# Assessing the impact of cGAS-STING pathway activation on adoptive cell therapy using a patient-derived 3D *ex vivo* tumoroid model.

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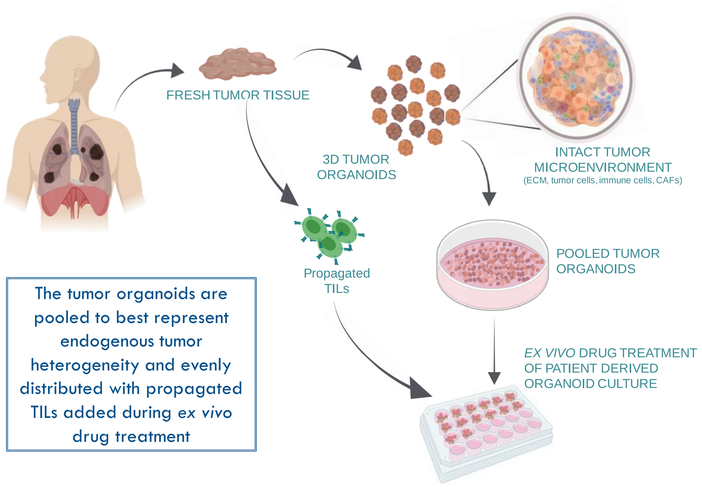


## Background

- 3D-ACT[SM] technology allows for the *ex vivo* testing of multiple cellular-based therapies alone or in combination with drugs for tumor cell killing and immune cell activation.
- Our proprietary processing method, which employs fresh patient derived tumor tissue, results in the generation of 3D tumor organoids that retain the intact tumor microenvironment. This model allows for accurate quantification of any drug-mediated changes to the complex cell-to-cell interactions within the tumor microenvironment.
- We have developed a high content imaging approach using a fresh patient 3D tumor organoid model with intact tumor stroma for assessment of adoptive cell therapy (ACT) effectiveness.

## Materials and Methods

- **Tumor tissue procurement:** 3D *ex vivo* studies were performed with fresh tumor tissue obtained from consented patients with colorectal (CRC) or kidney tumors (RCC). 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 for treatment with *ex vivo* expanded, cell tracker-labeled, tumor infiltrating lymphocytes (TILs) in combination with agonists of the cGAS-STING pathway – ADU-s100 and 2'3'-cGAMP, and the PD-1 inhibitor nivolumab (Nivo). For the *ex vivo* assays, 3D tumor organoids measuring 150 microns in size were prepared, mixed to replicate the endogenous tumor heterogeneity, and treated with the above compounds singly and in differing combinations.
- **High Content Imaging:** High content confocal imaging was used to detect tumor cell death within the tumor organoids and to identify treatment-induced tumor cell killing as well as penetration of cell tracker labeled TILs into the tumor microenvironment.
- **Flow Cytometry:** Immuno-phenotyping of resident and TIL cell populations were characterized using multiparameter flow analysis for cell surface antigens and intra-cellular markers of immune cell activation.
- **Multiplex Cytokine:** Culture media was collected over the course of the experiment to simultaneously analyze the differential release of cytokines and chemokines.

## Results

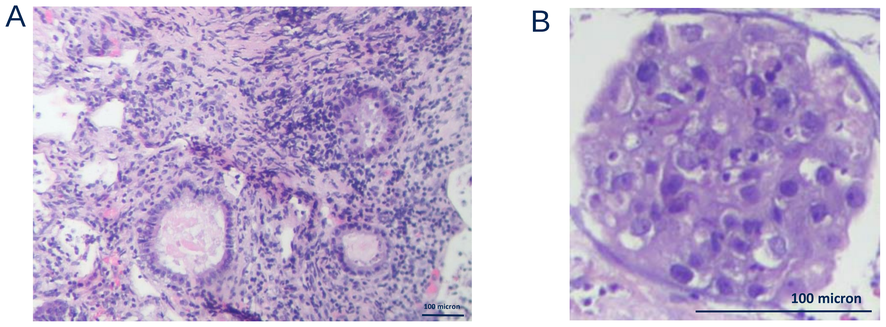


**Figure 1**. H&Es depicting the diverse microenvironment within the tumor tissue A: Original patient tumor; B: CRC tumoroid. Tumoroids are not produced using any sort of dissociation, propagation, or reassembly of the tumor tissue. The extracellular matrix (ECM) as well as ECM-cell and diverse cell-to-cell interactions remain intact. Scale Bar = 100µm

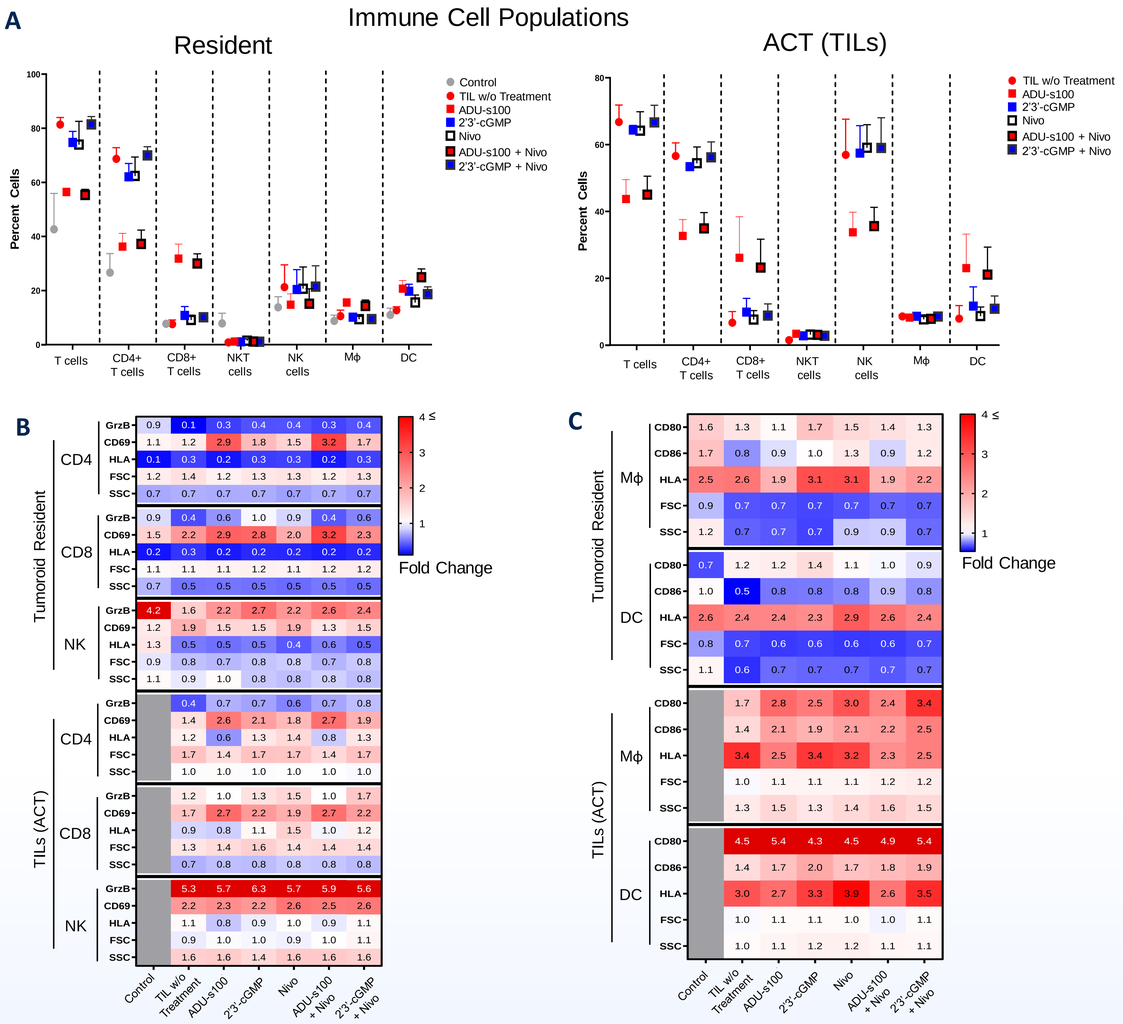


**Figure 2. Flow cytometric analysis**. Tumoroid resident and TIL (cell tracker +) immune cell populations were assessed for viability, immune cell profiling, and activation. **A.** Analysis of subsets within the CD45 population shows a consistent distribution of immune cells observed in both tumor resident and adoptive TIL cell (ACT) groups, with only an elevated percentage of NK cells present in ACT. Groups treated with ADU-s100 displayed reduced number of CD4+ T cells due to decreased viability following treatment **B&C.** Average fold change of activation marker MFI compared to total CD45[+] Cells (n=3). Tumoroid resident and TIL immune cell populations within the 3D tumor organoids, treated with Nivolumab (Nivo) alone or in combination with cGAS-STING agonists, consistently displayed increased expression in several activation markers in both lymphoid (**B**) and myeloid (**C**) cell subsets.

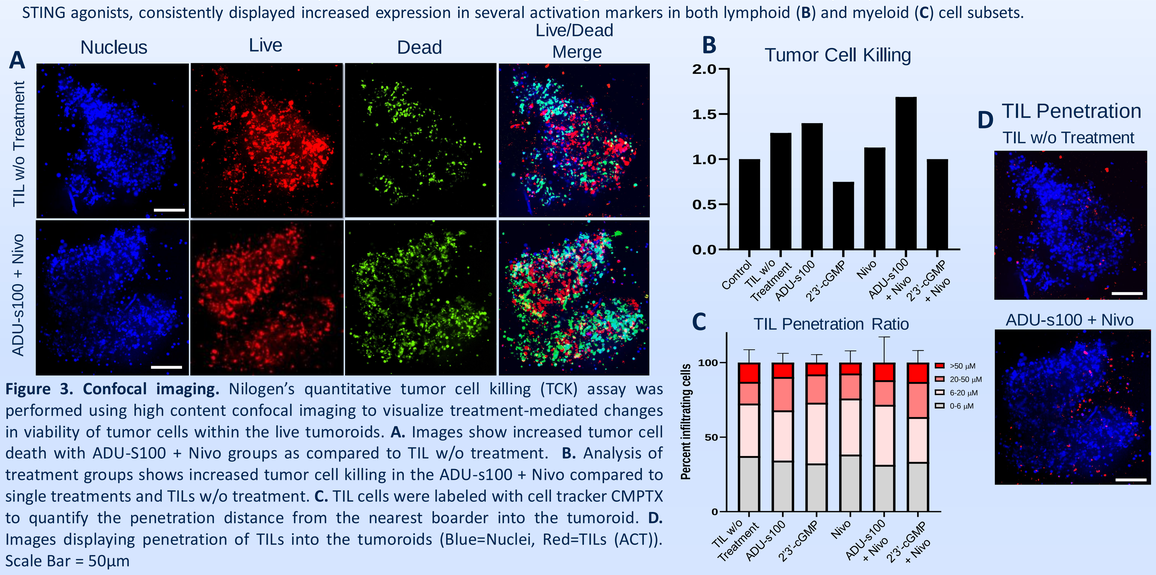


**Figure 3. Confocal imaging.** Nilogen's quantitative tumor cell killing (TCK) assay was performed using high content confocal imaging to visualize treatment-mediated changes in viability of tumor cells within the live tumoroids. **A.** Images show increased tumor cell death with ADU-S100 + Nivo groups as compared to TIL w/o treatment. **B.** Analysis of treatment groups shows increased tumor cell killing in the ADU-s100 + Nivo compared to single treatments and TILs w/o treatment. **C.** TIL cells were labeled with cell tracker CMPTX to quantify the penetration distance from the nearest boarder into the tumoroid. **D.** Images displaying penetration of TILs into the tumoroids (Blue=Nuclei, Red=TILs (ACT)). Scale Bar = 50µm

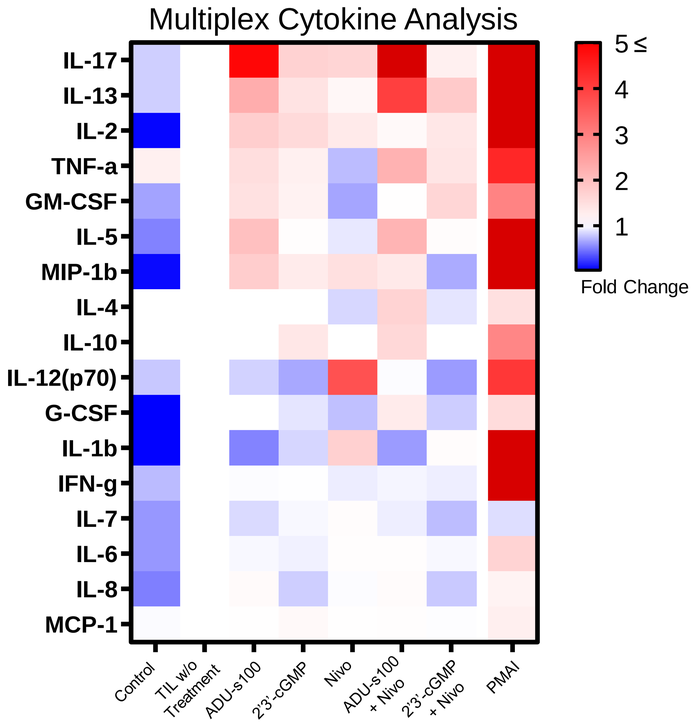


**Figure 4. Cytokine analysis**. 3D tumor organoids derived from fresh CRC patient tumors were treated with cGAS-STING agonists alone and combined with as immune checkpoint inhibitor, in the presence of adoptive TILs. After 48 hrs in *ex vivo* culture, supernatants were collected and analyzed by multiplex cytokine assay. Data is displayed as average fold change from TIL w/o treatment group (n=3). Nivo increased the secretion of several pro-inflammatory cytokines when combined with the ADU-s100 cGAS-STING agonists in an adoptive cell environment.

## Summary & Conclusion

- We successfully prepared unpropagated 3D tumor organoids from patient tumors which retain the unique heterogeneity of the endogenous tumor microenvironment.
- We demonstrated the efficacy of the 3D-ACT[SM] technology for the evaluation of the therapeutic effect of cellular-based oncology therapies in combination with other immune-oncology drugs. This platform provides a unique tool for monitoring the fate of cell therapies within the tumor microenvironment.
- High content confocal imaging allowed for the detection of cellular therapy penetration into the 3D tumor organoid and treatment induced tumor cell killing.
- In response to STING pathway agonists, we independently detected increased activation of both tumor-resident and adoptive T cells in colorectal carcinoma. Nivolumab enhanced the immuno-modulatory effects of ADU-s100 on T cell activation, cytokine production, and tumor cell killing - suggesting a potential synergistic interaction between these therapeutic agents. Combination of STING agonists and Nivolumab with adoptive cell therapy may have clinical benefit in colorectal cancer treatment.
- These results demonstrate that the 3D-ACT[SM] system, using *ex vivo* treated 3D tumor organoids, is an effective tool for the therapeutic assessment of adoptive cell-based therapies and novel drug combinations.

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