Evaluating the effectiveness of ADC targeted therapy in a patient-derived ex vivo tumoroid model, 3D-EXplore, for quantitative tumor cell killing

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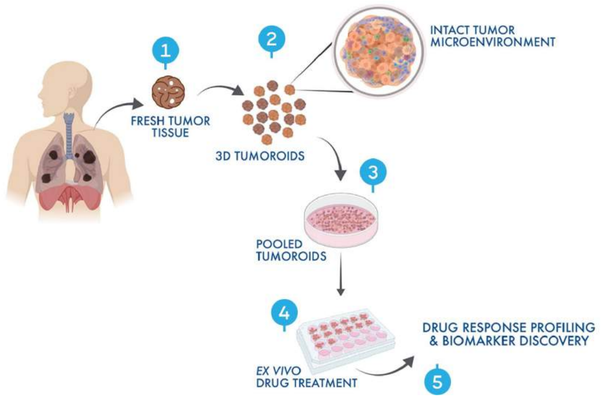
Background

- 3D-EXplore technology allows for the *ex vivo* testing of different classes of immuno-oncology therapeutics for tumor cell killing and tumor-resident immune cell activation.
- Our proprietary processing method, which employs fresh patient derived tumor tissue, results in the generation of 3D tumoroids that retain the intact tumor microenvironment. This model allows for accurate quantification of any antibody-drug conjugate (ADC) 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 tumoroid model with intact tumor stroma for assessment tumor cell killing.



Materials & Methods

- **Tumor tissue procurement:** 3D *ex vivo* studies were performed with fresh tumor tissue obtained from consented patients with colorectal (CRC) tumors. All experimental protocols were approved by the Institutional Review Board (IRB).
- **3D-EXplore platform:** Fresh tumor tissue obtained from patients was used to prepare 3D tumoroids for treatment with the pHrodo-labeled cetuximab. For the *ex vivo* assays, 3D tumoroids measuring 150 microns in size were prepared, mixed to replicate the endogenous tumor heterogeneity, and treated with the above compound. These tumoroids retain the unique stromal components present in the original patient tumor.
- **High Content Imaging:** High content confocal imaging was used to detect internalization of the pHrodo-labeled cetuximab, and to identify treatment-induced tumor cell killing with the 3D tumoroids. Upon internalization the pHrodo molecule fluoresces brightly at the low pH conditions found in lysosomes.
- **Flow Cytometry:** Expression of the ADC-target EGFR was characterized using multiparameter flow analysis for cell surface antigens and detection of internalization of the pHrodo-labeled cetuximab in 3D tumoroids.

Results

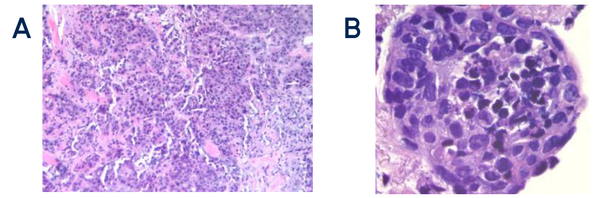


Figure 1. H&Es depicting the diverse microenvironment within the tumor tissue A: Original patient tumor; B: CRC tumoroid. There is no dissociation of the tumor tissue, propagation or re-assembly involved in the tumoroid preparation. The extracellular matrix (ECM) as well as ECM-cell and cell-cell interactions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

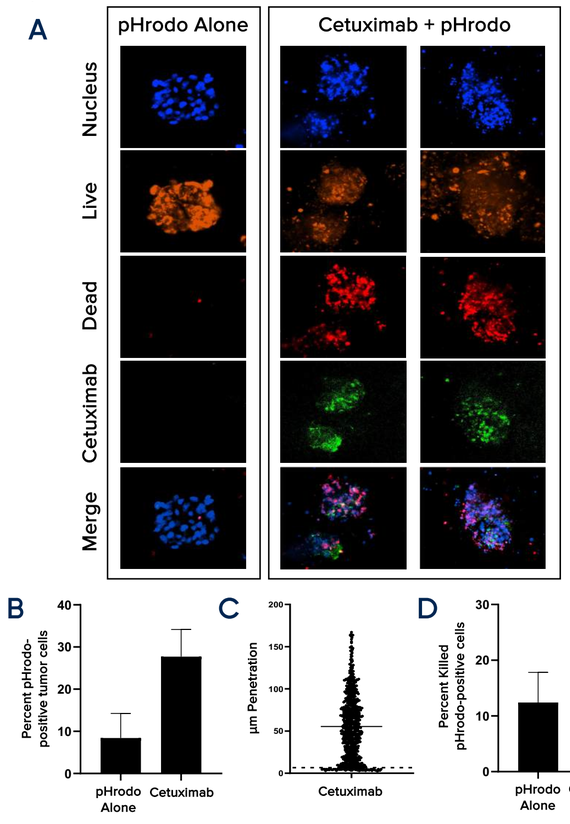


Figure 2. ADC mediated tumor cell killing. 3D tumoroids were assessed for ADC-mediated tumor cell killing. **A**. High content confocal microscopy shows penetration, and internalization into the lysosomal component of the pHrodo-labeled cetuximab in the tumoroid microenvironment. Merge images depict increased tumor cell death in response to ADC treatment compared to pHrodo-alone controls. **B-D**. Quantitative analysis of confocal imaging. We successfully detected (**B**) ADC internalization, (**C**) penetration of ADC into the tumoroid microenvironment, and (**D**) death of tumor cells due to ADC treatment. Scale Bar = 50µm

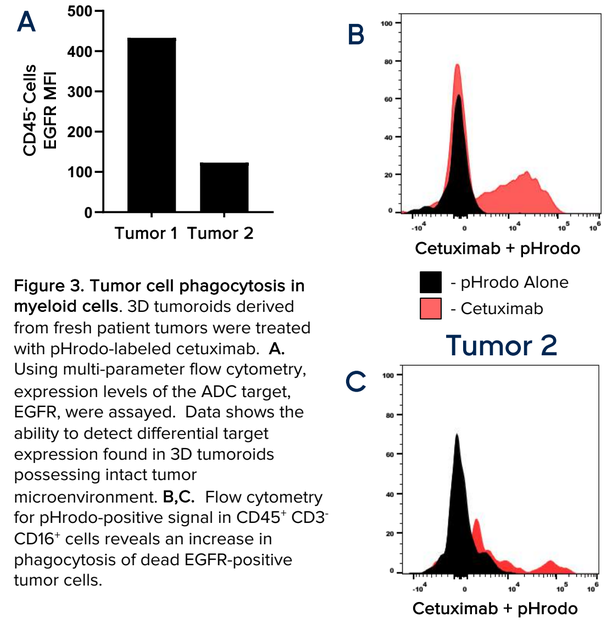


Figure 3. Tumor cell phagocytosis in myeloid cells. 3D tumoroids derived from fresh patient tumors were treated with pHrodo-labeled cetuximab. **A.** Using multi-parameter flow cytometry, expression levels of the ADC target, EGFR, were assayed. Data shows the ability to detect differential target expression found in 3D tumoroids possessing intact tumor microenvironment. **B,C.** Flow cytometry for pHrodo-positive signal in $CD45^+$ $CD3^-$ $CD16^+$ cells reveals an increase in phagocytosis of dead EGFR-positive tumor cells.

Summary & Conclusions

- We successfully prepared unpropagated 3D tumoroids from patient tumors which retain the unique heterogeneity of the original tumor microenvironment.
- We demonstrated the efficacy of the 3D-EXplore technology for the evaluation of the therapeutic effect of ADC-based immuno-oncology drugs.
- The effectiveness of ADC therapies rely on the binding of the antibody-drug conjugate to target antigens on tumor cells, internalization of the antibody-target complex, and release of the cytotoxic payload – directly killing the tumor cell.
- High content confocal imaging of the 3D tumoroid microenvironment allowed for detection of ADC penetration into the tumor microenvironment, binding to tumor cells, and induction of tumor cell killing in response to ADC treatment.
- High content confocal imaging coupled with multiparameter flow analysis allows for detection of the phagocytic activity of myeloid cell populations in the intact tumor microenvironment.
- These results demonstrate that the 3D-EXplore platform, using the *ex vivo* treated 3D tumoroid model, is an effective tool for the therapeutic assessment of antibody-drug conjugates in a clinically relevant *ex vivo* model.

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