Application of the 3D fresh patient tumoroid platform 3D-EXplore to assess the immunomodulatory and phagocytic activity of the receptor tyrosine kinase inhibitor, Sunitinib

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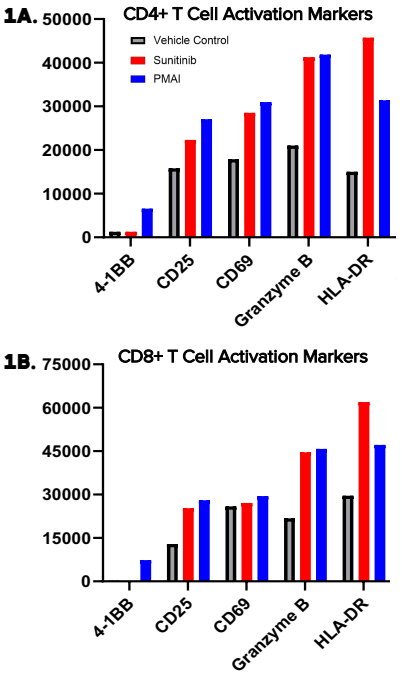
Background

- The receptor tyrosine kinase inhibitor (TKI) Sunitinib has proven useful in the treatment of a variety of tumors. Sunitinib has shown therapeutic activity in patients with metastatic renal cell carcinoma and currently represents a frontline therapy for this disease.
- The immune modulatory effect of Sunitinib on immune cells within the tumor microenvironment has been well documented.
- Here, we developed a novel 3D ex-vivo platform (3D-EXplore) using fresh patient tumor samples with intact stromal components and tumor immune microenvironment to assess the therapeutic efficacy of Sunitinib.

Materials & Methods

- **Tumor tissue procurement:** All tumor samples (n=10) were obtained with patient consent and relevant IRB approval. Unpropagated 3D tumoroids with intact TME measuring 150 µm in size were prepared from fresh tumor samples of renal cell carcinoma (RCC) using a proprietary technology developed at Nilogen Oncosystems.
- **3D-EXplore platform:** Tumoroids prepared from each patient's tumor sample were pooled to represent the tumor heterogeneity of the original primary tumor and treated ex vivo with Sunitinib for 48h to detect treatment-mediated changes in tumor immune cell composition including CD4 and CD8 T-cells, NK cells, and macrophages. Additionally, we analyzed treatment-mediated changes in T-cell activation and phagocytic activity of myeloid cells.
- **Flow Cytometry:** Multiparameter flow analysis was used at 48 hours to detect treatment-mediated changes in tumor immune cell composition including CD4 and CD8 T-cells, NK cells, and immune cell activation.
- **High Content Confocal Imaging:** Treatment-mediated changes in pHrodo bioparticle (pHrodo-BP) uptake was assessed by the confocal-based high-content real time imaging platform with Sunitinib and anti-CD47 treatments at 48 hours.

Results



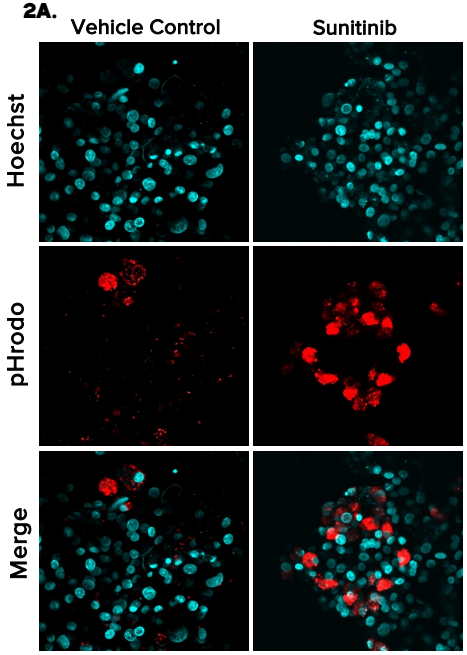
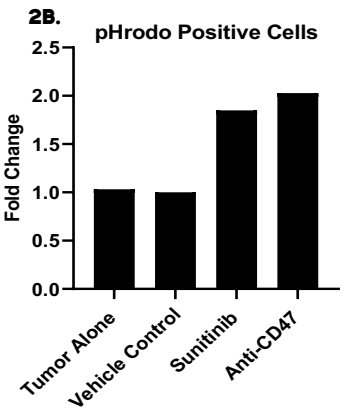


Figure 1. Sunitinib treatment of the RCC tumoroids ex vivo leads to activation of tumor resident CD4, CD8 and NKT cells. Flow cytometry analysis of tumor resident immune cell populations revealed that Sunitinib treatment caused an increase in the activation markers for both (B) CD4+ and (C) CD8+ T cells reveals increases in CD25, Granzyme B, and HLA-DR upon stimulation with Sunitinib (PMAI acting as positive control). Additionally, Granzyme B and HLA-DR were found to be increased in (D) NK-T cell, but not in (E) NK cell populations – compared to vehicle control. Data displayed as number of immune cells detected per $1x10^6$ viable CD45+ cells.



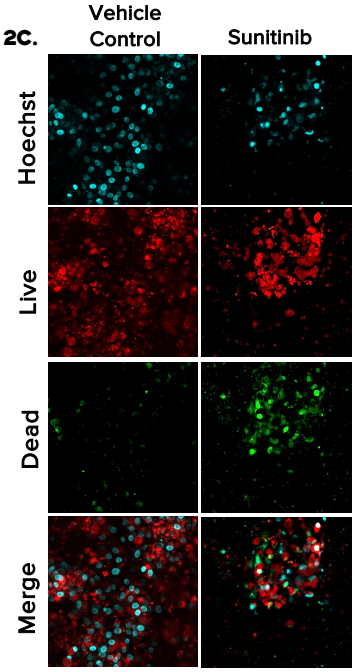


Figure 2: A and B: High Content Confocal imaging of ex vivo treated RCC tumoroids show increased phagocytic activity in tumor resident myeloid cells upon treatment with Sunitinib and anti-CD47 antibody targeting SIRPα-CD47 phagocytosis checkpoint. (A) 3D imaging and (B) analysis of RCC tumoroids shows a large increase in pHrodo-BP uptake with Sunitinib treatment comparable to anti-CD47 antibody treatment. (C) 3D imaging of thr confocal-based tumor cell killing (TCK) assay shows Sunitinib-mediated tumor cell killing in RCC tumoroids.

Summary & Conclusions

- **Our data shows that 3D tumoroids can be utilized to visualize increased phagocytic activity in tumor resident myeloid cells upon treatment with Sunitinib and an anti-CD47 antibody targeting SIRPα-CD47 phagocytosis checkpoint.**
- **Multiparameter flow cytometry analysis demonstrated that Sunitinib treatment leads to activation of tumor resident CD4, CD8 T-cells and NKT cells.**
- **Nilogen's proprietary tumor cell killing (TCK) assay utilizing 3D confocal analysis of tumoroids demonstrate that Sunitinib treatment ex vivo leads to a marked increase in the tumor cell killing activity in RCC tumoroids.**
- **These results demonstrate that Nilogen's 3D-EXplore platform using fresh tumor samples provides clinically relevant and actionable data to further enhance our understanding on drug mode of action and may help to improve patient outcome when utilized for personalized cancer therapy.**

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