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Differential T cell response within the tumor microenvironment observed across different indications

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INTRODUCTION:

Cytotoxic T-cells (CTLs) in the **T**umor **i**mmune **M**icro **E**nvironment (**TiME**) play an important role in mounting anti-tumor immune response. Immunotherapeutic (I/O) agents like checkpoint inhibitor aim to prevent anergy whilst T-cell agonists co-stimulate CTLs to reinvigorate them, leading to tumor cytotoxicity. The presence of CTLs within tumors does not always guarantee response to I/O agents. Farcast™ Tumor immune Micro-Environment (TiME) is a human histo-culture platform which preserves tumor and stroma along with the immune compartment, over a 48-72 hour culture period. In this study we investigate the abundance and functionality of the intratumoral CTLs post stimulation, across 4 different tumor indications, in order to understand the differential response across samples and indications to immune cell stimulation. These include Head and Neck Squamous cell carcinoma (HNSCC), Renal Cell Carcinoma (RCC), stomach adenocarcinoma (ca-Stomach) and Triple Negative Breast Cancer (TNBC).

METHODS:

Patient tissue samples: Fresh surgically resected HNSCC samples along with matched blood were collected from consented patients.

Table 1: Donor demographics and clinical summary

I.D	Gender	Age	Indication	Site Details	Tumor Grade	Stage	Prior treatment	Histopathology impression
H1	Female	48	HNSCC	Buccal Mucosa / Left	I	III	Naïve	SCC
H2	Male	65	HNSCC	Alveolus / Left	I	II	Naïve	SCC
H3	Male	55	HNSCC	Alveolus / Left	II	III	Naïve	SCC
H4	Male	46	HNSCC	Tongue	II	II	Naïve	SCC
S1	Male	60	Stomach	Stomach	I	II	5-FU+Cisplatin	Adeno- Moderately differentiated
S2	Female	60	Stomach	Antrum	III	III	Naïve	Adeno-High Grade
S3	Female	60	Stomach	Stomach / O.G. Junction	II	III	Naïve	Adeno- Moderately differentiated
S4	Male	56	Stomach	Antrum	I	I	Epirubicin+Oxaliplatin + Capecitabine	Adeno
R1	Female	60	RCC	Kidney / Left	II	II	Naïve	CCC
R2	Female	60	RCC	Kidney / Left	II	II	Naïve	CCC
R3	Male	60	RCC	Kidney / Left	I	NA	Naïve	RCC
B1	Female	60	Breast	Breast / Left	I	II	AC + Paclitaxel	TNBC: Infiltrating Ductal Carcinoma

Head and Neck Squamous Cell Carcinoma (**HNSCC**), Squamous Cell Carcinoma (**SCC**), Stomach Adenocarcinoma (**Stomach**), Adenocarcinoma (**Adeno**), Renal Cell Carcinoma (**RCC**), Clear Cell Carcinoma (**CCC**), Triple Negative Breast Cancer (**TNBC**), Not Available (**NA**)

Histo-Culture workflow: The tumor sample was processed to generate thin explants, without enzymatic digestion, to retain the tumor microenvironment. The tumor explants were cultured with media containing autologous plasma. The explants were treated with anti-CD3 (10 ng/ml) plus IL2 (100 units/ml) for 48-72 hours. Culture supernatant was collected every 24 hour and stored for cytokine analysis. Media was replaced every 24 hours.

Flow cytometry analysis: The tumor explants were dissociated post culture into single cells and stained with Live/Dead dye, and cocktail of immune cell lineage and activation marker antibodies. Data was acquired using BD LSR Fortessa Flow cytometer with appropriate compensation controls and analyzed using FlowJo software.

Cytokine Analysis: The cultured supernatants at T_0, T_{24}, T_{48}, T_{72} were tested for the presence of various cytokines using Luminex Magpix instrument and data was analyzed using MILLIPLEX™ Analyst software.

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

Statistical analysis: Data was organized using Excel (Microsoft Office 365) and statistical analysis was p=performed using Prism V9.0 (Graph Pad)

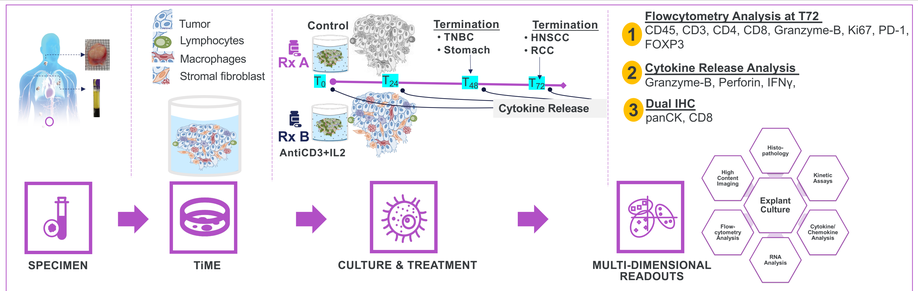

Fig. 1: Schematic representation of Farcast™ TiME Histo-culture platform work-flow and downstream assays used for treatment response evaluation. (Treatment arms: RxA – Media Control; RxB: treated with antiCD3+IL2)

RESULTS:

CD8+ immune cells were observed within the TIL populations across all indications at baseline

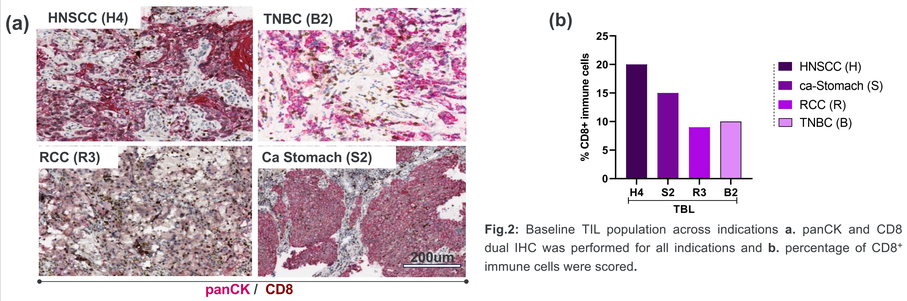

Fig.2: Baseline TIL population across indications **a.** panCK and CD8 dual IHC was performed for all indications and **b.** percentage of CD8+ immune cells were scored.

Live CD3+ immune cells were observed across all indications post culture

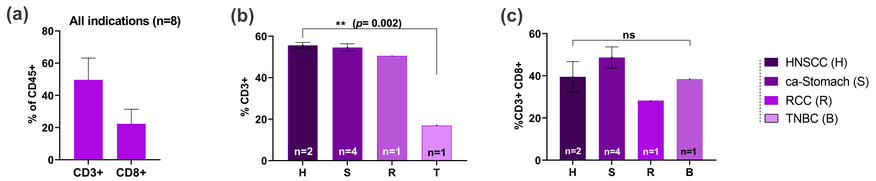

Fig. 3: Baseline immune cell phenotyping was carried out for different immune cell sub-populations across different tumor indications. **a.** Proportions of CD3+, CD8+ within CD45+ cells averaged across all indications. **b.** Proportion of T- cells in each indication. **c.** Proportion of CD8+ T cells within CD3+ in each indication. Data represented as Mean ±SEM, statistical analysis was performed using one way ANOVA.

ca-Stomach and RCC contained relatively higher proportions of activated (Granzyme B+) and exhausted (PD1+) CD8+ T-cells at baseline

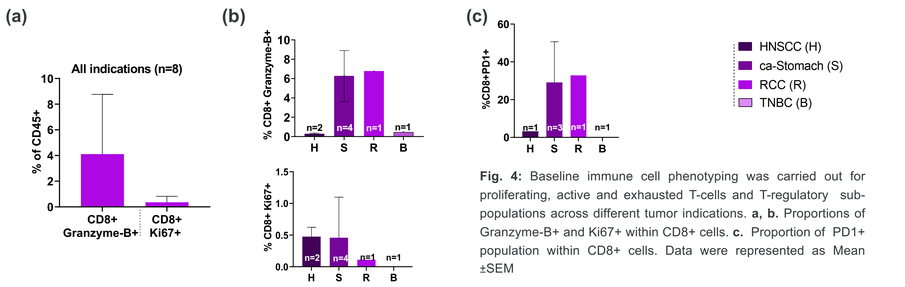

Fig. 4: Baseline immune cell phenotyping was carried out for proliferating, active and exhausted T-cells and T-regulatory sub-populations across different tumor indications. **a, b.** Proportions of Granzyme-B+ and Ki67+ within CD8+ cells. **c.** Proportion of PD1+ population within CD8+ cells. Data were represented as Mean ±SEM

Increase in T-cell proliferation and activity observed on anti-CD3 stimulation

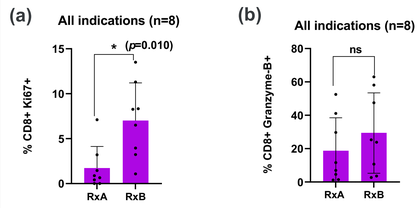

Fig. 5: T-cell response after stimulation with anti-CD3+IL2 across all indications **a.** Ki67+ CD8+ T- cell proportion was evaluated in the control (RxA) and anti-CD3+IL2 treated (RxB) arms. **b.** Granzyme-B+ CD8+ T-cell proportion was evaluated in the control and treated arms. Statistical significance was computed using two-tailed unpaired T-test.

Enhanced infiltration of CD8+ immune cells observed on treatment in HNSCC and CaBr

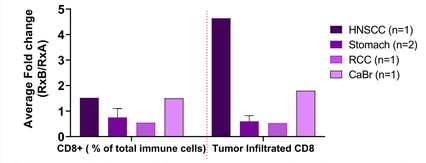

Fig.6: panCK and CD8 dual IHC was performed on all indications **Left panel:** CD8+ cells were scored as percentage of total immune cells. Data is represented as a fold change RxB/RxA. **Right panel:** Tumor Infiltrated CD8+ immune were scored. Data is represented as a Mean fold change ± SD of the treated arm with respect to the control.

Differential cytokine response levels were observed across indications in response to treatment: HNSCC showed a robust response followed by RCC

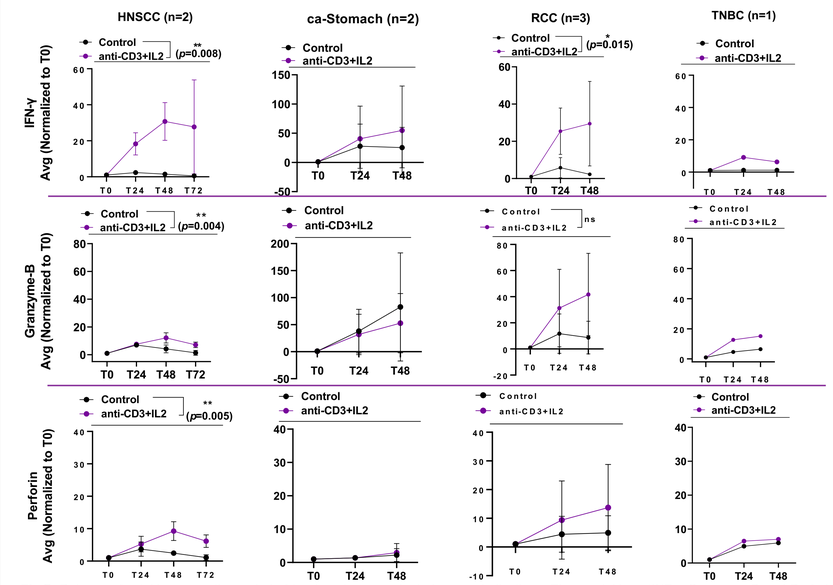

Fig.7: Release of cytokines over the period of treatment was evaluated across indications. Three cytokines (INFg, Granzyme-B and Perforin) were studied. Data is represented as Mean ±SD and statistical analysis performed using two-way ANOVA.

CONCLUSIONS

- Farcast histoculture platform contains live and functional Tumor Infiltrating Leukocytes
- Proportions of T-cells and their response to anti-CD3 stimulation varied across indications
- Differential T-cell response across indications could be attributed to baseline levels of exhaustion
- Farcast histoculture platform captures the complex interactions between tumor and intratumoral immune cell sub-populations with the potential to reproduce patient response to treatment

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