Neoantigen identification using ATLAS™ across multiple tumor types highlights limitations of prediction algorithms

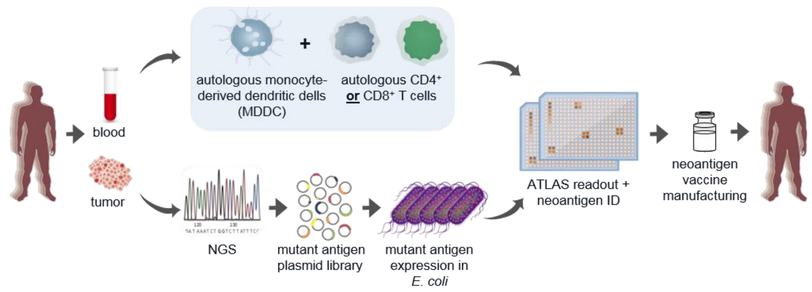
Jason R Dobson, Huilei Xu, Kyle Ferber, Johanna K Kaufmann, Judy Jacques, Christine McCoy, Michael S O'Keeffe, Yana Ostrovsky, Crystal Cabral, Erick Donis, Pamela Carroll, Theresa Zhang, Jessica B Flechtner, Wendy Broom

Genocea Biosciences, Cambridge, MA

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.

ATLAS Platform



Materials and Methods

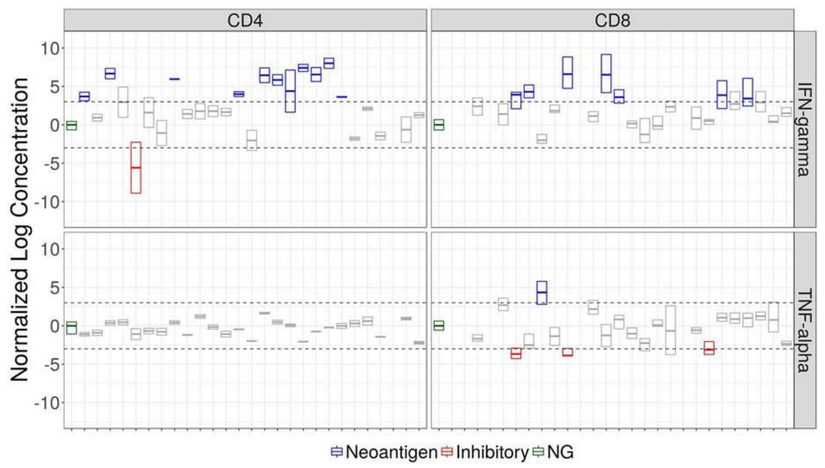
- Eleven patients with solid tumors were analyzed.
- Whole exome and RNA sequencing was performed on tumor biopsies and matched normal genomic DNA from which single nucleotide variants and insertion/deletions were identified, cloned into expression vectors and expressed in *E. coli* with and without 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

Indication	Mutations Profiled	HLA-A	HLA-B	HLA-C	HLA-DPA1	HLA-DPB1	HLA-DQA1	HLA-DQB1	HLA-DRB1
Colon Cancer	142	01/03	35/57	04/06	ND*	ND	ND	ND	ND
Colon Cancer	42	02/01	49/57	06/07	ND	ND	ND	ND	ND
Renal Cell Carcinoma	12	11/24	35/07	07/04	ND	ND	01/01	06/06	15/09
Colon Cancer	31	32/30	18/14	05/08	ND	ND	01/05	05/02	01/03
Colon Cancer	74	03/24	18/56	01/07	ND	ND	01/05	06/03	11/13
Colon Cancer	29	02/24	51/57	01/06	ND	ND	02/03	03/03	07/13
NSCLC	31	01/29	07/08	07/15	ND	ND	ND	05/02	03/10
NSCLC	86	02/33	45/53	16/04	02/02	01/30	04/01	06/04	13/03
Renal Cell Carcinoma	19	31/32	15/14	08/04	01/01	04/04	02/03	03/02	04/07
Prostate Cancer	14	01/32	08/39	07/07	01/01	04/02	04/05	02/04	03/08
Pancreatic Cancer	26	03/26	07/38	07/12	01/02	04/135	01/01	06/06	13/15

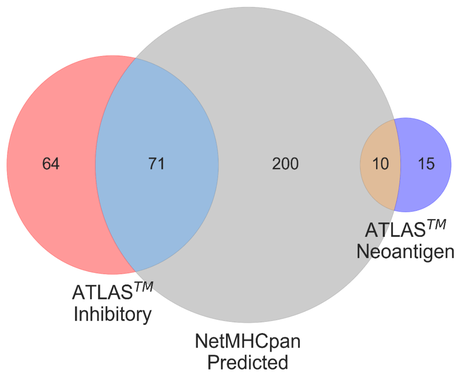
*ND = Not Determined

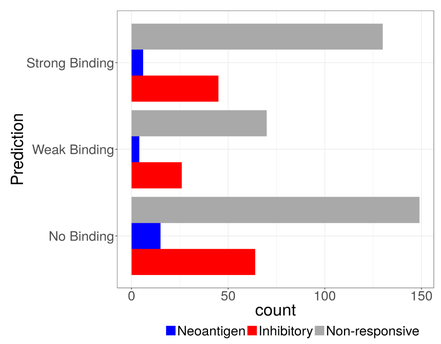
Exemplary neoantigen screen with ATLAS identifies patient-specific CD4⁺ and CD8⁺ T cell responses



Screening results from a pancreatic cancer patient. Displayed are the CD4⁺ and CD8⁺ T cell responses observed in response to each candidate neoantigen. Data are represented as box plots of 2 (CD4⁺), 4 (CD8⁺), or 12 (negative control) replicates. Horizontal dotted lines indicate cutoffs used to define neoantigens (>3 MADs) and inhibitory antigens (>-3 MADs) as described in methods.

An algorithm that predicts MHC class I binding does not accurately predict CD8⁺ T cell responses or type of response





Algorithm predictions confirmed by ATLAS	Neoantigens not predicted by algorithms but identified by ATLAS	Inhibitory antigens mischaracterized as neoantigens by algorithms
4%	60%	25%

A comparison between MHC class I binding predictions and T cell responses observed in ATLAS across all 11 subjects. **Left:** Comparison of ATLAS-identified responses to NetMHCpan predictions <500 nM cutoff. **Right:** Comparisons that stratify predictions by strong binding (<150 nM), weak binding (<500 nM), or non-binding (≥ 500 nM). Data show no enrichment of either stimulatory or inhibitory responses in CD8⁺ T cells across binding prediction groups.

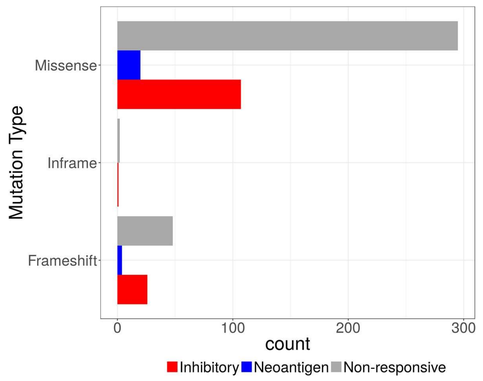
Results and Conclusions

- ATLAS empirically defines which potential neoantigens created by somatic mutations elicit 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 algorithmic approaches do not perform nearly as well (data not shown), are identified, providing 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).

Acknowledgements

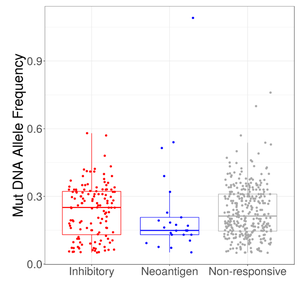
We would like to thank the patients who consented to participate in this study and their families.

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



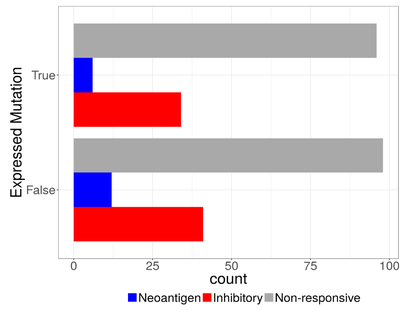
Frequency of CD8⁺ responses to candidate neoantigens by type of somatic mutation and response. There was no significant enrichment of response type by mutation type. Similar results seen with CD4⁺ T cell responses (not shown).

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



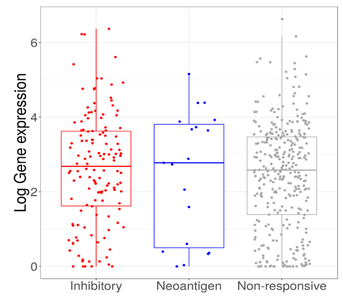
Mutant DNA allele frequency was derived from whole exome sequencing and compared to response type observed. An equivalent comparison for CD4⁺ T cells was made and was similar (data not shown).

Detection of a mutation in RNA does not predict a recall response in CD8⁺ T cells



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.

CD8⁺ T cell responses identified by ATLAS do not correlate with gene expression



For 10 samples, RNA-seq was performed on the tumor sample and quantitative gene expression values were calculated for each gene harboring a candidate neoantigen and compared to normalized cytokine measurements.