A Patient-driven *Ex Vivo* 3D Tumor Organoid Model to Assess Efficacy of Tumor Infiltrating T-cell Adoptive Cell Therapy

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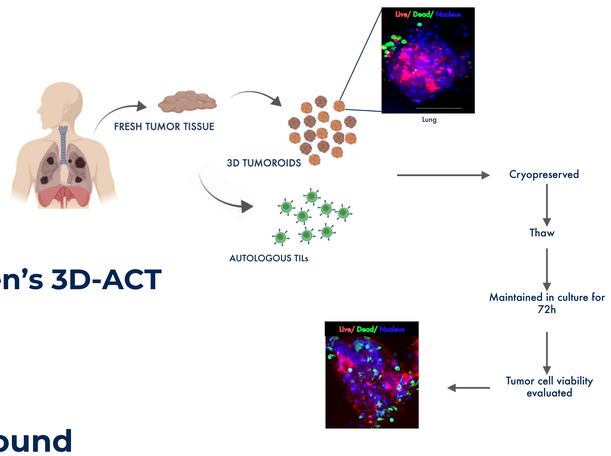

Nilogen's 3D-ACT

Background

- Adoptive cell transfer (ACT) of *ex vivo* expanded tumor-infiltrating lymphocytes (TILs) shows promising therapeutic efficacy in solid tumors.
- Improvement of anti-tumor efficacy of TIL ACT in solid tumors is critical to develop rational combination strategies and to identify biomarker(s) predictive of patients who would respond favorably to TIL therapy.
- We have developed a high content imaging approach using a fresh tumor organoid model with intact tumor stroma for quantitative assessment of autologous TIL infiltration and target tumor cell killing.

Materials and Methods

- **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 was used to prepare 3D tumor organoids and autologous tumor infiltrating lymphocytes (**TILs**). For the *ex vivo* assays, 3D tumor organoids measuring 150 microns in size were prepared and cryopreserved during the process of *ex vivo* propagation of autologous TILs.
- **High Content Imaging:** High content confocal analysis was used to quantify TILs infiltration into the tumor organoids and target tumor cell killing
- **Flow Cytometry:** TILs were characterized using multiparameter flow analysis, fluorescently labeled and exposed to fresh tumor organoids.
- **Multiplex Cytokine:** Culture media were collected over the course of the experiment to simultaneously analyze the differential release of cyto/chemokines.

Results

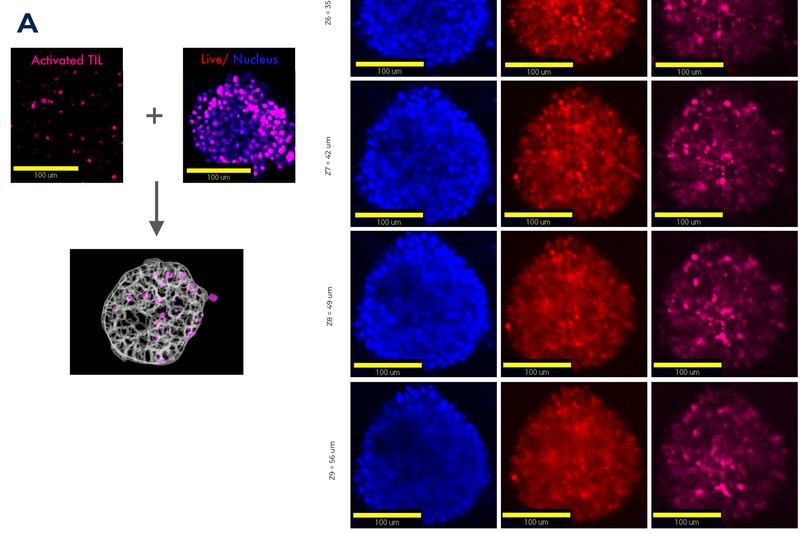

Figure 1. High Content Imaging of TIL infiltration in fresh patient 3D tumor organoids. A. Activated TILs were incubated together with 3D tumor organoids in a pre-determined ratio to see infiltration of TILs in tumor organoids. The mesh structure shows the infiltration of TILs throughout the tumor organoid. **B.** Tumor organoids (Red) and activated TILs (Pink) were prepared from the same lung tumor tissue and were incubated together at 37° C in the CO2 incubator for 72h. At the end point, the tumor organoid + TILs were stained with a nuclear marker (blue) and 3D confocal images were taken. Images from 4 different z-planes show infiltration of TILs throughout the tumor organoid.

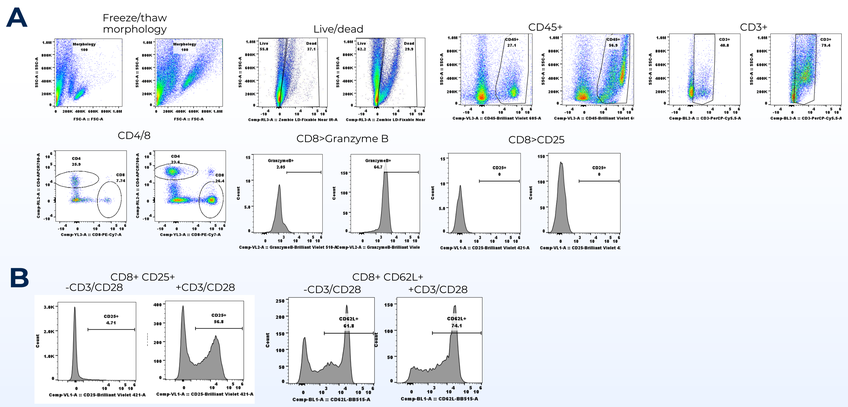

Figure 2. Flow cytometric analysis. 3D tumor organoids and autologous TILs were derived from fresh patient tumors and assessed for viability and activation. **A.** Morphology and viability were assessed for both tumor organoids (left) and TILs (right) after thawing **B.** TILs were incubated with +/- CD3/CD28 and activation was assessed by CD25 and CD62L activation markers. TILs treated with CD3/CD28 showed increased activation.

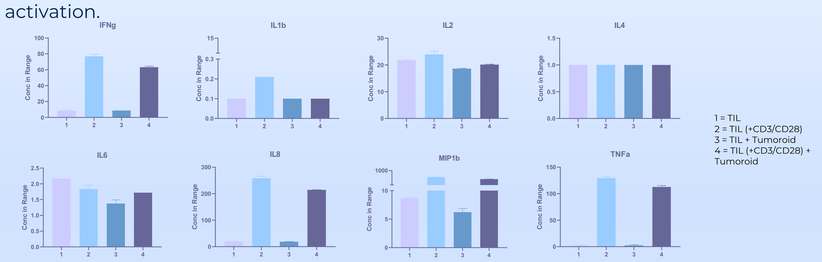

Figure 3. Cytokine analysis. 3D tumor organoids and autologous TILs were derived from fresh patient tumors and assessed for proinflammatory and anti-inflammatory cytokines.

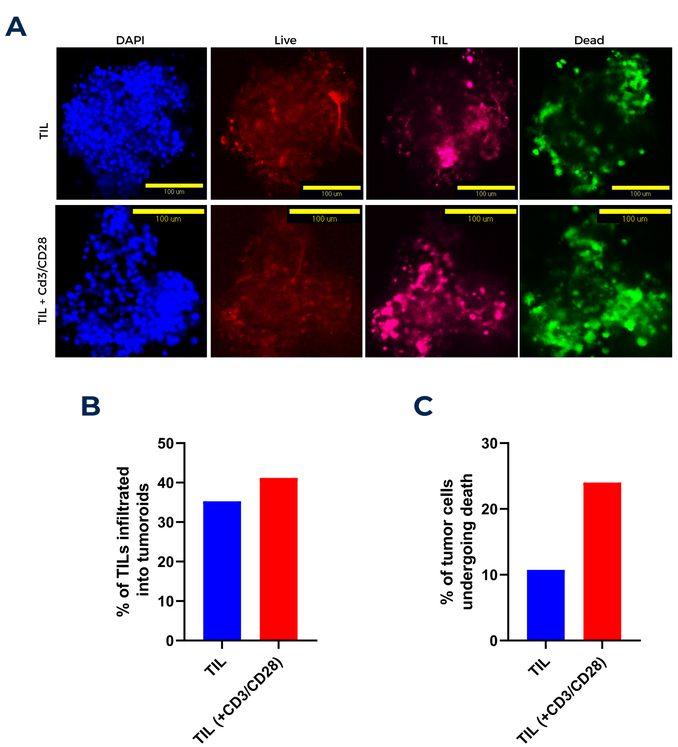

Figure 4. A. Tumor organoids were incubated with TILs (with and without CD3/CD28 stimulation). High through confocal 3D imaging showed increased infiltration of TILs and tumor cell killing as compared to controls. Computational confocal image analysis was done in 3D tumor organoids to quantify the number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

Summary & Conclusion

- We successfully prepared matched autologous TILs and unpropagated 3D tumor organoids from patient tumors.
- Using a custom image analysis algorithm, the characteristics of the tumor immune microenvironment and tumor cell viability were evaluated in previously cryopreserved tumor organoids. We were able quantify marker-specific cell number, cell viability and apoptosis.
- This analysis demonstrated that CD3/CD28 pre-activated TILs with increased activation phenotypes and enhanced pro-inflammatory cytokine release had increased infiltration into the 3D tumor organoids compared to untreated TILs and PBMCs. The data was correlated with a quantitative tumor cell killing assessment for tumor organoids.
- These results demonstrate that the 3D-ACT model using *ex vivo* expanded TILs and 3D tumor organoid models is an effective tool for the therapeutic assessment of autologous TILs and indicate that it can also be used to assess the efficacy of other cellular therapy applications.

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