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Using Big Data and Machine Learning to Understand T cell Dysfunction in Human Tumors

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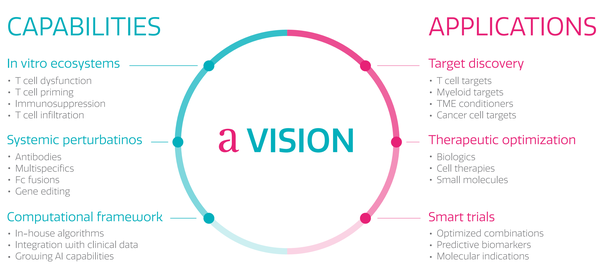
Background

Immune checkpoint blockade (ICB) elicits durable responses in some cancer patients, but novel targets and combination approaches are needed to address resistance and broaden clinical benefit. Agenus is addressing this need with our Virtual Systems for Immuno-Oncology (VISION) platform. VISION is based on a collection of in vitro ecosystems which we deeply and systematically interrogate to discover novel targets, optimize our therapies and design smarter clinical trials. Here, we present deep-learning model to predict response to anti-PD1 treatment in melanoma patients using disease relevant gene signatures acquired through VISION platform.

Agenus VISION platform supports smart, streamlined drug discovery and development



Methods

We developed a long-term human co-culture system comprised of primary T cells and cancer cells that enables controlled differentiation of naïve T cells to effector, memory and dysfunctional states. We longitudinally monitored T cell effector functions, protein and RNA expression across states and single cells. We identified gene signatures associated with each state. Parallelly, bulk RNAseq data from a cohort of melanoma patients treated with anti-PD1 was used for training and testing a deep neural network to differentiate responders and non-responders. We used a combination of gene signatures acquired from differential gene expression analysis of responders vs non-responders with VISION T cell state signatures to create a feature set for training and testing the model.

Results

T cells in our system become activated and then gradually progress to a terminally dysfunctional state driven by multiple cancer antigen exposures. T cell cytotoxicity is maintained over several antigen exposures before sharply decreasing

whereas cytokine secretion begins to decrease with only one prior antigen exposure. The expression of known T cell regulators and novel factors is altered over the time course, with known factors reflecting previous observations in vivo. This physiologically relevant system becomes the basis of a deep learning approach to develop a classifier model to differentiate responders and non-responders. Using the Agenus VISION T cell state signatures, we are able to train a binary classifier with high accuracy.

Conclusions

These findings demonstrate the potential of VISION systems to deeply interrogate response and resistance to current and next-generation I-O therapies. In this case, using deep learning neural networks and a physiologically relevant in-vitro T cell exhaustion system, we have defined a predictive biomarker signature for PD-1 response in melanoma. With a growing repertoire of virtual systems and capabilities, VISION is poised to advance Agenus' multi-faceted approach to fighting cancer with immunotherapy.

Disclosures
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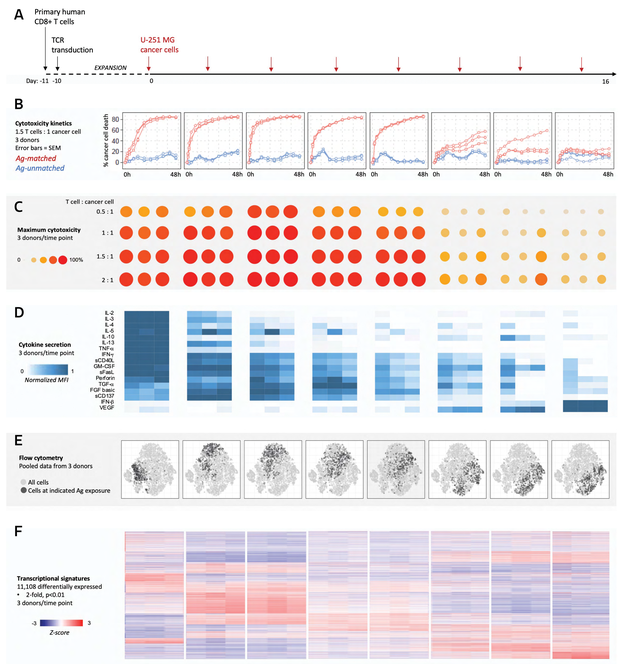
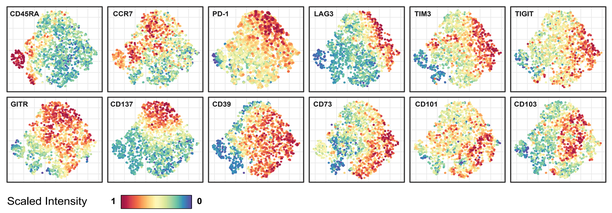
Figure 1. Chronic cancer antigen exposure drives CD8+ T cell dysfunction in vitro



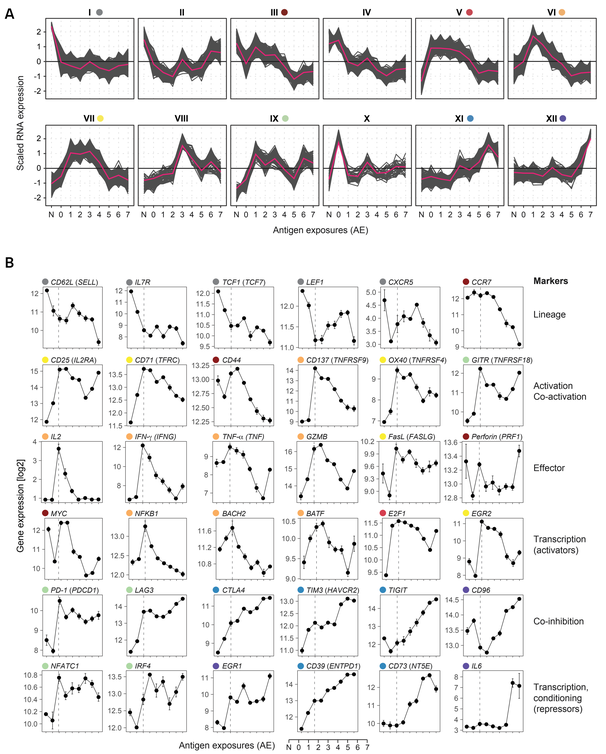
A) Method for driving T cell dysfunction in vitro. T cell preparation: PBMCs were isolated from three healthy donors on a Ficoll gradient and rested overnight. CD8+ T cells were isolated on magnetic beads, stimulated with CD3/CD28, transduced with NY-ESO-1 lentivirus, and expanded. TCR expression was confirmed by flow cytometry. Cancer cell preparation: U251 MG cell lines were transduced with lentivirus encoding a fusion protein of b2-microglobulin, HLA2-A2 and NY-ESO-1 or MART-1 peptide and selected on blasticidin. Antigen expression was confirmed with an NFAT reporter system in Jurkat T cells. For co-culture: A fixed number of irradiated U251 MG cells were cultured for 24h. CD8+ T cells were added to achieve the desired cancer:T cell ratio. The co-culture was monitored every 24h. If cancer cells were depleted, CD8+ T cells were transferred to a fresh well of irradiated U251 MG cells and the remainder were collected for downstream analyses. If cancer cells were not depleted, media was changed and reassessed the following day. **B)** *Cytotoxicity kinetics as monitored by live cell fluorescence microscopy.* **C)** *Maximum cytotoxicity across four T cell : cancer cell ratios.* **D)** *Secreted effector molecules measured by Luminex Bead Array, normalized to the maximum signal per molecule.* **E)** *tSNE visualization of flow cytometry measurements for twelve proteins related to T cell differentiation and function.* **F)** *RNA expression for 11,108 differentially expressed genes.*

Figure 2. Chronic cancer antigen exposure drives expression of activation and dysfunction markers on CD8+ T cells



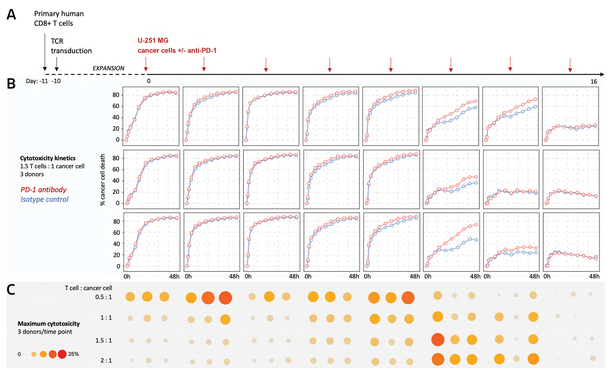
tSNE visualizations with false color representation indicating expression of individual proteins related to T cell differentiation and function.

Figure 3. Chronic cancer antigen exposure drives transcriptional signatures of activation and dysfunction in CD8+ T cells



A) K-means cluster profiles, with mean cluster expression shown in red and individual genes in grey. **B)** *Normalized log2 RNA expression for selected genes. Error bars = SEM across three donors.*

Figure 4. Chronic cancer antigen exposure ultimately drives CD8+ T cells to a anti-PD-1 refractory state



Correlation of Objective Response Rate of anti-PD1/PDL1 treatment in human tumors to A) *Tumor Mutation Burden,* **B)** *Fraction of PD1 high patients,* **C)** *CD8+ T-Cell Abundance,* **D)** *Univariate Agenus VISION signature,* **E)** *Bivariate Tumor Mutation Burden and Agenus VISION signature and* **F)** *Prediction of ORR by molecular subtypes using the bivariate model*

Figure 5. VISION gene signatures correlate with Objective Response Rate in anti-PD1/PDL1 treatment

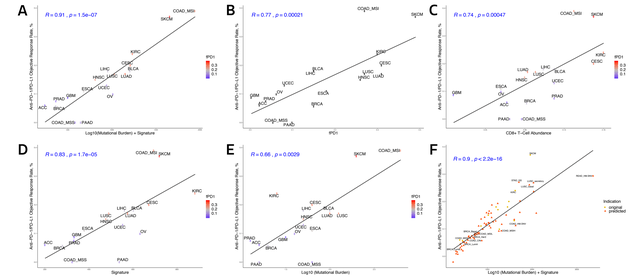


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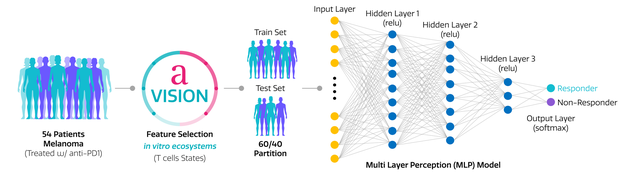
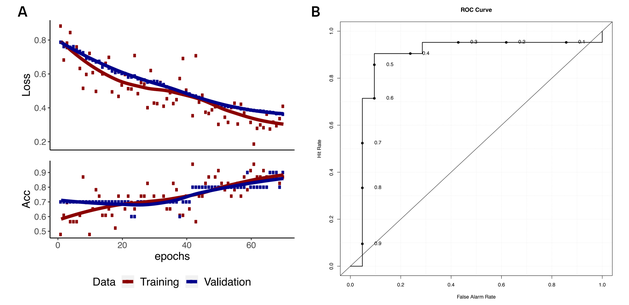
Figure 6. A deep-learning model to differentiate responders of anti-PD1 treatment in melanoma



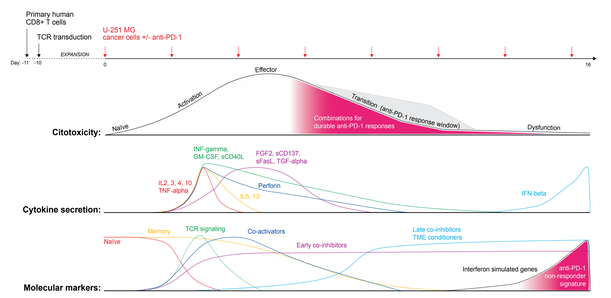
Layout describing development of a deep learning model to differentiate responders from non-responders of anti-PD1 treated melanoma patients from Hugo et. al. and Riaz et. al. Feature selection comprised of differentially expressed genes between the two groups filtered by Agenus VISION T cell state signatures. Data partition for training and validation was followed by modelling and performance evaluation.

Figure 7. Evaluation of Agenus deep-learning model performance



Performance evaluation of the model A) *Training and validation loss (top) and accuracy (bottom)* **B)** *ROC curve representing accuracy of the model with highest test accuracy of 90% in predicting responders and non-responders in melanoma patients treated with anti-PD1.*

Figure 8. I-O target discovery and translational insight from deep, integrative profiling of the tumor:immune interface in vitro



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