A novel *ex vivo* 3D tumor organoid model of fresh patient tumors (3D-ACT) to assess efficacy of cellular therapy in immuno-oncology

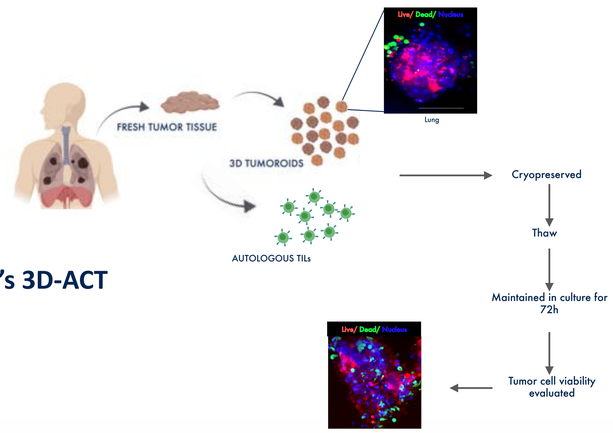
Vijayendra Agrawal Ph.D.[1], Mibel Pabon, Ph.D.[1], Tina Pastoor[1] , Jenny Kreahling, Ph.D.[1] and Soner Altiok, M.D., Ph.D.[1]

[1] Nilogen Oncosystems Tampa FL 33612

Background

- The spatial organization and dynamic interplay of the complex cell-to-cell interactions in patient tumors play an important role in cellular phenotypes that can result in permanent alterations in cellular functions and response to immuno-oncology (IO) treatments.
- To assess the therapeutic efficacy of IO treatments, including cellular therapeutics, it is imperative to develop models that preserve the stromal-stoichiometry of the tumor microenvironment.
- Nilogen's high content confocal imaging approach allows for a quantitative assessment of infiltration and target tumor cell killing activity of ex vivo expanded autologous tumor-infiltrating lymphocytes (TILs) in non-small cell lung cancer.

Materials and Methods



Nilogen's 3D-ACT

- **Tumor tissue procurement:** 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-ACT^{SM} platform:** Fresh tumor tissue obtained from patients were used to prepare 3D tumoroids and autologous tumor infiltrating lymphocytes (**TILs**). For the *ex vivo* assays, 3D tumoroids measuring 150 micron in size were prepared and cryopreserved during the process of *ex vivo* propagation of autologous TILs.
- **High Content Imaging:** Immune cell infiltration was evaluated by Nilogen's high-throughput, high-content technology.
- **Flow Cytometry:** TILs were characterized using multiparameter flow analysis, fluorescently labeled and exposed to fresh tumor organoids.
- **Multiplex Cytokine:** Culture media was collected over the course of the experiment to simultaneously analyze the differential release of cyto/chemokines.

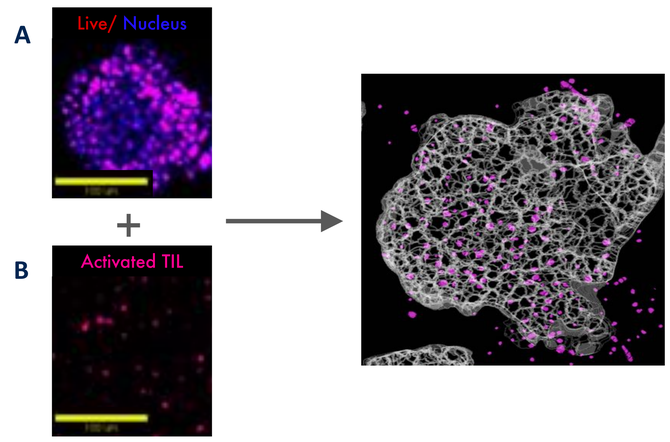


Figure 1. High Content Imaging of TIL infiltration in fresh patient 3D tumoroids. Tumoroids (A) and activated TILs (B) were incubated together in pre-determined ratio to see infiltration of TILs in tumoroids. The mesh structure shows the infiltration of TILs throughout the tumoroid.

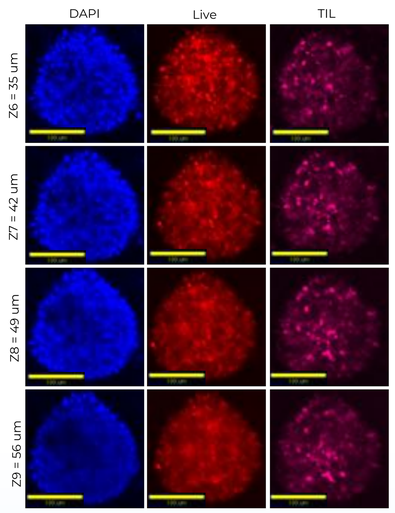


Figure 2. Confocal imaging of lymphoid cell infiltration into the autologous tumor organoids: Tumor organoids (Red) and activated TILs (Pink) were prepared from the same lung tumor tissue and were incubated together at 37C in the CO2 incubator for 72h. At the end point, the Tumoroid+ TILs were stained with nuclear marker (blue)) and 3D confocal images were taken. Images from 4 different z-planes shows infiltration of TILs throughout the tumoroid.

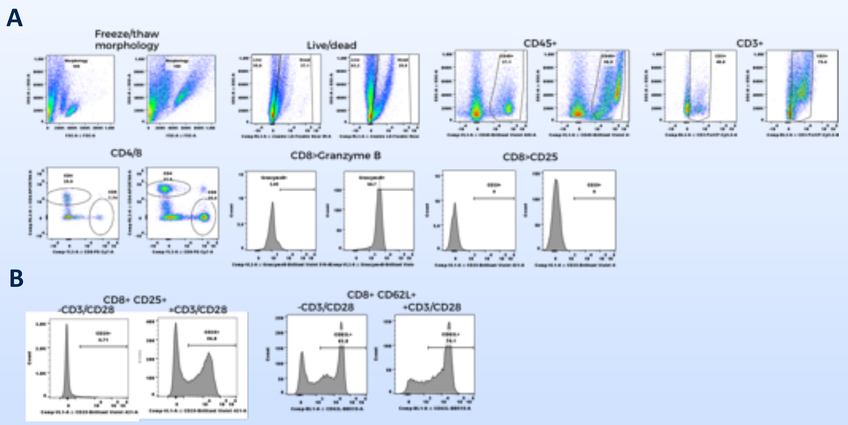


Figure 3. Flow cytometric analysis. 3D tumoroids and autologous TILs were derived from fresh patient tumors and assessed for viability and activation. **A.** Morphology and viability were assessed for both tumoroids (left) and TILs (right) after thawing **B.** TILs were incubated with +/- CD3/CD28 and activation was assessed by CD25 and CD62L activation markers, TILs treated with CD3/CD28 showed increased activation.

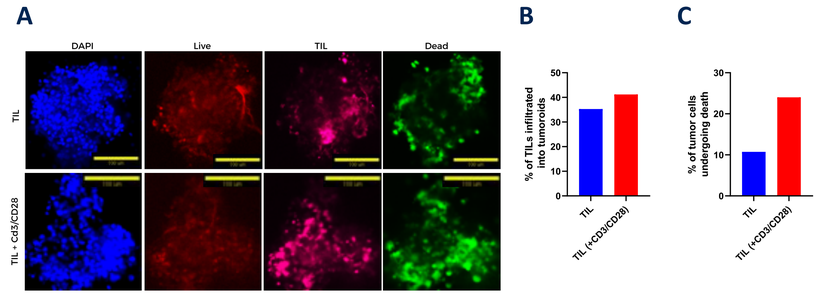


Figure 4. A. Tumoroids were incubated with TILs (with and without + CD3/CD28 stimulation). High through confocal 3D imaging showed increased infiltration of TILs and tumor cell killing as compared to controls. Computational confocal image analysis was done in 3D tumoroids to quantify the number of TILs infiltrated **(B)** and tumor cell killing **(C)**.

Results

- The characteristics of tumor immune microenvironment and tumor cell viability was evaluated in cryopreserved/thawed organoids using a custom image analysis algorithm that was developed for the collection of data in a structurally relevant environment on quantification of marker-specific cell number, cell viability and apoptosis in addition to structural and functional analysis of cells in intact 3D tumor organoids.
- High content confocal imaging analysis demonstrated that CD3/CD28 pre-activated TILs with increased activation phenotypes via flow cytometry and enhanced pro-inflammatory cytokine release (data not shown) had increased infiltration into the 3D organoids compared to untreated TILs. The data was correlated with quantitative tumor cell killing assessment for each tumor organoid.

Conclusion

- These results demonstrate that our 3D-ACT model using *ex vivo* expanded TILs and 3D tumoroids is an effective tool for the therapeutic assessment of autologous TILs.
- Additionally, this model can be used to assess efficacy of other cellular therapy applications in Immuno-oncology.
- Furthermore, implementation of this platform in the clinical studies may also allow determining the most effective combinatorial cellular therapy strategies for individual patients.