Optimization of checkpoint inhibitor-TKI combinations in renal cell carcinoma using an *ex vivo* 3D tumor organoid model of fresh patient tissue with intact TME

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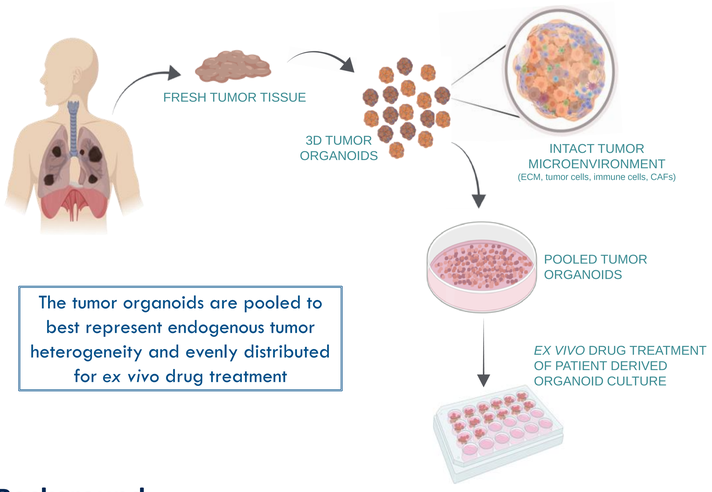

Background

- Since renal cell carcinoma (RCC) is highly resistant to systemic chemotherapy, modern tyrosine kinase inhibitors (TKIs) have been introduced resulting in improved outcomes in subsets of RCC patients.
- The endogenous tumor microenvironment (TME) plays an important role in the response to TKI therapies as well as other immuno-oncology drugs.
- Here we employed our *ex vivo* 3D-tumor organoid model, with unaltered TME, to identify the efficacy if TKI therapy on individual RCC tumors through both immune cell activation and tumor cell viability.
- We used flow cytometry and high content imaging of the fresh patient 3D tumor organoid model, with intact tumor stroma, for assessment of immune cell activation and tumor cell killing.

Materials and Methods

- **Tumor tissue procurement:** 3D *ex vivo* studies were performed with fresh tumor tissue obtained from consented patients with renal cell carcinoma (RCC). All experimental protocols were approved by the Institutional Review Board (IRB).
- **3D-Tumor organoid model:** Fresh tumor tissue obtained from patients was used to prepare 3D tumor organoids of uniform size and shape. For the *ex vivo* assays, 3D tumor organoids measuring approximately 150 microns in size were prepared and treated with various TKIs (Sunitinib, Sorafenib, or Axitinib) alone or in combination with a PD1 inhibitor, nivolumab.
- **Flow Cytometry:** Treatment mediated changes in tumor immune cell composition was determined with using immuno-phenotyping markers, in addition with staining for markers of immune cell activation.
- **High Content Imaging:** High content confocal microscopy was used to detect tumor cell death within the 3D tumor organoids and to identify treatment-mediated tumor cell killing.

Results

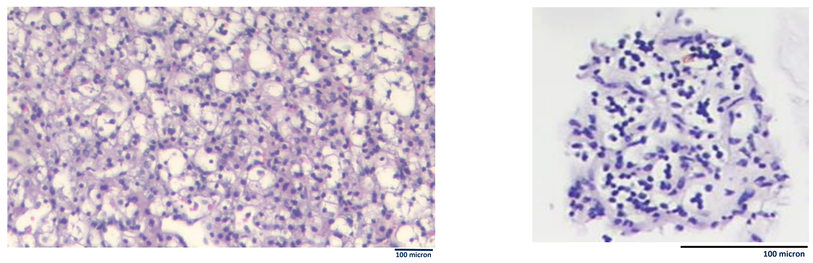

Figure 1. H&Es depicting the diverse microenvironment within the tumor tissue A) Original patient tumor; B) RCC tumoroid. There is no dissociation of the tumor tissue, propagation or re-assembly involved in the tumoroid preparation. The extracellular matrix (ECM) as well as ECM-cell and cell-cell interactions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

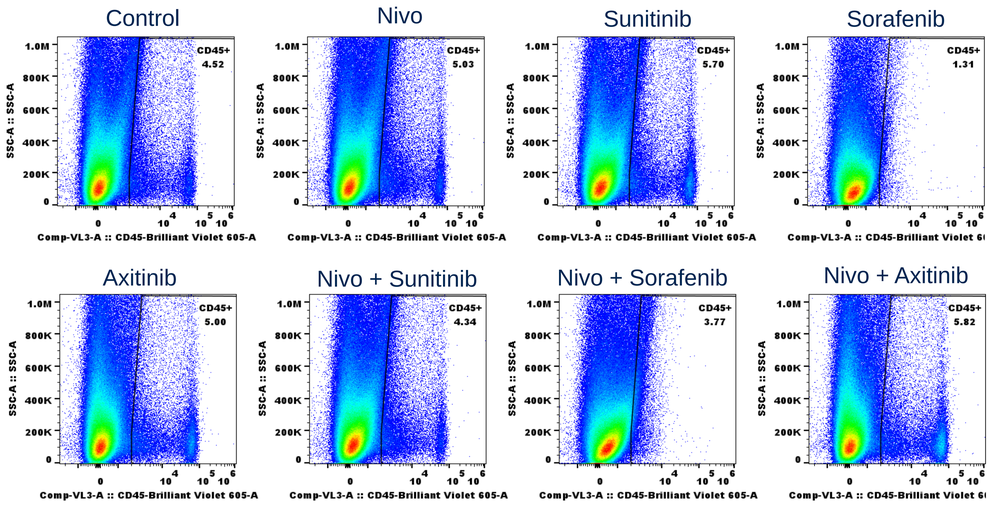

Figure 2. Flow cytometric analysis. 3D tumor organoids derived from fresh patient tumors were assessed for viability and phenotypic immune cell markers. Viable CD45+ populations show a relatively even distribution of tumor organoids amongst different treatment conditions. Interestingly, Sorafenib resulted in a decrease in CD45+ populations that was not observed with other treatment conditions.

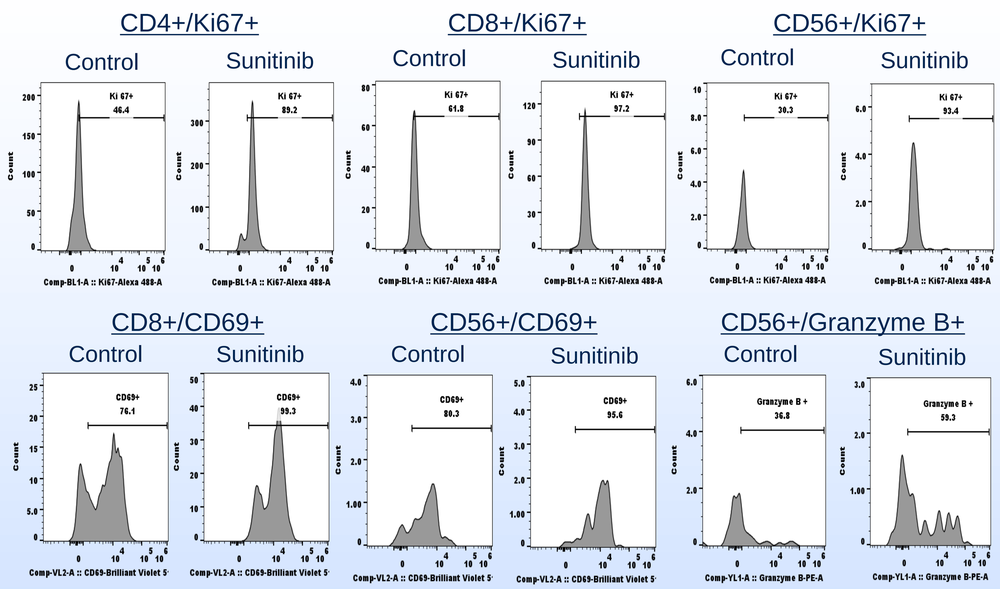

Figure 3. Flow cytometric histogram analysis of activation markers. 3D tumor organoids derived from fresh patient tumors were assessed for immune cell activation markers. Treatment with sunitinib results in increased Ki67+ staining in both CD4+ and CD8+ T-cell subsets, as well as CD56+ granulocytic populations. Most of these cell populations also show an increase in expression of CD69+ and Granzyme B+ activation markers. Similar changes, to a lesser extent, were observed with Axitinib, but not with Sorafenib (data not shown). Nivo did not enhance Sunitinib or Axitinib effect. No changes were observed on Treg and Macrophage populations (data not shown).

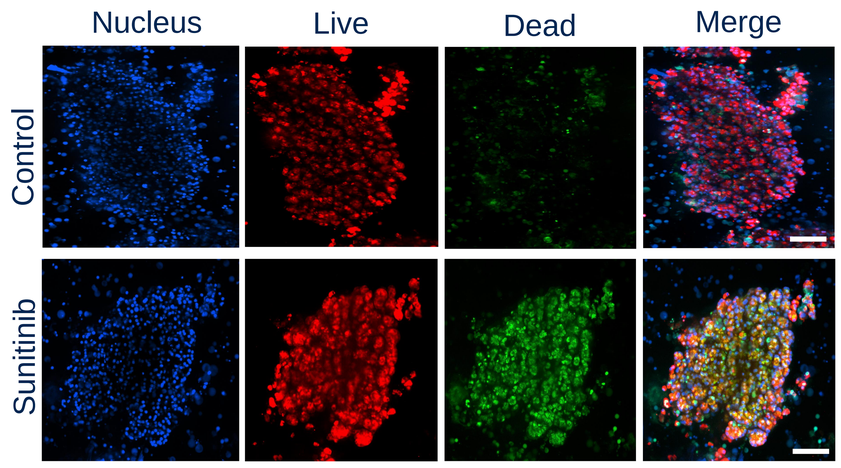

Figure 4. High content confocal imaging provided visualization of the tumor cell killing in response to *ex* vivo treatment of the 3D tumor organoids. Imaging showed increased tumor cell death in organoids treated with Sunitinib as compared to controls. Scale bar = 50µm

Summary & Conclusion

- Nilogen's physiologically relevant 3D tumor organoid model was generated from freshly resected patient tumors.
- Flow cytometry and high content confocal imaging of the unpropagated 3D tumor organoids demonstrate the importance of the heterogeneric cell populations within the tumor microenvironment, and their impact on TKI/nivolumab treatments in renal cell carcinoma.
- Flow cytometric analysis of immune cell populations show positive activation markers for several groups, including CD4+ and CD8+ T-cell subsets, as well as CD56+ granulocytes.
- Imaging of the 3D tumor organoids using high content confocal microscopy show increased tumor cell killing due to TKI treatment.
- Our data show that Sunitinib and Axitinib enhance activation of tumor-resident T-cells in RCC. They also demonstrated tumor cell killing in intact tumoroids, likely involving both direct effect on tumor cells as well as cytotoxic effect of activated T-cells.
- These results demonstrate that the proprietary 3D tumor organoid model, retaining the original tumor microenvironment, is an effective tool for the therapeutic assessment of various immuno-oncology drugs.
- Additionally, this approach can be used to identify the most effective drug and drug combinations in renal cell carcinoma and may improve personalized immunotherapy for individual patients in clinical studies.

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