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

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Introduction:
Tumor 3D-histoculture provides a near-native Tumor immune Micro Environment (TME), making it well suited for evaluating immune cells in a tumoral microenvironment. Prior studies using TiME in human 3D tumor models demonstrated the utility of the platform in discerning the activity of immune cells in response to treatment. The aim of this study was to evaluate the utility of this platform in demonstrating the activity of ADCC in anti-cancer drug testing for human solid tumors (S). In this study, we investigated the utility of the platform in demonstrating the activity of ADCC in a human tumor histo-culture platform (TiME) using PBNC or Granzyme CULTURE using Cetuximab. Fresh tumor explants were cultured with media and autologous plasma with or without Peripherial Blood Nuclear Cells (PBNC) that included lymphocytes, monocytes, NK cells and dendritic cells. TiME was tested from the matched donor blood samples before treatment and after treatment. The TME platform was cultured with treated with 1×10^6 genetix (anti-G722) at control and in control and in culture for 72 hrs. Media was replaced every 24 hours. Response was evaluated using flow cytometry, cytokine-chemokine and LDH release assays.

Tumor explant culture:
Tumor explants were dissociated post culture into single cells and stained with LIVE/DEAD and propidium iodide, and residual of fluorescence tagged antibodies (Lineage markers, CD3, CD8, CD4, CD16, CD56, CD193, CD14). Data was acquired using BD LSR Fortessa Flow cytometer with appropriate compensation controls and analysed using Flowjo software.

Cytokine Analysis:
The cultured supernatants of Ti, T24, T48, T72 were tested for the presence of cytokines using a multiplexed human cytokine Bio-Plex panel (Bio-Rad). The values for each analyte were normalized to G-CSF (IL-3, IL-6, IL-10, TNF-alpha). Data was analysed using MILLIPLEX™ Analyst software.

LDH Assay:
The cultured supernatants at Ti, T24, T48, T72 were used to determine the LDH activity using a colorimetric assay at 490nm wavelength and reading was taken in a Bio-Rad plate reader with 1% triphenyltetrazolium chloride (TTC) and cleaved Caspase 3/3H was performed with 5μm sections obtained from the FFPE block using IHC and automated scanning system.

Farcast® TiME Histo-culture platform:
The Farcast® TiME platform uses organotypic tumor models to study the effects of antibodies and drugs on a tumor environment. The platform consists of tumor explants, autologous T cells, and PBNCs. The tumor explants can be treated with antibodies or drugs, and the response of immune cells can be evaluated using flow cytometry, cytokine-chemokine, and LDH release assays.

Results:

**Granzyme-B+NK cells**
- Showed increased expression in the control compared to treatment groups.
- The expression was highest in the S1 group, followed by S2, S3, and S4.
- This suggests that treatment with PBNC or Granzyme CULTURE using Cetuximab resulted in an increase in Granzyme-B+NK cell expression.

**CD56+CD16+ NK cells**
- Increased expression was observed in the S1 group compared to the control.
- Treatment with PBNC or Granzyme CULTURE using Cetuximab resulted in an increase in CD56+CD16+ NK cell expression, with the highest expression observed in the S1 group.

**Cytotoxicity**
- The cytotoxicity of the immune cells was evaluated using LDH release assay.
- The cytotoxicity was highest in the S1 group, followed by S2, S3, and S4.

**Cytokine release**
- The release of various cytokines was evaluated using bio-plex panel.
- The cytokine release was highest in the S1 group, followed by S2, S3, and S4.

**Cytokine signature**
- The cytokine signature was analyzed using flow cytometry.
- The signature suggested an increase in the release of pro-inflammatory cytokines.

**Conclusion:**
- The study demonstrated ADCC response in the explant/Farcast TiME co-culture platform leading to specific inhibition of effector sub-populations.
- Farcast™ TiME provides a unique platform to explore drug response mechanisms that involve immune cell infiltration into the tumor micro-environment, like heterogeneous adaptive cell and CAR-T Therapies.