28

Profiling of T Cell Responses to Tumor-Associated Antigens in Lung Cancer Patients Treated With Checkpoint Inhibitors

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

Genocea Biosciences, Inc., Cambridge, MA, USA

Introduction

- Successful treatment of lung cancer patients with immune checkpoint inhibitor (ICI) therapies has reinforced the importance of T cells in anti-tumor efficacy.
- Despite significant progress, ICI therapy is effective in only a subset of treated patients. It is therefore imperative to understand the profile of T cell responses to better understand the heterogeneity of response to therapy.
- ATLAS is a T cell antigen discovery platform where putative antigens are expressed as individual clones that can be processed by any patient's antigen presenting cells (APCs) and presented as peptide epitopes in the context of their own MHC class I or II molecules. After addition of the patient's own CD4⁺ or CD8⁺ T cells, a multiplex cytokine readout of T cell activation can be measured to each tumor-associated antigen (TAA).
- This is a proof-of-concept study to apply the ATLAS technology to characterize and profile T cell responses to TAAs of a diverse sample of lung cancer patients undergoing ICI therapy.

Patient Characteristics

Lung Cancer Subtype	ICI Therapy	Clinical Outcome	Age	Sex	Race	Frequency (%) of Stimulatory Responses		Frequency (%) of Inhibitory Responses	
						CD4⁺	CD8⁺	CD4⁺	CD8⁺
Adenocarcinoma	Nivolumab	Responder	68	F	White	9.1	NA	18.2	NA
Adenocarcinoma	Nivolumab	Responder	70	M	Black	Failed QC	0.0	Failed QC	17.8
Adenocarcinoma	Nivolumab	Responder	52	F	Black	13.2	16.2	7.9	5.4
Small Cell Carcinoma	Nivolumab	Responder	64	F	Black	7.0	4.8	11.6	26.2
Subtype not specified	Nivolumab	Responder	57	F	White	NA	25.8	NA	35.5
Adenocarcinoma	Nivolumab	Unknown	52	M	White	0.0	0.0	14.0	4.8
Adenocarcinoma	Nivolumab	Unknown	63	M	Black	6.6	38.9	9.2	9.7
Adenocarcinoma	Nivolumab	Unknown	72	M	Black	NA	22.2	NA	59.3
Adenocarcinoma	Pembrolizumab	Unknown	56	M	White	0.0	0.0	13.2	10.3
Adenocarcinoma	Pembrolizumab	Unknown	67	M	White	12.5	NA	8.3	NA
Large Cell Carcinoma	Nivolumab	Unknown	48	F	White	9.2	30.6	0.0	0.0
Squamous Cell Carcinoma	Nivolumab	Unknown	66	F	White	4.5	NA	18.2	NA
Squamous Cell Carcinoma	Nivolumab	Unknown	68	F	White	4.9	6.8	2.4	18.6

Conclusions

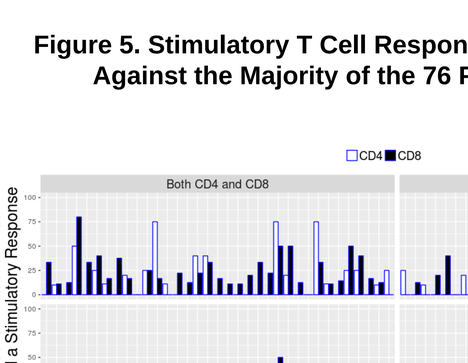
- ATLAS is a high-throughput screening platform that is capable of empirically interrogating recall CD4⁺ and CD8⁺ T cell responses across HLA-diverse individuals.
- Stimulatory CD4⁺ and CD8⁺ T cell responses to multiple TAAs were detected in lung cancer patients undergoing ICI therapy.
- Multiple TAAs induced responses that were "inhibitory" in these subjects.
  - Most, but not all, were from CD8⁺ T cells.
  - The mechanism of action and role in response or non-response to ICI therapy will be explored.
- Two TAAs induced more frequent stimulatory CD4⁺ T cell responses than previously described vaccine antigens, indicating these TAAs may represent candidates for the development of a therapeutic vaccine.
- Future steps include a longitudinal screening study of a larger sample of patients undergoing ICI therapy to examine:
  - Changes in response profiles to TAAs from pre- to post-therapy.
  - Differences in TAA response profiles of responders and non-responders.
    - Differences pre-therapy could lead to a biomarker panel for predicting response.
    - Differences post-therapy could lead to earlier detection of non-response.

Acknowledgements

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

Methods: The ATLAS™ Platform

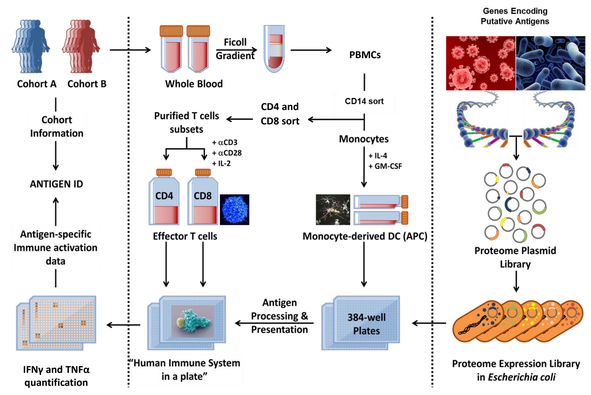
Figure 1. cLLO Facilitates MHC Class I* Presentation by MDDC



*MHC class II presentation to CD4⁺ T cells facilitated through conventional endocytic route of processing of *E. coli* not co-expressing cLLO.

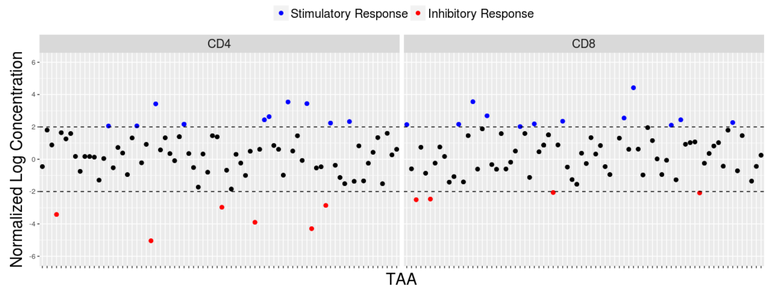
- 76 full-length TAA genes were cloned into the ATLAS expression vector and sequence verified. Each TAA was recombinantly expressed in *E. coli*.
- Blood samples were collected from 13 consenting patients undergoing ICI therapy.
- Peripheral blood mononuclear cells (PBMCs) were enriched by density gradient centrifugation. CD4⁺ and CD8⁺ T cells were sorted and non-specifically expanded, and monocytes were differentiated into monocyte-derived dendritic cells (MDDCs).
- Library clones were screened in duplicate using 500,000 *E. coli*, 1,000-5,000 MDDCs, and 80,000 T cells per well.
- Assay supernatants were harvested at 18-24hr and stored at -80°C.
- Supernatant cytokine levels were analyzed using a custom Meso Scale Discovery (MSD) 4-plex human kit.

Figure 2. ATLAS Technology Workflow



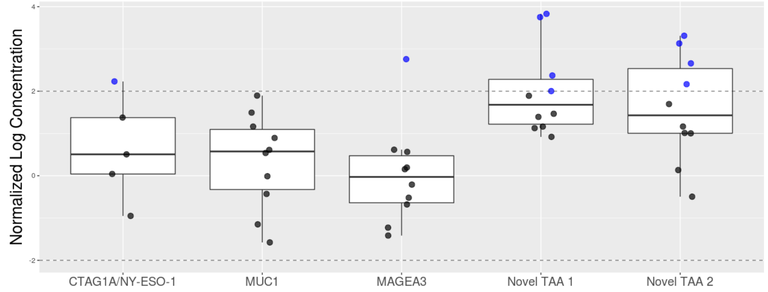
Results

Figure 3. Empirical Determination of Responses to Profiled TAAs



An exemplary lung cancer patient's IFN-γ CD4⁺ (left) and CD8⁺ (right) response profiles to 76 TAAs. Responses are reported as natural log cytokine concentrations back-calculated from the MSD standard curve and normalized to responses to a negative control protein. Each symbol represents a unique TAA. Responses are defined as TAAs that elicit a median response that exceeds two median absolute deviations (MADs) of the negative control replicates, indicated by the horizontal dotted line. Stimulatory responses (blue symbols) fall above the upper 2xMAD cutoff, Inhibitory responses (red symbols) fall below the lower 2xMAD cutoff.

Figure 4. Frequent CD4⁺ T Cell Responses to Novel TAAs Compared to Previously Described TAAs

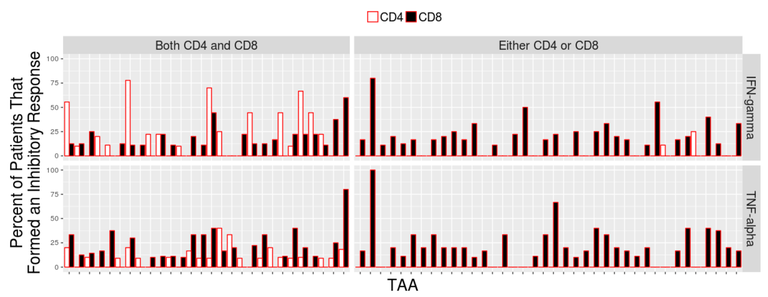


Across patients, IFN-γ CD4⁺ responses to two novel TAAs appear to be stronger than responses to NY-ESO-1, MUC1, and MAGEA3, three TAAs that have been utilized in cancer vaccines for lung cancer patients in clinical trials. Each point represents a patient's response to that TAA, normalized to the patient's response to an irrelevant negative control protein. Stimulatory responses, those that fall above the 2xMAD cutoff indicated by the upper horizontal dotted line, are colored blue. Both the median normalized concentration and the proportion of stimulatory responses to these two TAAs are higher than those of the three other TAAs. CD8⁺ responses to these five TAAs were more similar across patients (not shown).

Figure 5. Stimulatory T Cell Responses Are Observed Against the Majority of the 76 Profiled TAAs

The percent of patients that developed a stimulatory T cell response to each TAA is shown separately for CD4⁺ (white bars) and CD8⁺ (black bars) T cells. IFN-γ responses are displayed in the top two panels, and TNF-α responses are displayed in the bottom two panels. Antigens to which patients develop both a CD4⁺ and a CD8⁺ T cell response (left panels) are differentiated from antigens to which patients develop either a CD4⁺ or a CD8⁺ T cell response (right panels).

Figure 6. Inhibitory T Cell Responses Are Common Against Profiled TAAs and Are Detected More Frequently in the CD8⁺ T cell Subset



For each profiled TAA, the percent of patients that developed an inhibitory T cell response, defined as a response that is 2xMAD lower than the response to the negative control protein, is shown for CD4⁺ (white bars) and CD8⁺ (black bars) T cells. IFN-γ responses are displayed in the top two panels, and TNF-α responses are displayed in the bottom two panels. Antigens to which patients develop both a CD4⁺ and a CD8⁺ T cell response (left panels) are differentiated from antigens to which patients develop either a CD4⁺ or a CD8⁺ T cell response (right panels).