Characterization of the Immune Landscape and Analysis of Tumor Response After Anti-PD-1 Blockade in a 3D *Ex-Vivo* System of Non-Small Cell Lung Cancer

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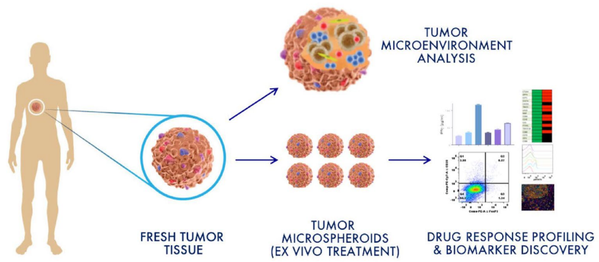
Nilogen's Drug Discovery Platform



Background

- Cell lines and animal models have had limited success in translation and recapitulation of the tumor microenvironment.
- Nilogen Oncosystems' 3D-EX^{SM} *ex vivo* drug screening platform utilizes fresh patient tumor tissue embedded in its natural environment allowing for an accurate measure of response to immunotherapeutics and correlates can be found among different platforms.

Materials and Methods

- 3D *ex-vivo* studies were performed with fresh tumor tissue obtained from consented patients with non-small cell lung cancer (NSCLC), all experimental protocols were approved by the Institutional Review Board (IRB).
- 3D tumor spheroids were treated in their intact immune microenvironment with the PD-1 inhibitor Keytruda (pembrolizumab) at 10ug/mL for 48 hours.
- Culture media was collected over the course of the experiments to simultaneously analyze the differential release of cyto- and chemokines.
- Treatment-mediated changes in T-cell activation, checkpoint proteins and immune cell populations were monitored by flow cytometry and NanoString's PanCancer Immune Profiling platform.

Results

- *Ex-vivo* treatment of the NSCLC 3D spheroids with Keytruda, showed significant changes in the CD8 and CD4 T-cell populations and their activation status via flow cytometry in anti-PD-1 responders.
- Upon cytokine analysis, the T-cell activation seen via flow cytometry is further exemplified by the simultaneous increase in IFN-γ and TNF-α in anti-PD-1 responders.
- Furthermore, using advanced Nanostring analysis of signature genes that are related to various immune cell subsets including, but not limited to, cytotoxic T-cells, CD4 T-cells, NK cells, we observed that these immune cell subsets increase in response to Keytruda treatment.

Flow Cytometric Analysis

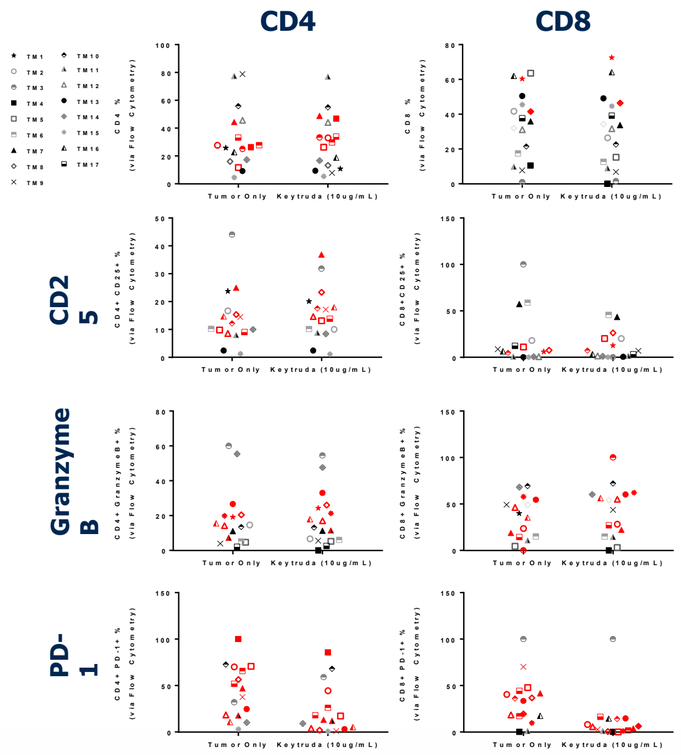


Figure 1. Flow cytometric analysis of T-cell activation, in Keytruda treated 3D spheroids. 3D spheroids derived from fresh patient NSCLC tumors (17) treated or untreated with Keytruda (10ug/mL) for 48 hours. At 48 hours, expression of CD3+/CD4+ or CD3+/CD8+, CD25 (proliferation marker), Granzyme B (T-cell activation marker), and PD-1 occupancy were assessed. Each symbol is one patient, responders (in red) and non-responders (black/grey). Significant changes were seen in the expression of CD25 and Granzyme B in both CD4 and CD8 T-cells. PD-1 occupancy can be observed, demonstrating Keytruda binding to its target protein.

Cytokine Analysis

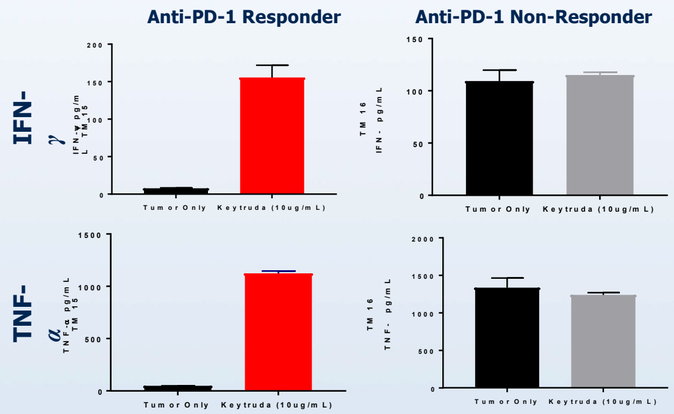


Figure 2. Multiplex analysis of cytokines in Keytruda treated 3D spheroids. 17-plex cyto-chemokine analysis was performed. Expression of IFN-γ and TNF-α in an anti-PD-1 responding vs non-responding patient. Culture media obtained from *ex vivo* experiments were analyzed using the Bioplex Multiplex Assay for cytokine secretion. All experiments were performed in duplicate, and the means and standard deviations were plotted. Combination of Phorbol myristate acetate (PMA) and Ca^{2+} ionophore (I) was used as positive control to activate TILs (data not shown).

Gene Expression Analysis

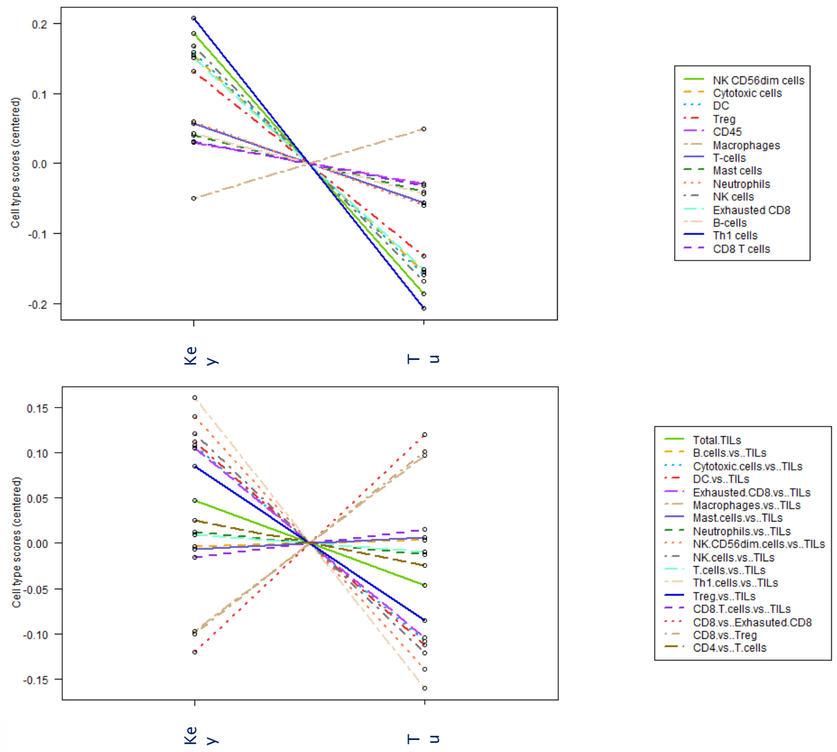


Figure 3. Immune gene expression analysis in Keytruda treated 3D spheroids. Advanced Nanostring analysis data shows trend plot cell type scores of of three patients that responded to anti-PD-1 treatment vs three non-treated patients.

Summary

- Nilogen Oncosystems' 3D-EX^{SM} platform can accurately assess the response of the tumor microenvironment after treatment in NSCLC.
- Increased T-cell activation closely correlates with genes involved in T-cell activation and an increase in pro-inflammatory cytokines like IFN-γ and TNF-α.
- Nilogen Oncosystems' 3D-EX^{SM} platform provides of a comprehensive analysis of the tumor immune microenvironment for a better understanding of the mechanism of action of immuno-oncology drugs that may aid in developing biomarkers that can be used for patient selection.