Abstract # 52

Understanding Cetuximab Response and Immune Modulation in Colorectal Cancer using Patient-Derived Organotypic Tumor Spheroids

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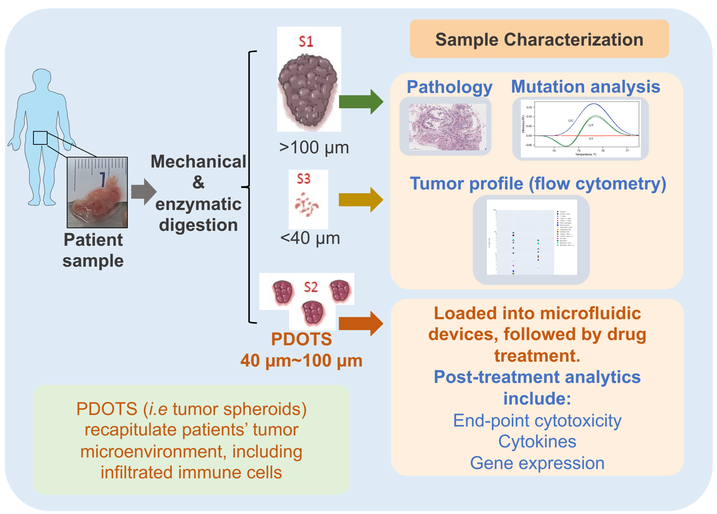
Background

Cetuximab, an epidermal growth factor receptor (EGFR) specific monoclonal IgG1 antibody, holds considerable importance in the treatment against Kirsten Rat Sarcoma virus (KRAS) wild type ($KRAS^{wt}$) colorectal cancer (CRC). Cetuximab triggers internalization of EGFR, which in turn suppresses the downstream signaling pathways including IL6/STAT3, PI3K/AKT, MTORC1. KRAS mutations are associated with resistance to Cetuximab treatment in CRC, thus anti-EGFR therapies are only approved for $KRAS^{wt}$. Cytotoxic response by Cetuximab is also known to be mediated by antibody-dependent cellular cytotoxicity (ADCC). Here, an *ex vivo* patient-derived organotypic tumor spheroids (PDOTS) platform[1] provides insight into the response, mechanistic pathways and resistance dynamics to Cetuximab treatment in PDOTS and supports a better understanding of the variability in patient response.

Our patented *ex vivo* microfluidic platform



The platform conserves native tumor milieu

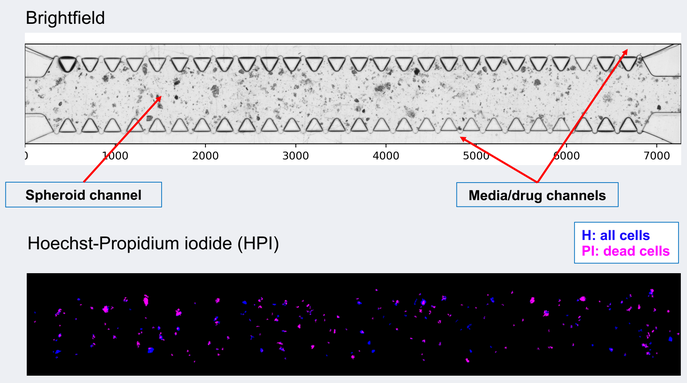


Fig. 1: Brightfield and HPI images of CRC spheroids embedded in matrix and cultured in a singular channel of a microfluidic device.

Live tumor samples collected from patients are dissociated into spheroids within 24 hours of resection using a mechanical and enzymatic digestion process. Samples are fractionated into three different sizes: S1, S2 and S3 fractions. Patient derived organotypic spheroids (S2 or PDOTS) 40 µm - 100 µm diameter are loaded onto microfluidic devices, mixed with a matrix with complete medium to maintain the patients' native tumor microenvironment (TME). Tumor cell profile was determined using flow cytometry on single cell S3 fraction at baseline.

Experimental design

PDOTS isolated from n=32 CRC patient tumor specimens were used to evaluate the therapeutic efficacy of Cetuximab. KRAS mutations were determined pre-treatment by high-resolution melting analysis. PDOTS were treated with Cetuximab or isotype-matched IgG1 control (300 µg/mL). On day 3 post-treatment, cytotoxicity was measured using Hoechst/Propidium Iodide staining. Automated image analysis was used to report cytotoxicity measurements. Cytokines secreted by spheroids were collected at day 3 and measured using Ella (ProteinSimple, Biotechne). Bulk gene expression analysis was performed on purified RNA isolates derived from post-treatment PDOTS using PanCancer IO360 panel on nCounter® SPRINT Profiler (Nanostring Technologies).

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[1]Jenkins RW, Aref AR, *et al. Ex Vivo* Profiling of PD-1 Blockade Using Organotypic Tumor Spheroids. Cancer Discov. 2018 Feb;8(2):196-215.
[2]De Roock W, Piessevaux H, *et al.* KRAS wild-type state predicts survival and is associated to early radiological response in metastatic colorectal cancer treated with cetuximab. Ann Oncol. 2008 Mar;19(3):508-15.
[3]Gelfo V, Mazzeschi M, *et al.* A Novel Role for the Interleukin-1 Receptor Axis in Resistance to Anti-EGFR Therapy. *Cancers.* 2018, *10*, 355.

Noteworthy findings:

- Among CRC samples derived from $KRAS^{wt}$ tumors, 6 out of 16 (37.5%) exhibited a cytotoxic response to Cetuximab compared to the IgG control, slightly higher than the ~21% response rate observed in CRC patients.
- No $KRAS^{mut}$ samples tested responded to Cetuximab treatment.
- There was evidence of rapid initiation of ADCC in CRC PDOTS samples responsive to Cetuximab.
- Among treatment-responsive CRC samples, suppression of pathways downstream of EGFR signaling was evident at day 3 post-treatment.
- Data generated from the PDOTS platform are consistent with clinical findings[2], indicating the platform's effectiveness in maintaining patients' tumor microenvironment and unraveling the complexity of tumor-targeted therapies.

Results

Cetuximab cytotoxic response on day 3

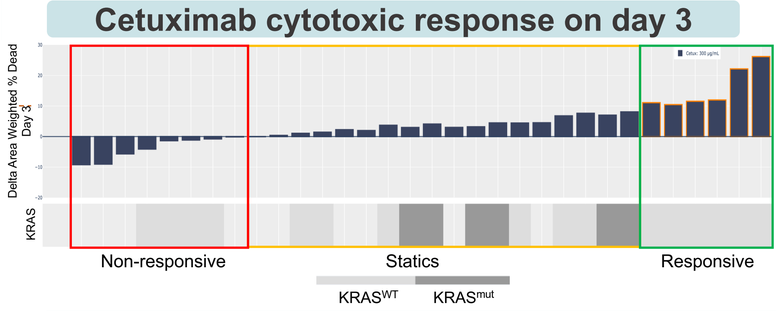


Fig. 2: Cetuximab cytotoxic response on day 3, expressed as area weighted (AW) % dead. AW % Dead is calculated by dividing the sum of dead area of all spheroids in a given channel by the sum of total area of all spheroids. With n=32 treated with 300 µg/mL Cetuximab, 18.75% showed significant response ($p<0.2$). Samples were categorized into treatment-responsive (R) and non-responsive (NR) groups. All responsive samples were $KRAS^{WT}$.

Cytokine profiles

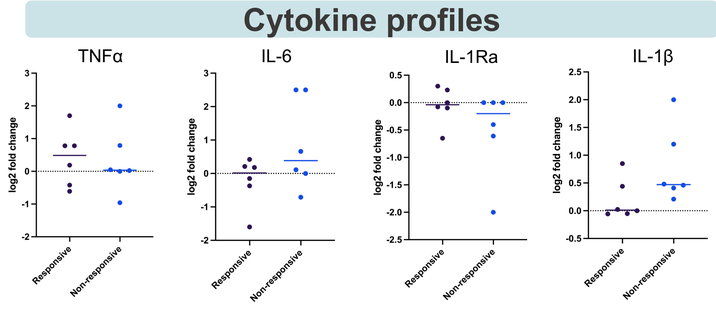
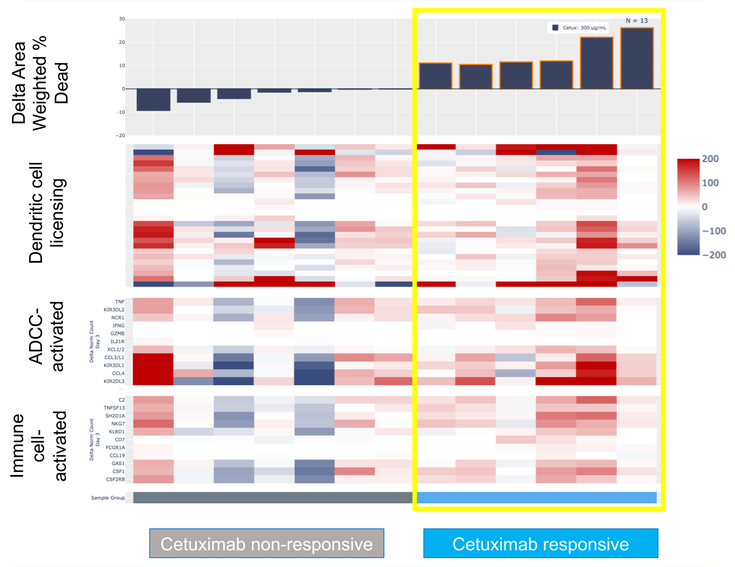


Fig. 3: Cytokine profiles compared between Cetuximab-responsive and non-responsive PDOTS at day 3, measured from spheroid supernatants. TNFα was upregulated in 3/6 responsive PDOTS. IL-1β and IL-6 were elevated in non-responsive compared with responsive PDOTS samples.

Evidence of immune cell activation



Genes from top to bottom (Note: 2 patients with no GE data recorded were excluded from the set)
DC licensing: *HLA-C, HLA-A, CD80, IL2, CD86, CD8A, CD40LG, CD27, CD70, CD28, IFNG, GZMB, HLA-DQA2, HLA-DQB1, CD8B, A2M, TLR4, HLA-DPB1, HLA-DPA1, CD40, CD4, IL4, PRF1, HLA-DQA1, HLA-DRA, HLA-B*
ADCC-activated: *TNF, KIR3DL2, NCR1, IFNG, GZMB, IL21R, XCL1/2, CCL3/L1, KIR3DL1, CCL4, KIR2DL3*
Immune cell-activated: *C2, TNFSF13, SH2D1A, NKG7, KLRD1, CD7, FCGR1A, CCL19, GAS1, CSF1, CSF2RB*

Fig. 4: Heatmaps for specific pathways for Cetuximab-responsive and non-responsive PDOTS at day 3 (Delta norm count shown). Samples from the treatment-responsive group displayed elevated levels of genes pertaining to activation of ADCC. Additionally, we measured an increase in expression of genes responsible in the process of dendritic cell licensing and immune cell activation.

Molecular pathways of tumor suppression

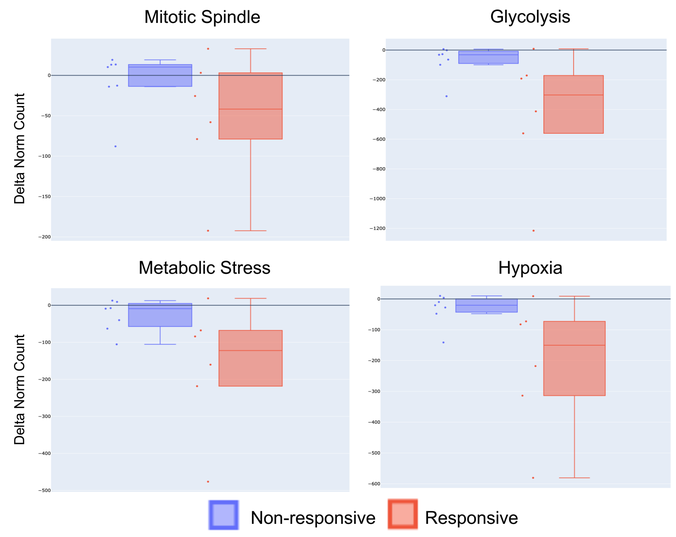


Fig. 5: Gene expression profiles compared between Cetuximab-responsive and non-responsive PDOTS. Treatment-responsive group (in red) showed evidence of tumor suppression by downregulation of genes responsible for formation of mitotic spindle, glycolysis, metabolic stress and hypoxia, compared to the non-responsive group (in blue).

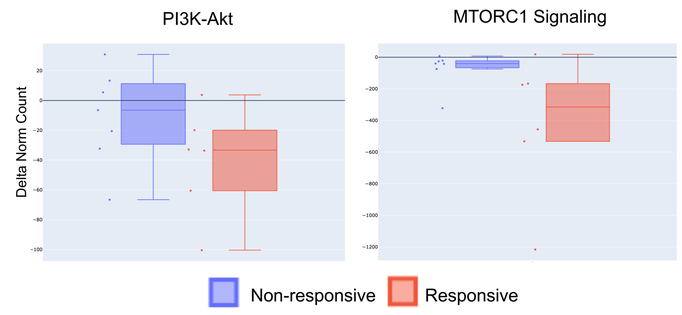


Fig. 6: Gene expression profiles for specific pathways between Cetuximab-responsive and non-responsive PDOTS. Pathways downstream of EGFR signaling, PI3K-Akt and MTORC1 signaling, were suppressed in the treatment-responsive group (red), compared to the non-responsive group (in blue).

Differentially expressed genes in Cetuximab-responsive CRC PDOTS

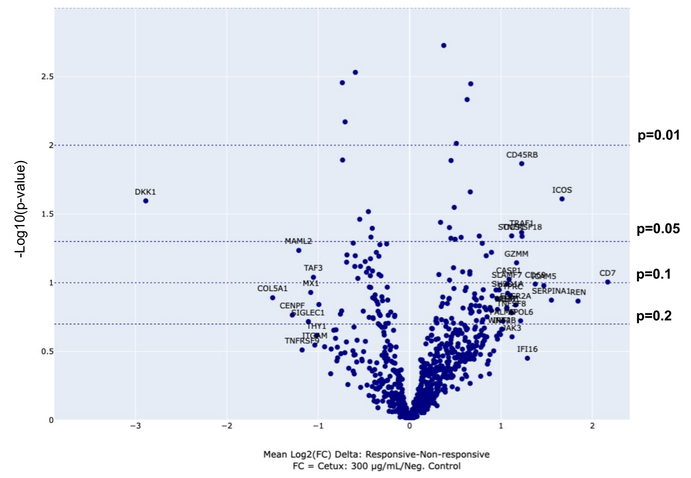


Fig. 7: Volcano plot representing statistically significant expressed transcripts from Nanostring panel. Differentially expressed genes in treatment-responders relative to negative control are associated with immune cell activity. For every transcript, $-\log_{10}$(p-value) was plotted against the mean $\log_2$(fold-change) between responsive and non-responsive samples.

Genes (top-right):
CD45RB, SOCS1, TRAF1, ICOS, TNFRSF18, GZMM, CASP1, SLAMF7, SH2DIA, PTPRC, CD69, ICAM5, SERPINA1, REN, CD7, UBA7, TREM1, TNFSF8, FCGR2A, APOL6, PALMD, IRF4, WNT2B

Conclusions

Data generated from Xsphera's platform are consistent with clinical findings from Cetuximab response in CRC patients and support evidence of Cetuximab-mediated suppression of EGFR downstream signaling pathways. IL-1β has been reported to impair the therapeutic efficacy of Cetuximab[3], which aligns with our data. Our findings on upregulation of DC-licensing and activation of ADCC indicate involvement of immune cells in cytotoxic response of CRC tumor cells to Cetuximab. These insights could guide the development of more personalized therapeutic strategies, potentially improving the effectiveness of treatments like Cetuximab.