Poster # 70

Beyond PD-L1: novel PD-1 biomarkers identified by driving T cell dysfunction in vitro

Simarjot Pabla, Tenzing Khendu, Benjamin Duckless, Andrew Basinski, Cailin E. Joyce, Thomas Horn, Lukasz Swiech, David Savitsky, Jennifer S. Buell, Dhan Chand

[1]Agenus Inc. or subsidiary thereof (current or former employee), Lexington, MA

Agenus Inc. or subsidiary thereof (current or former employee), Lexington, MA 02421, USA.

Agenus VISION Platform

Integrated discovery & development platform drives concepts to clinic

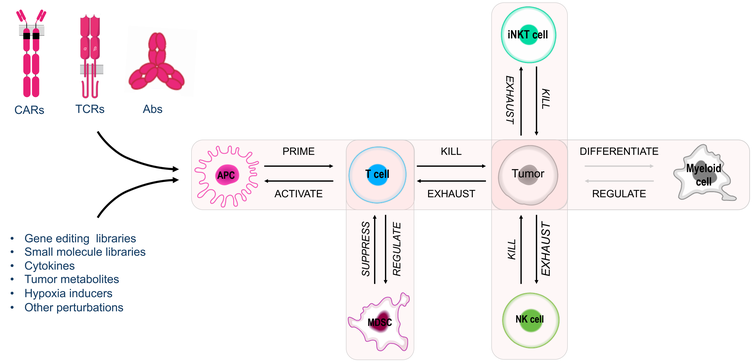

Figure 1: Representation of VISION platform as an integrated tool to recapitulate tumor immune interactions allowing perturbations to interrogate concepts and accelerate them to clinic.

T cell dysfunction system drives CD8+ T cells to dysfunction through chronic cancer antigen exposure

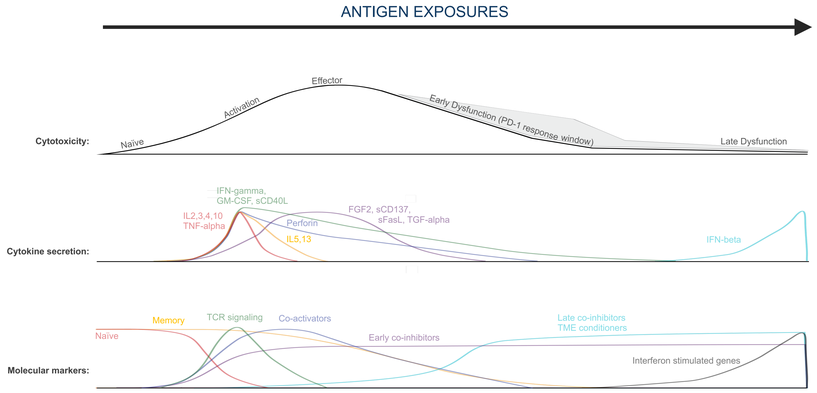

Figure 2: Method for driving T cell dysfunction in vitro. T cell preparation: PBMCs were isolated from three healthy donors on a Ficoll gradient and rested overnight. CD8+ T cells were isolated on magnetic beads, stimulated with CD3/CD28, transduced with NY-ESO-1 lentivirus, and expanded. TCR expression was confirmed by flow cytometry. Cancer cell preparation: U251 MG cell lines were transduced with lentivirus encoding a fusion protein of b2-microgobulin, 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. **Top Panel)** Cytotoxicity kinetics as monitored by live cell fluorescence microscopy. **Middle Panel)** Secreted effector molecules measured by Luminex Bead Array, normalized to the maximum signal per molecule. **Bottom Panel)** Transcriptomic landscape of T cell dysfunction across antigen exposures.

VISION PLATFORM PROVIDES CLINICAL INSIGHTS

VISION T cell dysfunction signature correlate with Objective Response Rate in anti-PD1/PDL1 treatment

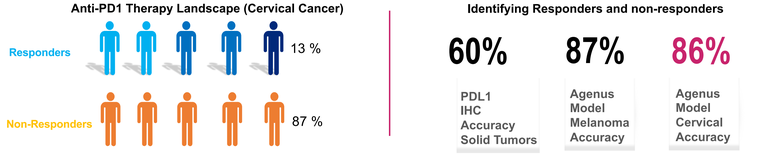

Figure 3: (Left Panel) Average response rate to anti-PD1 therapies. (Right Panel) Accuracy of predicting response to anti-PD1 on a per patient basis using either PD-L1 IHC or Agenus' VISION machine learning models using melanoma (Riaz et. Al and Hugo et. al) and cervical (Agenus C-550 and C-700) patients treated with anti-PD1.

VISION deep-learning model identifies anti-PD1 responders in melanoma and cervical cancer

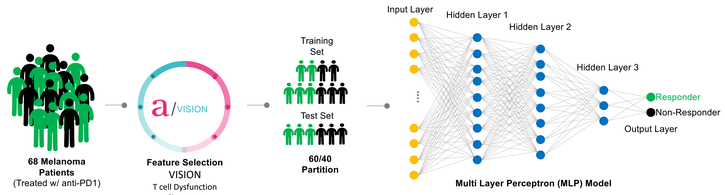

Figure 4: Development of a deep learning model to differentiate responders from non-responders in a population of anti-PD1 treated melanoma patients. Melanoma was selected for initial training and validation due to the availability of public, clinically-annotated tumor RNA-seq datasets from large numbers of patients (Hugo et al. (n=25) and Riaz et al. (n=43)). Feature selection comprised of Agenus VISION T cell state signatures combined with differentially expressed genes between the two groups. Data partition for training and validation was followed by modelling and performance evaluation.

VISION T cell dysfunction signature correlates with Objective Response Rate in anti-PD1/PDL1 treatment across indications

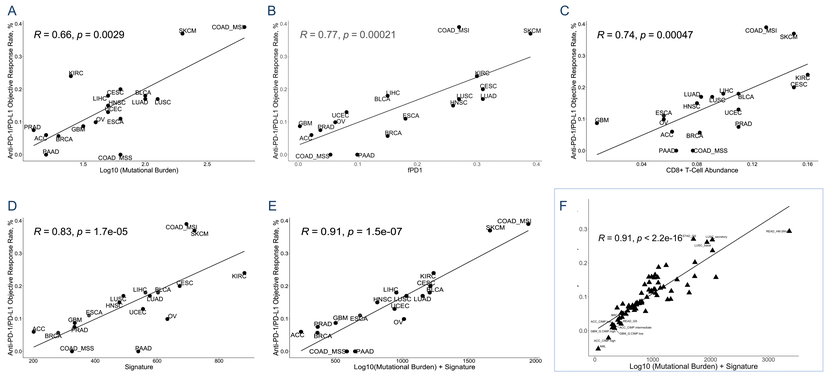

Figure 5: Correlation of Objective Response Rate of anti-PD1/PDL1 treatment in human tumors to **A)** Tumor Mutation Burden, **B)** Fraction of PD1 high patients (fPD1), **C)** CD8+ T-Cell Abundance, **D)** VISION T cell dysfunction signature, and **E)** Bivariate Tumor Mutation Burden and VISION T cell dysfunction signature.

F) Prediction of ORR for TCGA molecular subtypes using the bivariate model. TCGA indications were split into previously defined molecular subtypes (e.g. for BRCA - HER2, LumA, LumB, and basal) to validate correlations seen at the indication-level.

VISION PLATFORM DEFINES RATIONAL COMBINATIONS

Single cell transcriptomics of T cells during early dysfunction identifies T cell subsets that respond to anti-PD1

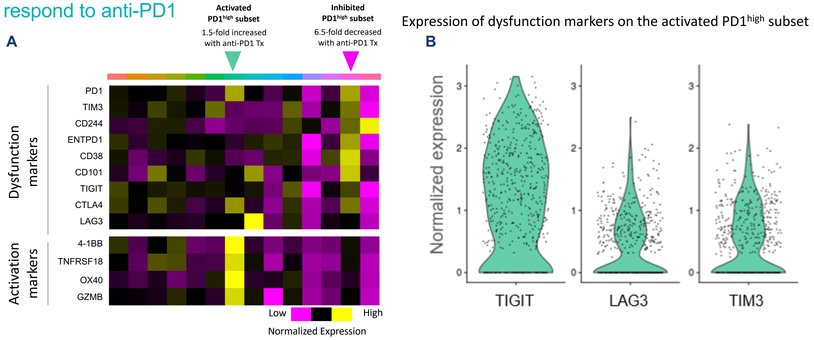

Figure 6: A) Heatmap showing mean expression of select activation and dysfunction markers based on single cell RNA-sequencing across 14 T cell clusters that are present during the early dysfunction state. (B) Violin plot depicting single cell expression levels for select dysfunction markers within the activated PD1high subset.

Combination of PD-1 and TIGIT blockade enhanced T cell cytotoxicity of tumor cells relative to monotherapies

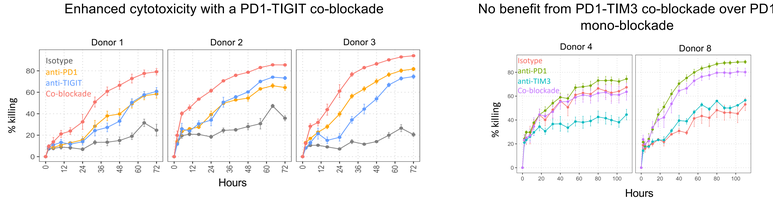

Figure 7: A) Tumor killing capacity of anti-PD1 and co-blockade **B)** Tumor killing capacity of PD1 and anti-TIM3 co-blockade.

Conclusions

- **Agenus' VISION platform combines deep in vitro profiling and AI-based approaches to predict clinical outcomes, plus rational targets & combinations.**
- **We defined a predictive biomarker signature that outperforms standard PD-L1 IHC.**
- **We identified a potential mechanism underlying the effective combination of anti-PD1 and anti-TIGIT antibodies in the clinic.**

References:

- Riaz et. AL., Tumor and Microenvironment Evolution during Immunotherapy with Nivolumab.
- Hugo et. al., Genomic and Transcriptomic Features of Response to Anti-PD-1 Therapy in Metastatic Melanoma. Cell. 2016 Mar 24;165(1):35-44.
- Morrison, C., Pabla, S., Conroy, J.M. et al. Predicting response to checkpoint inhibitors in melanoma beyond PD-L1 and mutational burden. j. immunotherapy cancer 6, 32 (2018).

Correspondence:

Simarjot Pabla
simarjot.pabla@agenusbio.com

Dhan Chand
dhan.chand@agenusbio.com

Presented at Society for Immunotherapy of Cancer (SITC), November 10 - 15, 2020, Virtual Meeting