Selective infiltration of Antibody-Dependent Cellular Cytoxicity (ADCC) mediating immune cells in response to treatment in a human tumor histo-culture platform

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Introduction:

Tumor 3D histo-culture provides a near native Tumor immune Micro-Environment (TiME), making it best suited for evaluating response to immunotherapy drugs. Farcast™ TiME is a human 3D tumor histo-culture platform that preserves TiME and maintains functional fidelity of intra-tumoral immune cells (IIC). In this study we investigated the utility of this platform in demonstrating treatment induced Antibody-Dependent Cellular Cytotoxicity (ADCC) mechanism driven by IICs alone versus co-culture with autologous peripheral blood immune cells (**P**eripheral **B**lood **N**ucleated **C**ells, PBNCs) .

Methodology:

Patient tissue samples: Fresh surgically resected Head and Neck Squamous Cell Carcinoma (HNSCC) tissue samples were collected from consented patients. A matched blood sample from the patient was also collected.

Histo-Culture workflow: The tumor sample was processed to generate thin explants, without enzymatic digestion, to retain the tumor microenvironment. Tumor explants were cultured with media and autologous plasma with or without **P**eripheral **B**lood **N**ucleated **C**ells (PBNCs) that included lymphocytes, monocytes, NK cells and neutrophils. PBNCs isolated from the matched donor blood were stained with a tracking dye (CellTrace™ CFSE dye, Thermo Fisher) to distinguish them from IICs The explants were treated with 184 µg/ml Cetuximab (anti-EGFR) or vehicle control in and cultured for 72 hrs. Media was replaced every 24 hours. Response was evaluated using flow cytometry, cytokine-chemokine and LDH release assays.

Flow cytometry analysis: Tumor explants were dissociated post culture into single cells and stained with with Live/Dead dye, and cocktail of fluorescence tagged antibodies (Lineage markers: CD45,CD3,CD8,CD15,CD56); Activation markers: CD16, Granzyme-B). Data was acquired using BD LSR Fortessa Flow cytometer with appropriate compensation controls and analysed using FlowJo software.

Cytokine Analysis: The cultured supernatants at T0, T24, T48, T72 were tested for the presence of various cytokines using a 18plex (Cytokine names: GM-CSF, IL-8(CXCL8), G-CSF(CSF-3), IL-1beta, Granzyme-B, IFNg, IL-10, Perforin and TNFa) assay kit (Thermo Fisher) and Luminex Magpix instrument. Data was analysed using MILLIPLEX™ Analyst software.

LDH Assay: The cultured supernatants at T0, T24, T48, T72 were used to determine the LDH release using a colorimetry assay kit (Cayman Chemical) and reading was taken in a Biotek plate reader.

IHC: Cleaved Caspase 3 IHC was performed with 5µm sections obtained from the FFPE block using Ventana IHC automated staining system. Scoring was performed by certified pathologists.

Farcast™ TiME Histo-culture platform:

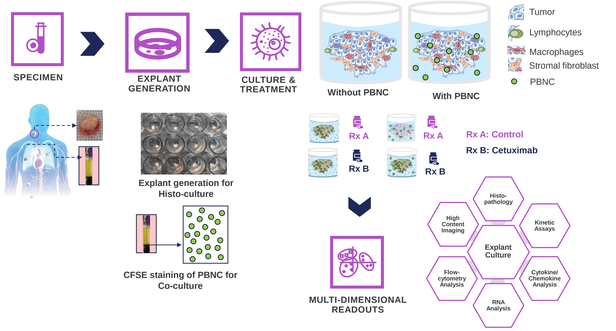

Fig. 1: Schematic representation of the Farcast™ TiME Histo-culture platform and multi-dimensional assays for various downstream evaluation

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Mechanism of cetuximab-mediated immune activity

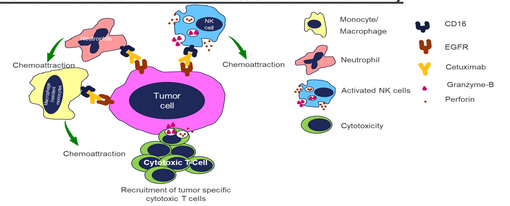


Fig.2 : Schematic representation of cetuximab-mediated immune activity demonstrating the involvement of different cell types (Cancer Treat Rev. 2018 Feb; 63: 48–60).

Patient Demography:

Sample ID	Age	Gender	Tumor	Grade	Site	Pre-treatment	EGFR levels in tumor at Baseline
S1	45	F	Primary	II	Tongue	2 cycles of paclitaxel and carboplatin	89%
S2	51	F	Primary	II	Alveolus	None	69%
S3	68	M	Primary	I	Lip	None	77%
S4	60	F	Primary	II	Pharynx	5 cycles of cisplatin	56%
S5	50	F	Primary	II	Supra Glottis	None	79%

Results:

IICs determined level of PBNC infiltration

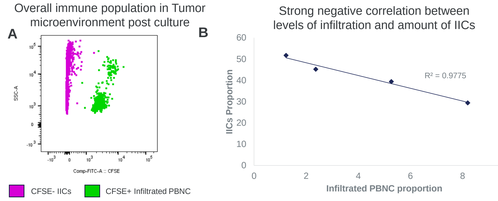


Fig.3: Evaluation of CFSE+ PBNC infiltration post 72 hrs of co-culture using flowcytometry analysis. A. Representative image showing CFSE+ infiltrated PBNC and IICs. B. Correlation of IICs vs infiltrated PBNC.

PBNC co-culture elicited enhanced ADCC response

Increased Granzyme-B secretion in response to treatment, on co-culture, observed in most samples

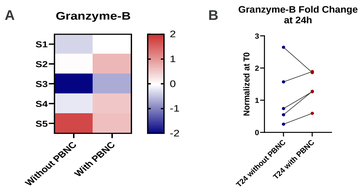


Fig.4: Evaluation of early ADCC response of Granzyme-B release, to cetuximab treatment with and without PBNC co-culture A. Heatmap of log2 fold change Granzyme-B relative to untreated control across the period of 72h of culture. B. Graph demonstrating the trend of Granzyme-B release within the first 24h of cetuximab treatment.

Heterogeneous infiltration of PBNC sub-population across samples on cetuximab treatment

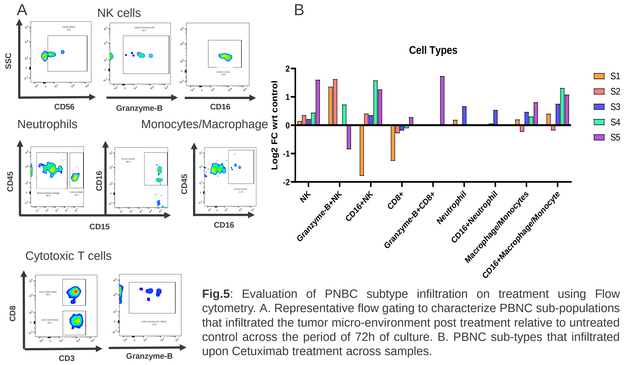


Fig.5: Evaluation of PNBC subtype infiltration on treatment using Flow cytometry. A. Representative flow gating to characterize PBNC sub-populations that infiltrated the tumor micro-environment post treatment relative to untreated control across the period of 72h of culture. B. PBNC sub-types that infiltrated upon Cetuximab treatment across samples.

Heterogeneous cytokine release across samples in response to Cetuximab treatment and Cytotoxicity

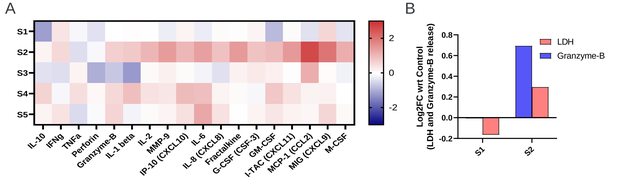


Fig.6: Evaluation of cytokine and LDH release over 72h culture period across samples. A. Heatmap was generated based on Log2 fold change relative to untreated control. B. LDH and Granzyme-B release in the sample S2 showing best cytokine release response and sample S1 showing least release response.

Common cetuximab mediated early response features across samples

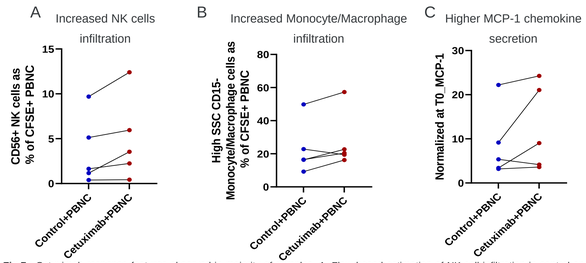
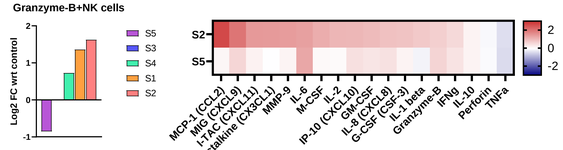


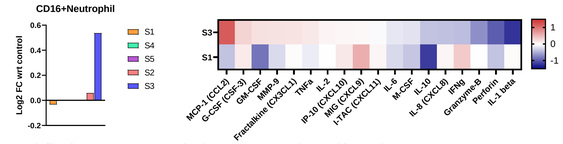
Fig.7 : Cetuximab response features observed in majority of samples. A. Flow based estimation of NK cell infiltration in control and cetuximab treated arms (fold change: 1.6 ± 0.8). B. Flow based estimation of Monocyte/Macrophage infiltration in control and cetuximab treated arms. C. MCP-1 chemokine release expressed as average across the 72h period of culture in control and cetuximab treated arms (Average fold change: 1.7 ± 0.9).

Cytokine release response mediated by different ADCC effector cell populations

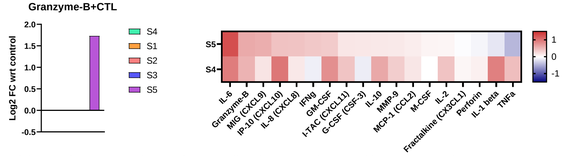
A. Highest NK cells mediated response in S2 and least response in S5



B. Highest Neutrophil mediated response in S3 and least response in S1



C. CTL infiltration response to cetuximab treatment was observed in S4 alone



D. Highest active monocyte/macrophage mediated response in S4 and least response in S1

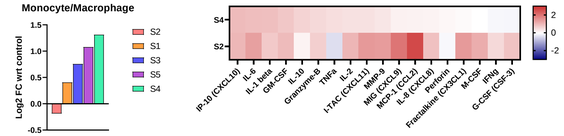


Fig.8 : Differential cytokine release patterns across samples mediated by different ADCC effector cell populations that infiltrated the TME. Data is shown as log2 fold change (left panel) the A. NK cells, B. Neutrophils, C. CTLs and D. Monocyte/Macrophages across samples and a heatmap (right panel) comparing cytokine release pattern for samples with the corresponding highest and lowest effector cell populations.

Conclusions:

- The study demonstrated ADCC response in the explant/PBNC co-culture platform leading to specific infiltration of effector sub-populations.
- Farcast™ TiME thus provides a unique platform to explore drug response mechanisms that involve immune cell infiltration into the tumor micro-environment. like heterologous adoptive cell and CAR-T therapies.

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