632

DO NOT POST

Genome-scale neoantigen survey using ATLAS™ in a non-small cell lung cancer patient identifies unique vaccine candidates that are not predictable by algorithms

Lila Ghamsari[1], Emilio Flano[1], Biao Liu[1], Judy Jacques[1], Zheng Yan[1], Aula Alami[1], Christine Kelley[1], Johanna K Kaufmann[1], Wendy Broom[1], Jonathan Havel[2], Vladimir Makarov[2], Taha Merghoub[3], Jedd D. Wolchok[4], Matthew Hellman[4], Timothy Chan[2], Jessica B. Flechtner[1]

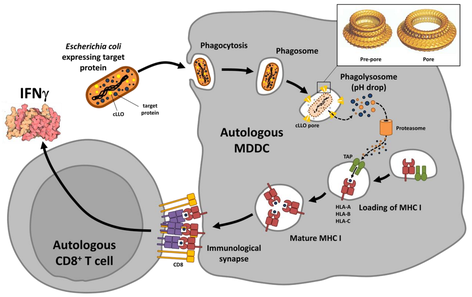
[1]Genocea Biosciences, Cambridge, MA, USA; [3]Ludwig Collaborative Laboratory, [4]Department of Medicine, [2]Memorial Sloan Kettering Cancer Center, New York, NY, USA

Introduction

- Despite the unprecedented efficacy of checkpoint blockade (CPB) therapy in treating some cancers, a large number of patients still fail to respond to these interventions. Recent data indicate that the combination of CPB and neoantigen vaccines may prolong survival in some cancer patients.
- Current approaches to neoantigen prioritization involve deep sequencing of tumor samples, followed by selection of epitopes based on prediction algorithms primarily examining MHC class I binding affinity and processivity. However this strategy has proved challenging with the majority of candidates lacking immunogenic activity in patients.
- ATLAS™ is a T cell antigen discovery platform in which putative antigens are expressed as individual clones that can be processed by any subject's antigen presenting cells (APCs) and presented as peptide epitopes in the context of their own MHC class I or II molecules.
- We identified both MHC class I and II neoantigens inducing T cell cytokine responses in a non-small cell lung cancer (NSCLC) patient. Approximately 50 % of MHC class I neoantigens were not predicted by multiple *in silico* methods; MHC class II neoantigens can not be effectively predicted using current *in silico* methods.

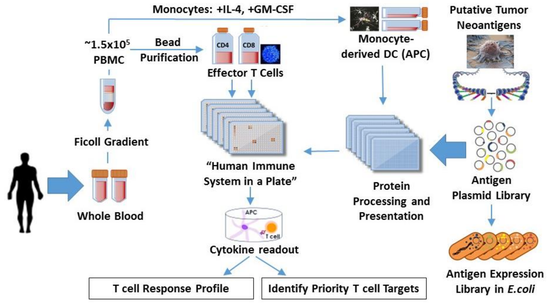
Methods: The ATLAS™ Platform

Figure 1. Lysteriolysin O (cLLO) facilitates MHC class I presentation by MDDC



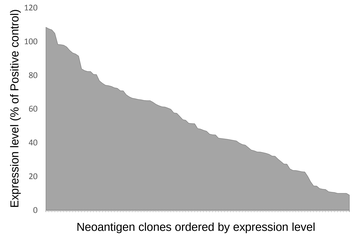
- MHC class II presentation to CD4⁺ T cells facilitated through conventional endocytic route of processing of *E. coli* not co-expressing cLLO[2].
- Whole exome sequencing of a tumor biopsy from a NSCLC patient identified 202 somatic mutations. Each somatic mutation was recombinantly expressed in *E. coli* to create a screening library.
- Protein coding sequences were synthesized covering ~100 amino acids with the mutation centrally located, cloned into the ATLAS™ expression vector, and sequence verified. Pre and post-treatment peripheral blood mononuclear cells (PBMC) were collected from one patient treated with, and responding to, anti-PD-1 therapy (pembrolizumab).

Figure 2. ATLAS™ technology workflow



- PBMC were enriched by density gradient centrifugation. CD4⁺ and CD8⁺ T cells were sorted and non-specifically expanded, and CD14⁺ monocytes were differentiated into dendritic cells (MDDC).
- Library clones were screened in duplicate using 2,000 MDDC and 80,000 T cells per well, at an *E. coli*:MDDC ratio of 250:1; 20 replicates of *E. coli* expressing Neon Green were included as negative controls.
- Assay supernatants were harvested at 24 hr and cytokines levels analyzed using a Meso Scale Discovery custom kit, for detection of IFNγ and TNFα.

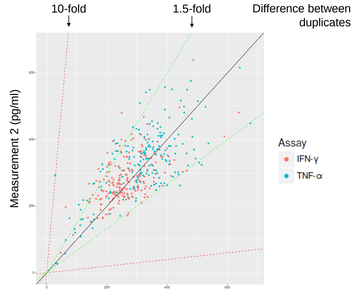
Figure 3. Expression verification



The expression level of each neoantigen was detected using a surrogate T cell assay that identifies a C-terminal fusion tag SIINFEKL ($OVA_{257-264}$ class I epitope).

Neoantigen Identification

Figure 4. Good alignment between replicate measurements for each cytokine



Example data for CD8⁺ T cells post-treatment:

- Biological analysis: > 3 SD of mean of negative control
  - 7 antigens with significant biologically relevant IFNγ responses compared to negative controls **– most robust hits**
- Statistical analysis: Wilcoxon rank sum, $p < 0.05$
  - 57 antigens have IFNγ responses statistically different from negative control **– 28 % overall hit rate**

Acknowledgements

We would like to thank the patient and their family who consented to participate in this study.

Results

Figure 5. Multiple neoantigens were identified through CD8⁺ T cell responses pre- and post-checkpoint blockade therapy

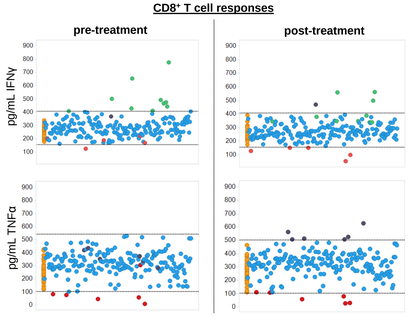
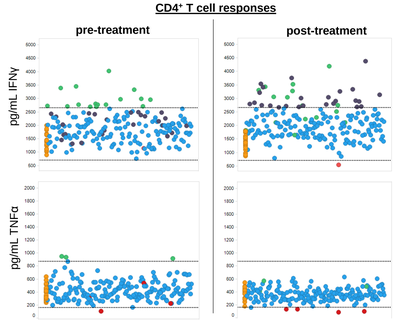


Figure 6. Increased breadth of CD4⁺ T cell IFNγ responses to potential neoantigens post-checkpoint blockade therapy



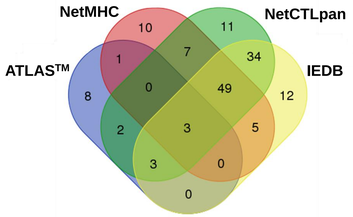
- Non-specifically expanded CD4⁺ or CD8⁺ T cells were screened in duplicate against autologous MDDC individually pulsed with *E. coli* expressing each of 195 or 201 somatic mutations, respectively. Clones that induced mean IFNγ or TNFα responses that were statistically different from background (Wilcoxon Rank Sum, $p < 0.05$) and exceeded 3 standard deviations (SD) of the mean of the negative control Neon Green clones (N = 20) were considered antigens (indicated by horizontal dotted line).

NeoAg pre-treatment
NeoAg post-treatment
Suppressive NeoAg
Negative controls (NG)

Table 1. Mutations identified as neoantigens based on IFNγ and TNFα responses

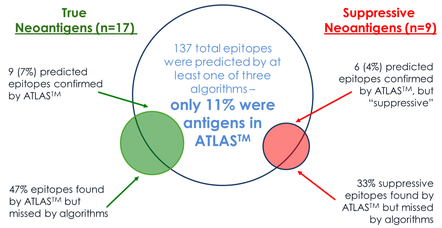
T cell responses	Pre-treatment	Post-treatment	Both
CD8⁺	5 %	5 %	1 %
CD4⁺	10 %	17 %	5 %

Figure 7. Limited overlap between CD8⁺-specific T cell neoantigens identified by ATLAS™ and epitope prediction algorithms



- MHC class I epitopes were predicted for all screened neoantigens using three commonly used algorithms: NetMHC, NetCTLpan and IEDB, and using patient-specific haplotypes HLA-A*02:01/*32:01, HLA-B*40:01:02/*45:01:01, HLAC*06:02/*03:04[1].
- All of the unique epitopes identified using these algorithms carry the tumor somatic mutation.

Figure 8. Epitope predictions had a high false positive rate and missed relevant and inhibitory antigens



Conclusions

- ATLAS was able to detect neoantigen-specific immune responses in NSCLC patients both pre- and post-CPB therapy
- Based on ATLAS, not all T cell antigens are stimulatory; suppressive responses to T cell antigens were also observed; while these epitopes may emerge from predictive algorithms, their suppressive activity cannot be predicted
- CD4⁺ and CD8⁺ T cell antigens are mostly unique with only 3 % of putative neoantigens eliciting responses from both T cell subsets
- Breadth of CD4⁺ T cell responses based on IFNγ secretion increased upon checkpoint therapy
- Current MHC class I epitope prediction algorithms have a high false positive rate, and missed a large number of relevant and suppressive antigens

References

1. Rizvi, et al. Mutational landscape determines sensitivity to PD-1 blockade in non–small cell lung cancer (2015) Science. 348(6230): 124-8.
2. Hu, et al. Escherichia coli expressing recombinant antigen and listeriolysin O stimulate class I-restricted CD8+ T cells following uptake by human APC (2004) J Immunol 172(3): 1595-601.