Assessment of immunotherapeutic efficacy of Entinostat by using the 3D-EXplore ex vivo platform utilizing tumoroids of fresh patient tumor samples with intact tumor microenvironment

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Background

- **Entinostat, a selective histone deacetylase 1/3 inhibitor has been shown to promote a robust anti-tumor response and increased neoantigen expression and antigen-specific T cell activation in preclinical tumor models.**
- **Entinostat plays a role in neutralizing myeloid derived suppressor cells (MDSC) to improve anti-tumor immune response and is currently being tested in later-stage clinical trials in various types of cancer.**
- **Using a novel 3D ex-vivo platform with fresh patient tumor samples we assessed the therapeutic efficacy of Entinostat with intact stromal components and tumor immune microenvironment..**

Materials & Methods

- **Tumor tissue procurement:** All tumor samples were obtained with patient consent and relevant IRB approval. All 3D *ex vivo* studies were performed with fresh renal cell carcinoma (RCC) tissue.
- **3D-EXplore platform:** Unpropagated 3D tumoroids with intact TME measuring 150 µm in size were prepared from fresh tumor samples of RCC tissues using proprietary technology developed at Nilogen Oncosystems. Tumoroids prepared from each patient's tumor sample were pooled to represent the tumor heterogeneity and treated ex vivo with Entinostat for 48h to detect treatment-mediated changes in tumor immune cell composition including lymphoid and myeloid markers and their activation status.
- **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, macrophages, and markers of immune cell activation.
- **High Content Confocal Imaging:** Treatment-mediated tumor cell killing was detected using high content real-time confocal imaging with Entinostat treatment at 48 hours.

Result

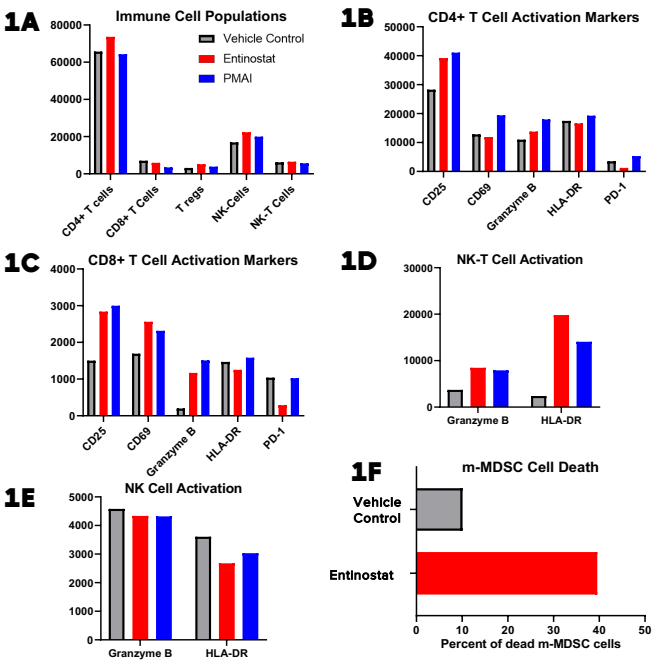


Figure 1. Entinostat treatment in the RCC tumoroids ex vivo leads to activation of tumor resident CD4, CD8 and NKT cells. Flow cytometry analysis of tumor resident immune cell populations revealed increases in the activation markers CD25 and Granzyme B in both (1B) CD4+ and (1C) CD8+ T cells upon stimulation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

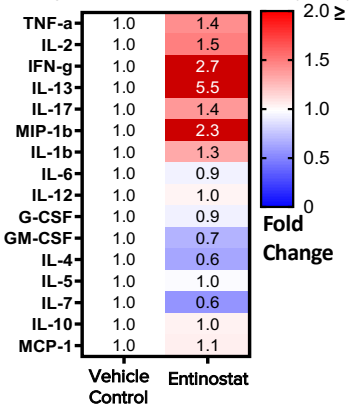
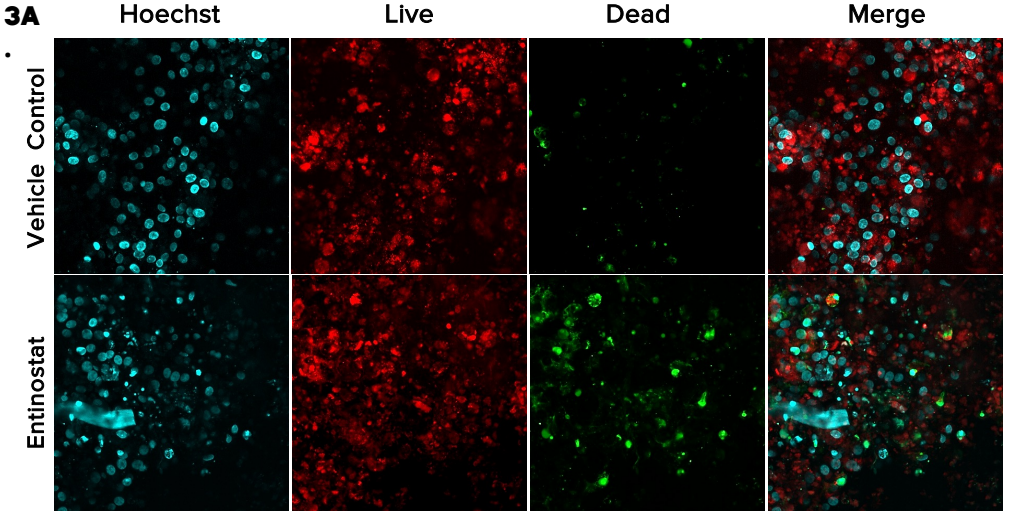


Figure2. Cytokine production. (Left) Multiplex Cytokine analysis was used to detect treatment mediated changes in cytokine production within conditioned culture media of tumoroids treated with Entinostat. Data is displayed as the fold change of treatment groups from vehicle control.



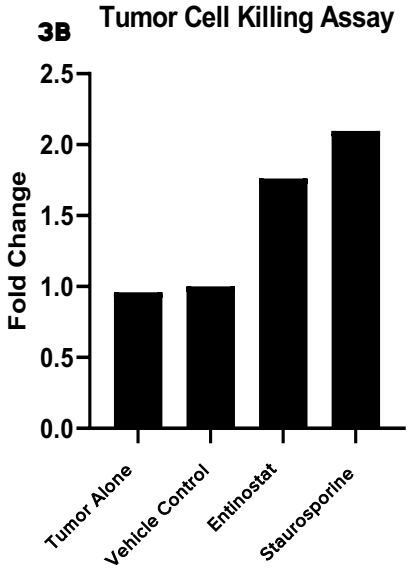


Figure 3: High Content Confocal Imaging analysis of tumor cell killing. High Content Confocal imaging of *ex vivo* Entinostat-treated RCC tumoroids shows increased tumor cell killing activity. (A) 3D imaging and (B) analysis of treatment-mediated TCK in RCC tumoroids. The data shows that Entinostat treatment ex vivo leads to a 75% increase in the tumor cell killing activity in RCC tumoroids. Staurosporine was used as a positive control.

Summary & Conclusions

- **These results demonstrate that Entinostat treatment leads to a broad range of immune cell activation and MDSC inhibition in 3D RCC tumoroids.**
- **These data provide a mechanistic rationale utilizing our 3D tumoroid platform for discovery of potential biomarkers of drug sensitivity and to identify novel immunotherapeutic approaches.**
- **Clinical applications of 3D-EXplore may help to identify RCC patients who may likely benefit from Entinostat treatment.**
- **Nilogen's 3D-tumoroid platform provides a novel model to analyze treatment-mediated changes within the complex tumor microenvironment.**

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