD and the p-value between the test and negative control was ≤0.05 by the Kruskal-Wallis test.

**Eclipse predictions:** NetMHCScan 4.0 (MHC class I) and NetMHCIPan 3.1 (MHC class II) algorithms were used to predict epitopes based on predicted binding affinity to HLA molecules. Briefly, peptide lengths input for analysis were SLPs for NetMHCScan 4.0 and 15 aa for NetMHCIPan 3.1. A single selection was based on patient HLA allele type and ≥2% net threshold was used to predict peptide-MHC binding. MHC protein sequences were analyzed only for mutations that were screened by ATLAS.

**Summary**

- By recalling both stimulatory and inhibitory neoantigen-specific CD4+ and CD8+ T cell responses, the ex vivo ATLAS platform is a powerful tool with which to identify neoantigens.
- Compared to in silico methods, ATLAS revealed a significant proportion of neoantigens that were not predicted.
- Post-vaccination, algorithm-predicted neoantigens were not more immunogenic than those that were not predicted.
- ATLAS-identified inhibitory antigens may have a detrimental effect if included in a vaccine formulation (see poster P678).

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**The proportion of stimulatory and inhibitory responses to neoantigens may predict immunotherapy outcomes**

**ATLAS neoantigens missed by algorithms elicit T cell responses upon vaccination confirming accuracy of ATLAS in neoantigen identification**

**Discussion**

ATLAS identified neoantigens that were missed by algorithms elicit T cell responses post vaccination thus validating the capability of ATLAS to identify neoantigens (A) Comparison of the percentage of predicted and ATLAS-identified and not predicted but ATLAS-identified neoantigens that generated a T cell response using ex vivo and IVS assays as described in methods at baseline and day 50 post vaccination. Data segregated by T cell type for entire patient cohort with exclusion of CD4 responses for patient C for whom cell HLA typing data were unavailable. (B) IVS results represented as total IFN-γ or TNFα specific SFC (exemplifying cells) from CD4+ or CD8+ T cells stimulated with SLPs at day 0 and day 50 post vaccination. Stacked graphs illustrating the combined SFCs for 20,000 T cells for CD4+ and CD8+ (MHC class II) algorithm-predicted and ATLAS-identified (gray) or not predicted ATLAS-identified (orange) for individual patients. Class II predictions are not yet available for patient C, therefore total SFCs for IFNγ or TNFα is plotted (bar) independently of algorithm prediction.