A novel 3D *ex vivo* platform of fresh patient tumor tissue with intact tumor microenvironment for immuno-oncology drug development and biomarker discovery

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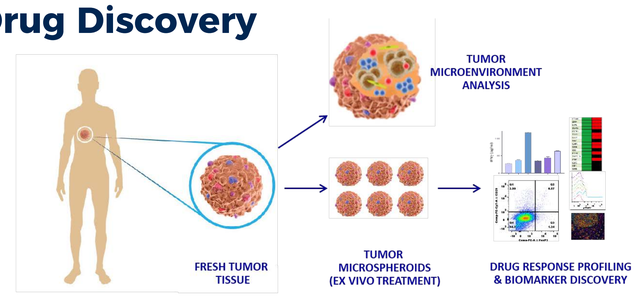
Introduction

- The tumor microenvironment is complex and unique for each tumor, hence not every patient responds to the same immunotherapy.
- Among the most promising approaches in activating therapeutic anti-tumor immunity is the blockade of immune checkpoints.
- Cell lines and humanized mouse models provide limited information and there is an unmet need to recapitulate the microenvironment of patient tumors.
- Nilogen's 3D *ex-vivo* platform allows the simultaneous analysis of different components of the intact tumor microenvironment.

Nilogen's Drug Discovery Platform



Methods

- 3D *ex-vivo* studies were performed with fresh tumor tissue obtained from consented patients with non-small cell lung cancer.
- 3D tumor microspheroids were treated in their intact immune microenvironment with the PD1 inhibitor Keytruda at 10mg/ml for 36 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 PanCancer Immune Profiling platform.
- Immunohistochemical studies were performed to identify PD-L1 expression and immune cell composition in tumor samples.

Results

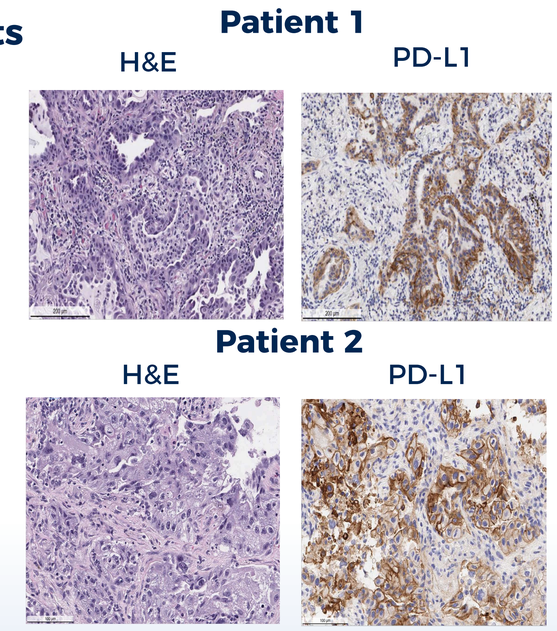


Figure 1. Characterization of immune checkpoint markers in lung cancer patient tumor samples. H&E and PD-L1 analysis of fresh tissue from two patients with adenocarcinoma of the lung.

Results

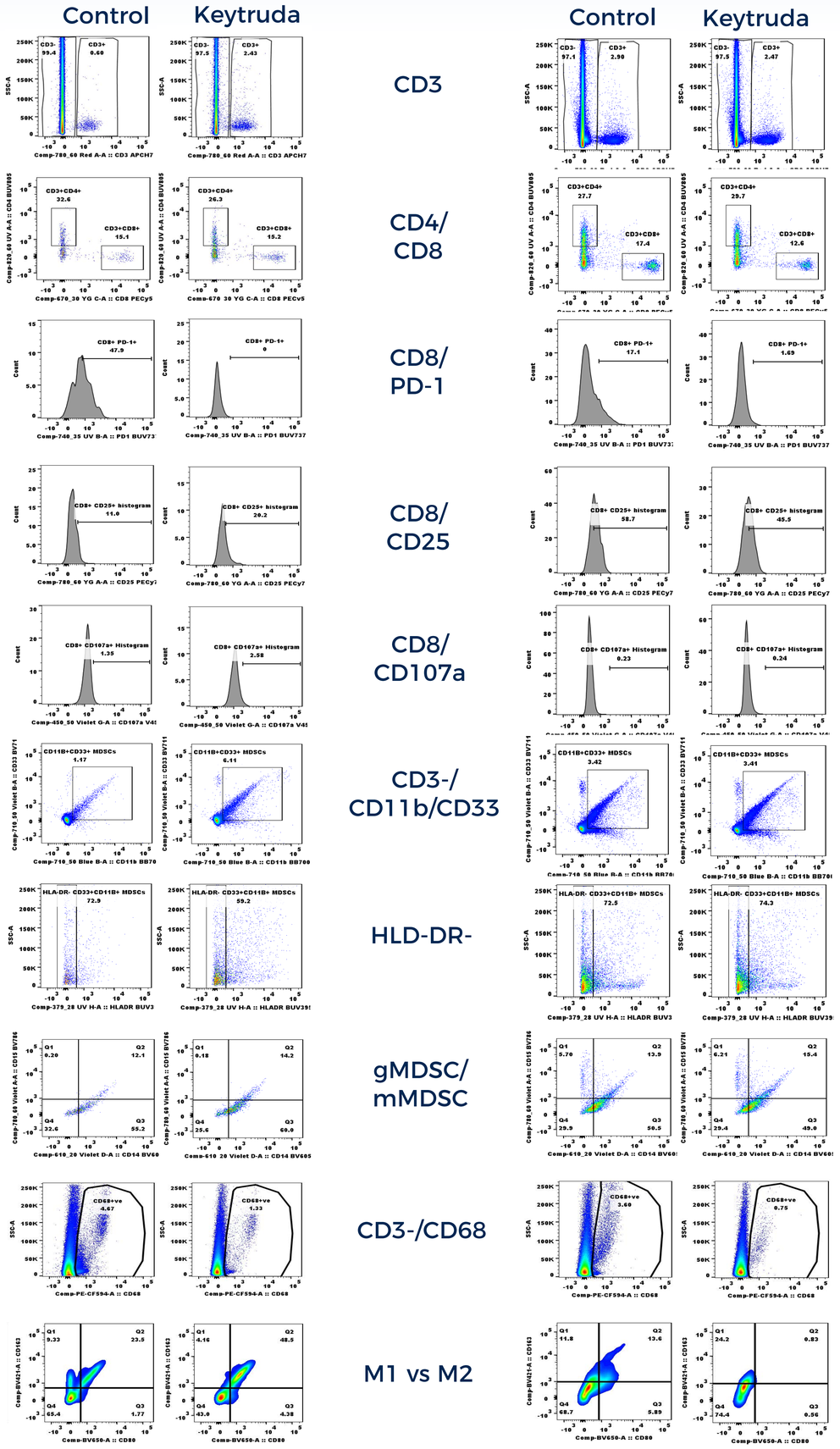


Figure 2. Flow cytometric analysis of T-cell activation, MDSC populations and macrophage polarization in Keytruda treated 3D microspheroids. Expression of CD3, CD4/CD8, T-cell activation markers in CD8 cells, MDSCs and macrophage polarization was performed after treatment with Keytruda in 2 patients. Patient 1 shows an increase in T-cell activation markers, as well as M1 macrophage polarization, while no significant changes were observed in patient 2. PD1 occupancy can be observed in both patients, demonstrating Keytruda binding to its target protein.

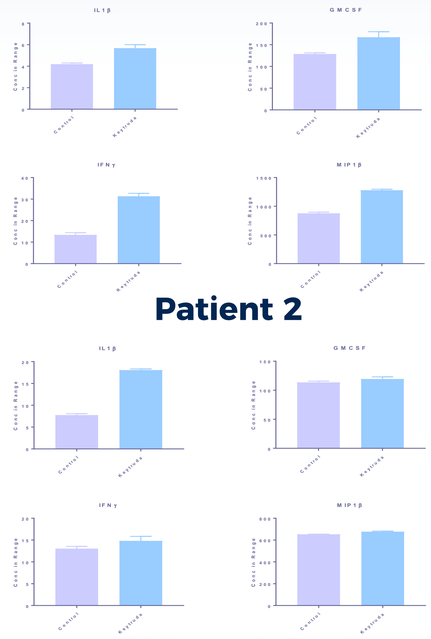


Figure 3. Multiplex analysis of cytokines in Keytruda treated 3D microspheroids. 17-plex cyto-chemokine analysis was performed. Expression of IL1β, GM-CSF, IFNγ, and MIP1β in two patients is shown. Culture media obtained from ex vivo experiments were analyzed using the Bioplex Multiplex Assay for cytokine 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).

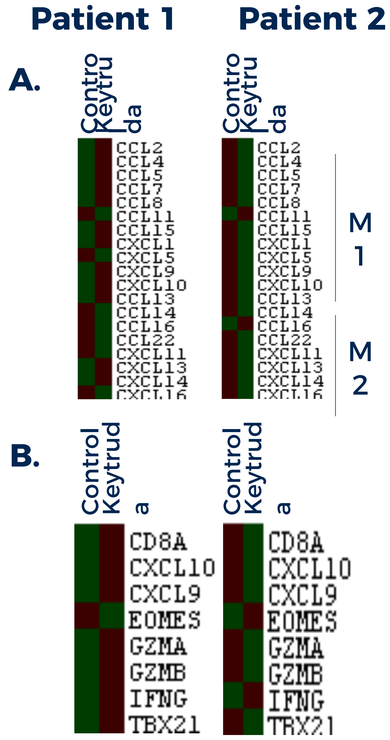


Figure 4. Immune gene expression analysis in Keytruda treated 3D microspheroids. (A) Expression of genes involved in M1 and M2 macrophage polarization in two patients. Patient 1 shows an increase in M1 genes when compared to patient 2. **(B)** Expression of key genes involved in T-cell activation in two patients. An increase in these genes is observed in patient 1 when compared to patient 2.

Summary

- Nilogen's *ex-vivo* system reliably demonstrates drugs effect on the tumor immune microenvironment in fresh patient samples.
- Keytruda treatment leads to T-cell activation, M1 macrophage polarization and changes in MDSC subpopulations in select patients. There was a close correlation between flow analysis, cytokine release and NanoString immune gene profiling.