Employing *ex vivo* 3D-Screen^{SM} technology for the development of rational combinations of immuno-oncology drugs in tumor organoids of fresh patient tissue

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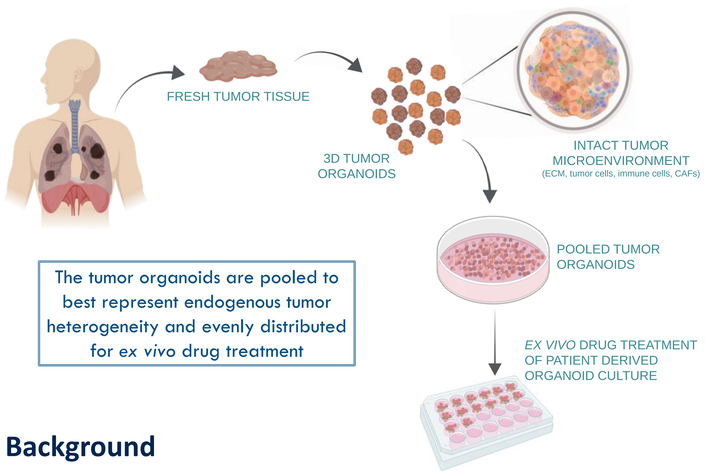

The tumor organoids are pooled to best represent endogenous tumor heterogeneity and evenly distributed for ex vivo drug treatment

Background

- 3D-Screen^{SM} technology allows for the *ex vivo* testing of multiple drugs and drug combinations 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 that may result in changes in tumor cell viability and immune cell profile.
- We have developed a high content imaging approach using a fresh patient 3D tumor organoid model with intact tumor stroma for assessment tumor cell killing.

Materials and Methods

- **Tumor tissue procurement:** 3D *ex vivo* studies were performed with fresh tumor tissue obtained from consented patients with bladder, colorectal (CRC) or kidney tumors (RCC). All experimental protocols were approved by the Institutional Review Board (IRB).
- **3D-Screen^{SM} platform:** Fresh tumor tissue obtained from patients was used to prepare 3D tumor organoids for treatment with the immune checkpoint inhibitor Nivolumab and agonists of the cGAS-STING pathway – ADU-s100 and 2'3'-cGAMP. 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.
- **Flow Cytometry:** Immuno-phenotyping of TILs was 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

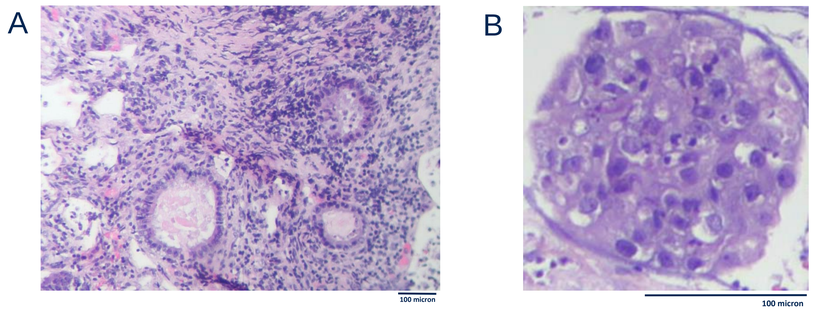

Figure 1. H&Es depicting the diverse microenvironment within the tumor tissue A: Original patient tumor; B: CRC tumoroid. There is no dissociation of the tumor tissue, propagation or re-assembly involved in the tumoroid preparation. The extracellular matrix (ECM) as well as ECM-cell and cell-cell interactions remained intact. Scale Bar = 100µm

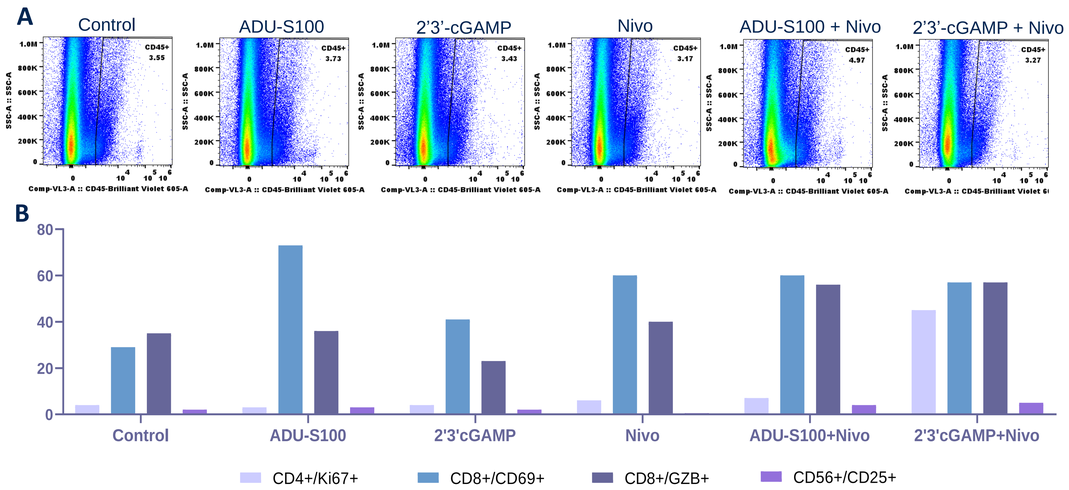

Figure 2. Flow cytometric analysis. 3D tumor organoids derived from fresh CRC patient tumor were assessed for viability and activation. **A.** Analysis of CD45 populations shows equal distribution of processed tumor tissue to each *ex vivo* treated well. **B.** Flow cytometric analysis of immune cell activation markers. 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 T-cell subsets as well as granulocytes.

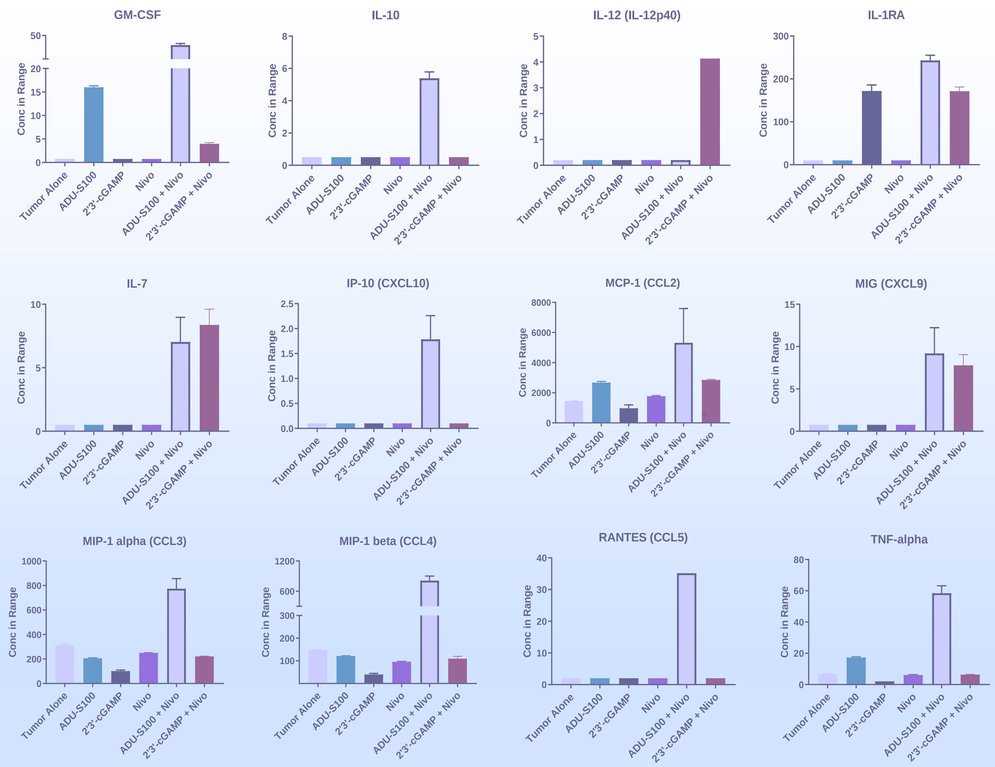

Figure 3. Cytokine analysis. 3D tumor organoids derived from fresh CRC patient tumors were treated with combinations of a checkpoint inhibitor and cGAS-STING agonists. After 48 hrs in *ex vivo* culture, supernatants were collected and analyzed by multiplex cytokine assay.

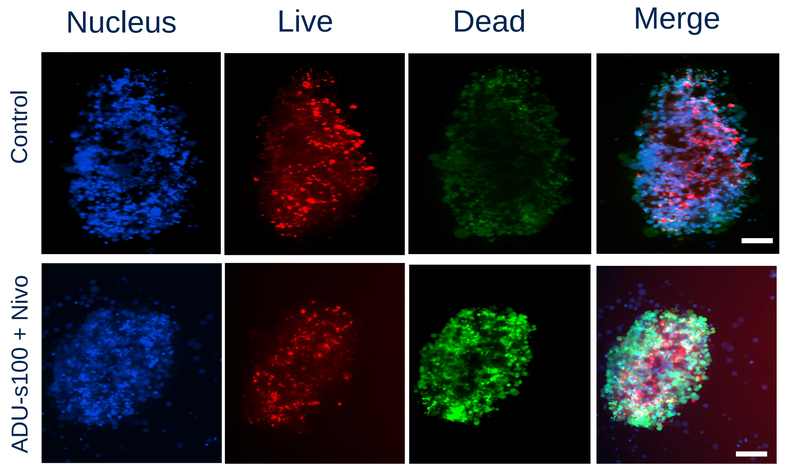

Figure 4. Confocal imaging. Nilogen's TCK (tumor cell killing) assay was performed using high content confocal imaging to visualize treatment-mediated changes in viability of tumor cells within the live tumoroids. Images show increased tumor cell death with the combination of ADU-S100 + Nivo as compared to controls. Scale Bar = 50µm

Summary & Conclusion

- We successfully prepared unpropagated 3D tumor organoids from patient tumors which retain the heterogeneity of the endogenous tumor microenvironment.
- Our results show that STING pathway agonists lead to activation of tumor-resident T-cells in Colorectal carcinoma. Nivolumab enhanced immune-modulatory effects of STING activators, suggesting a potential synergistic interaction between these therapeutic agents. Combination of STING agonists with Nivolumab may have clinical benefit in colorectal cancer treatment.
- We demonstrated the efficacy of the 3D-Screen^{SM} technology for the evaluation of the therapeutic effect of different immuno-oncology drugs alone and in combination.
- High content confocal imaging of the 3D tumor organoid microenvironment allowed for detection of changes in tumor cell killing in response to varying treatment conditions.
- This analysis demonstrated enhanced cytokine release and increased tumor cell death in the 3D tumor organoids, compared to controls.
- These results demonstrate that the 3D-Screen^{SM} system, using the *ex vivo* treated 3D tumor organoid model, is an effective tool for the therapeutic assessment of multiple drugs and drug combinations.

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