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, plays a critical role in treating Kirsten Rat Sarcoma virus wild-type (K-ras^{wt}) colorectal cancer (CRC). Understanding the response, mechanistic pathways, and resistance dynamics to cetuximab can be facilitated using an *ex vivo* platform of patient-derived organotypic tumor spheroids (PDOTS). This platform can provide valuable insights into the variability of patient responses to this drug.

Methods PDOTS were generated from n=32 CRC patient tumor specimens to evaluate the therapeutic efficacy of cetuximab. These PDOTS were loaded with extracellular matrix into microfluidic devices and treated with cetuximab (300 µg/ml) or an IgG1 control (300 µg/mL). Cytotoxicity was assessed on Day 3 post-treatment using Hoechst/Propidium Iodide staining and automated image analysis, reporting area-weighted% dead (AW% Dead) or% Live Area. K-Ras mutations were determined by PCR analysis, and immune activation was evaluated by measuring changes in cytokine release and gene expression. Integrated analyses of patient specific responses was performed using the Xsphera Cloud analytic platform.

Results Among the 16 K-RAS^{wt} samples, 6 of 16 (37.5%) exhibited a cytotoxic response to cetuximab compared with the IgG control, being slightly higher than the ~21% response rate observed clinically. None of the patient tumors displaying a cytotoxic response harbored K-RAS mutations. EGFR pathway suppression was not evident on day 3 and a longer treatment duration may be required for pathway suppression to be measurable. Cetuximab-induced immune activation relative to control was observed among a number of patient tumor specimens. The samples were further categorized into cytotoxicity-responsive (R) and non-responsive (NR) groups. PDOTS from responsive patient tumors demonstrated elevated levels of pro-inflammatory cytokines and increased expression of genes associated with antibody-dependent cellular cytotoxicity (ADCC) compared to the NR group. Among the responsive tumors, 3 out of 6 showed elevated TNF-α levels, while 2 out of 6 exhibited a concomitant increase in mature NK cells, indicating the rapid initiation of ADCC within the tumor microenvironment and potentially influencing the cetuximab response.

Conclusions Utilizing the PDOTS platform to evaluate patient tumor responsiveness to cetuximab unveils the variability of patient responses and highlights the role of rapid immune responses, particularly ADCC, in shaping the drug response within the tumor microenvironment. Our findings align with clinical observations, demonstrating the platform's effectiveness in preserving the tumor microenvironment and unraveling the complexity of tumor-targeted therapies. These insights can guide the development of personalized therapeutic strategies, potentially enhancing the effectiveness of treatments like cetuximab.

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Ethics Approval The study used excised patient tumor specimens from established vendors. The vendors obtained ethics approval by the Institutional Review Boards at each clinical site and Xsphera Biosciences maintains an approved IRB [wcgIRB# 120200016]. The patients are required to give informed consent before collection of the specimen. Patient ID is anonymized prior shipping specimens.

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