# Integrated Comprehensive Analysis of Immune Cell subsets and Assessment of Keytruda Response in Head and Neck Squamous Cell Carcinoma, Urothelial Cell Carcinoma, and Renal Cell Carcinoma *Ex-vivo*

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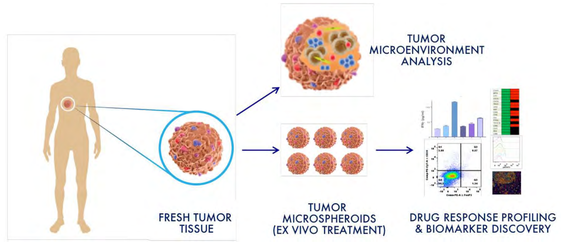
## Background

- PD-1 inhibitors show dramatic impact on subsets of tumors, which does not always correlate with PDL-1 expression.
- In this study, we used an integrated comprehensive strategy to interrogate tumor immune cell compositions and T-cell activation in intact tumors before and after *ex-vivo* treatment with the PD-1 inhibitor Keytruda (pembrolizumab) utilizing Nilogen Oncosystems' 3D-EX$^{SM}$ drug screening platform.

## Materials and Methods

- 3D *ex-vivo* studies were performed with fresh tumor tissue obtained from consented patients with H&N, RCC, and UC.
- 3D tumor spheroids were treated in their intact immune microenvironment with the inhibitor anti- PD-1- Keytruda (10ug/mL) for 48 hours. At 48 hours, the phenotype (activation/proliferation/checkpoint) of the tumor immune microenvironment was assessed via flow cytometry.
- 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.

## Nilogen's Drug Discovery Platform




## Summary

- There are dramatic changes in the immune cell composition in each tumor type.
- 3D-EX$^{SM}$ platform can successfully detect checkpoint inhibitor-mediated activation in T-cells within spheroids prepared from surgical samples obtained from fresh patient tumors.
- 3D-EX$^{SM}$ platform is versatile and provides valuable information on the mechanisms involved in drug sensitivity and resistance.
- This approach might aid in the development of rational mechanism-based combination strategies to block compensatory signalling mechanisms to impart clinical benefit.

## Cytokine Analysis

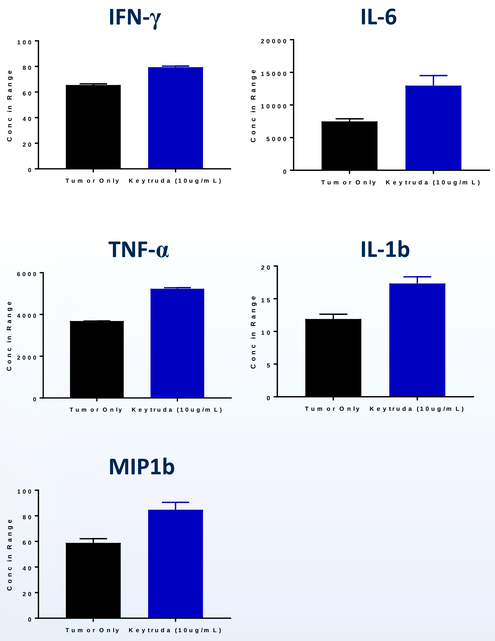



**Figure 1. Bioplex analysis of cytokines in Keytruda treated 3D tumor spheroids.** Expression of cytokines and chemokines IL1b, IL-6, IFN-γ, and MIP1b, and TNF-α renal cell carcinoma. Culture media obtained from *ex vivo*-treated 3D microspheres were analyzed using the Bioplex Multiplex Assay for cytokine secretion. All experiments were performed in duplicate, and the means and standard deviations were plotted. Combination of Phorbol myristate acetate (PMA) and Ca2+ ionophore (I) was used as positive control to activate TILs (data not shown).

## Gene Expression Profile


**Figure 2. Immune gene expression analysis in Keytruda treated 3D spheroids.** Data above is representative Nanostring gene expression data from renal cell carcinoma (similar data can be seen in urothelial cell carcinoma and head and neck squamous carcinoma), expression of key genes involved in T-cell activation and effector function as well as T-cell checkpoints.

## Flow Cytometric Analysis

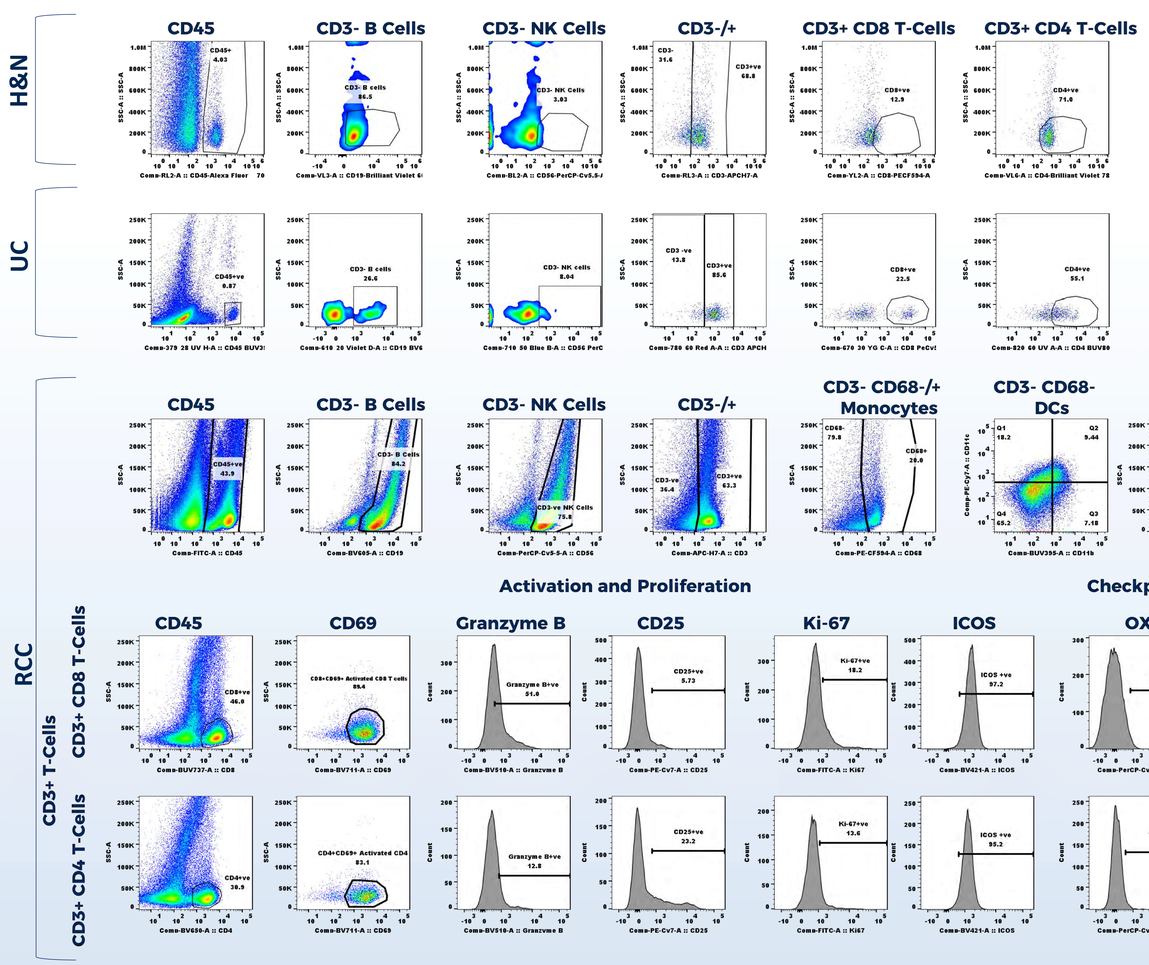



**Figure 3. Flow cytometric analysis of immune cell composition in tumors. One representative panel is shown for each tumor.** Comparison between head and neck squamous cell carcinoma, urothelial, and renal cell carcinoma revealed a significant heterogeneity in immune cell composition. Represented in the renal cell carcinoma, T-cell activation status and checkpoint expression between different tumor types as assessed by activation and proliferation of CD8 and CD4 T-cells was assessed by flow cytometry.