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ex vivo ATLAS™-identification of neoantigens for personalized cancer immunotherapy in mouse melanoma

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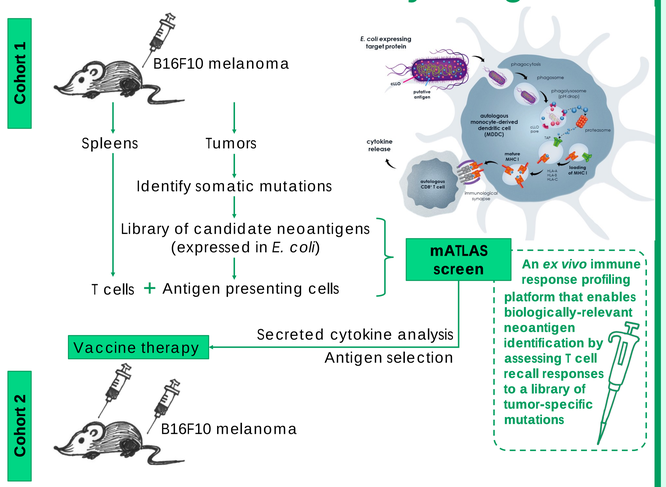
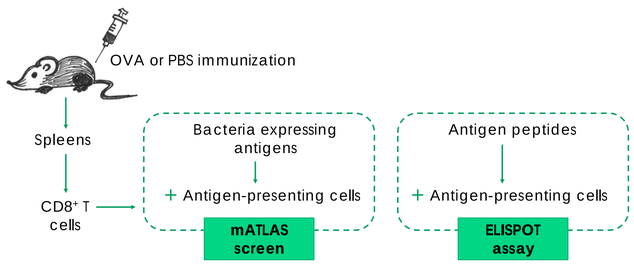
1. Mouse ATLAS Study Design

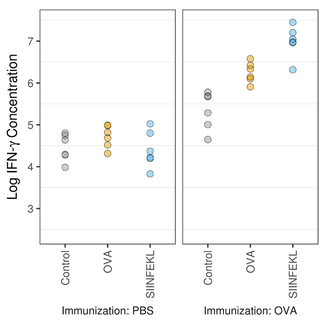


Tumors and spleens were collected from B16F10-bearing C57BL/6 mice. Tumor, cell line, and normal tissue were subjected to whole exome sequencing to identify somatic mutations that were synthesized as DNA, and transformed individually into bacteria with listeriolysin O (cLLO) for CD8⁺ T cell screening. Mouse antigen presenting cells (APCs) were cultured overnight with individual bacterial clones and subsequently cultured with T cells sorted from the spleens of B16F10 tumor-bearing mice. Supernatant was collected, and cytokine secretion was measured by MSD assay. Neoantigens were selected and vaccine manufacturing is currently in progress.

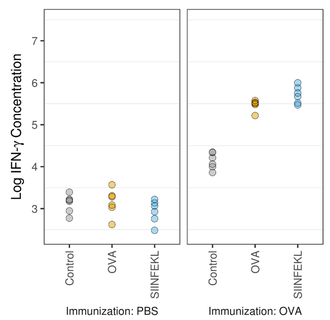
2. mATLAS Assay Validation



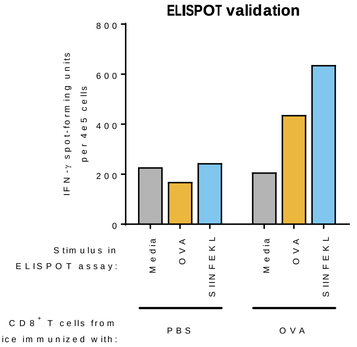
mATLAS validation: Dendritic cells



mATLAS validation: Macrophages



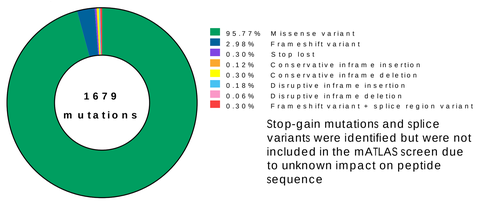
ELISPOT validation



Wildtype mice were immunized with full-length chicken ovalbumin (OVA) protein with an adjuvant or phosphate-buffered saline (PBS) control. CD8⁺ T cells were sorted and cultured from resected spleens and utilized in ELISPOT and mATLAS assays. The ELISPOT assay was performed with mouse dendritic cells and either full-length OVA or the SIINFEKL peptide (a well characterized CD8⁺ T cell epitope within the OVA protein). The mATLAS assay was performed with either mouse dendritic cells or mouse macrophages and bacteria expressing either full-length OVA, the SIINFEKL peptide, or a background control.

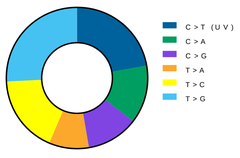
3. B16F10 Mutations

Types of mutations identified and screened



Stop-gain mutations and splice variants were identified but were not included in the mATLAS screen due to unknown impact on peptide sequence

Types of single-nucleotide variants identified and screened

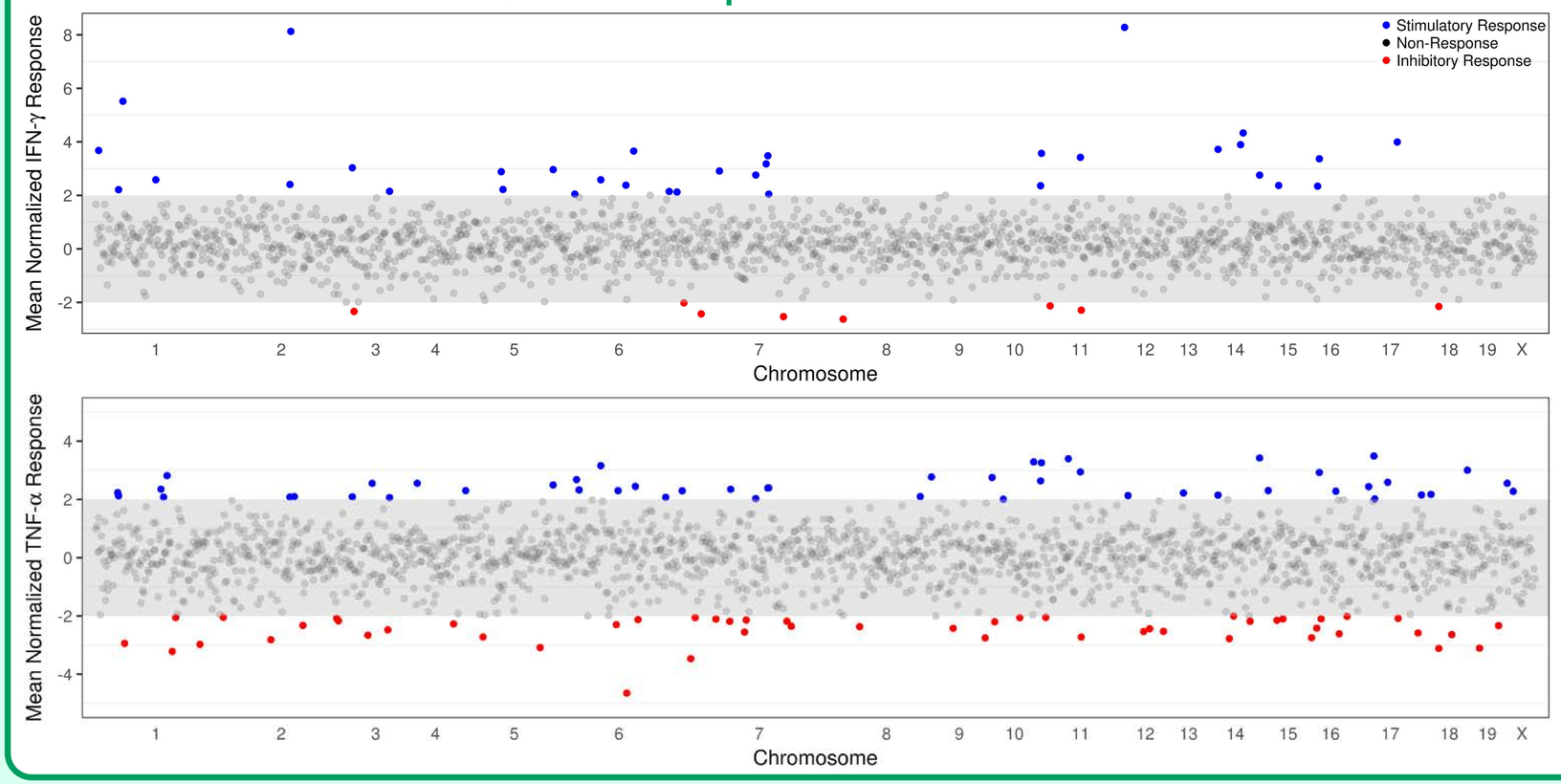


Examples of known mutations identified and screened

Oncogenes	Tumor suppressors	Immunogenic neoantigens*
Braf	Pten	Obsl1
Fgfr2	Trp53	Pbk
	Brca2	

*B16F10 mutations shown to be immunogenic for CD8 T⁺ cells by S. Kreiter et al, Nature 520 (30 April 2015)

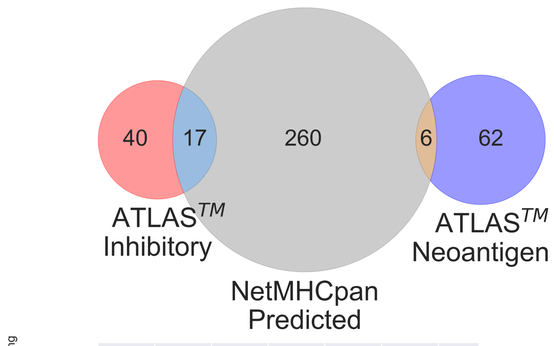
4. mATLAS CD8⁺ T Cell Responses to B16F10 Mouse Melanoma Mutations

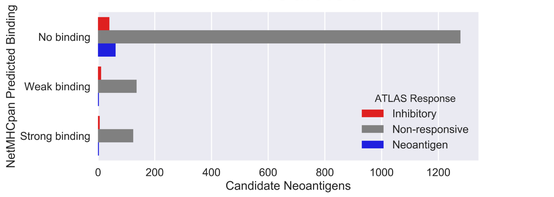


mATLAS assay:
Mouse antigen presenting cells were cultured with bacterial clones expressing individual B16F10 candidate antigens and were subsequently cultured with T cells sorted from the spleens of B16F10-bearing mice. Supernatant was collected, and cytokine secretion was analyzed by MSD assay. Replicates: candidate antigens N=2, background control N=18.

Empirical determination of responses to profiled candidate antigens:
For each MSD screening plate and cytokine, a multilevel model was fit to the log-transformed data. The estimated mean response to a background control protein was subtracted from the mean response to each candidate neoantigen and the estimated residual standard deviation was divided by this difference to arrive at a normalized log cytokine concentration. Normalized cytokine concentrations can be interpreted as standard deviations above or below the background control. Values greater than 2 were deemed "stimulatory responses" (blue points), and values less than -2 were deemed "inhibitory responses" (red points).
IFNγ responses: 2.0% stimulatory, 0.5% inhibitory
TNFα responses: 2.9% stimulatory, 3.1% inhibitory
Response by both cytokines: 0.9% stimulatory, 0.1% inhibitory

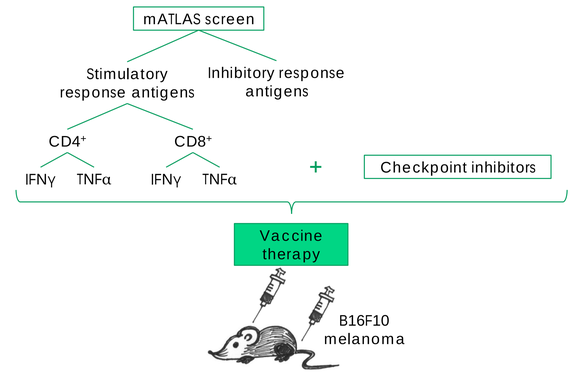
5. mATLAS vs. Algorithm Prediction





mATLAS screen results were compared to the NetMHCpan 4.0 algorithm that predicted binding affinity of peptide sequences to H2-K^b and D^b molecules from C57BL/6 mice. Strong binding was defined as <150nM and weak binding as <500nM. Venn diagram depicts all binding for NetMHCpan predicted, <500nM.

6. Future Mouse Research Studies



1. Explore preclinical vaccine studies with mATLAS-identified stimulatory response antigens
2. Explore the mechanism of mATLAS-identified inhibitory response antigens
3. Determine the effect of mATLAS-identified vaccine therapy in combination with checkpoint inhibition
4. Interrogate the balance of mATLAS-identified CD4⁺ and CD8⁺ stimulatory antigens for vaccine therapy
5. Examine the importance of magnitude of preexisting antitumor T cell response for vaccine therapy
6. Assess the impact of mATLAS-identified dual cytokine T cell responses

Conclusions

- Mouse ATLAS identified stimulatory and inhibitory neoantigens, analogous to recent human ATLAS neoantigen screening data
  - 99% of identified mutations in B16F10 mouse melanoma were screened
  - 68 (4%) stimulatory and 57 (3%) inhibitory neoantigens were identified
- MHC-binding prediction algorithms fail to identify the majority of mATLAS-identified stimulatory neoantigens
  - 2% of neoantigens predicted by algorithms were empirically confirmed
  - 91% of mATLAS-identified stimulatory neoantigens were not predicted
  - 6% of algorithm-predicted neoantigens were inhibitory
- Additional studies will explore the efficacy of mATLAS-identified neoantigen vaccines and the mechanism of mATLAS-identified inhibitory response antigens
- Phase 1/2a clinical development of GEN-009, a targeted personalized cancer vaccine using ATLAS-identified neoantigens, is expected to begin in 2H 2018