Abstract 155

Effect of treatment with immune checkpoint inhibitors on different immune sub-populations in the tumor microenvironment

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Background

Spatial contexture of tumor microenvironment (TME) influences immunotherapy response across multiple cancer indications. In this study, we investigated the roles of different immune cell types in the TME in determining response to a combination treatment comprising of Nivolumab and Ipilimumab using the Farcast™ TruTumor histoculture Head and Neck Squamous Cell Carcinoma (HNSCC) platform.

Methodology

Patient tissue samples: Head and Neck Squamous Cell Carcinoma (HNSCC) tissue samples (n=4) were collected along with matched blood from consented patients post-surgery.

No.	SAMPLE ID	AGE	GENDER	TUMOR GRADE	TUMOR STAGE	TUMOR SITE
1	P1	68	Male	2	IV	Right submandibular region
2	P2	60	Male	1	IV	Buccal Mucosa
3	P3	65	Female	1	III	Lateral border of tongue
4	P4	52	Male	2	II	Tongue

Table 1: Demography of the patient sample used in this study

Histoculture: Tissue explants were generated and allotted to arms and cultured for 72 hours. Four HNSCC samples were treated with anti-PD1 Nivolumab at a concentration of 132 µg/ml and Ipilimumab at a concentration of 90.8 µg/ml for 72 hours.

H&E & IHC: H&E and Cleaved Caspase 3 IHC was performed with 4µm sections obtained from the FFPE block using Leica automated multi-stainer system and Ventana IHC automated staining system, respectively. Scoring was performed by certified pathologists.

Multiplex Immunohistochemistry (mIHC): Using 4µm FFPE sections, mIHC (comprising anti-CD68, anti-CD163, anti-CD8, anti-CD4, anti-CD57, anti-FoxP3, anti-panCK and DAPI nuclear counterstain) was performed using TissueGnostics TSA mIHC kits for detection. Data was analyzed using StrataQuest analysis software.

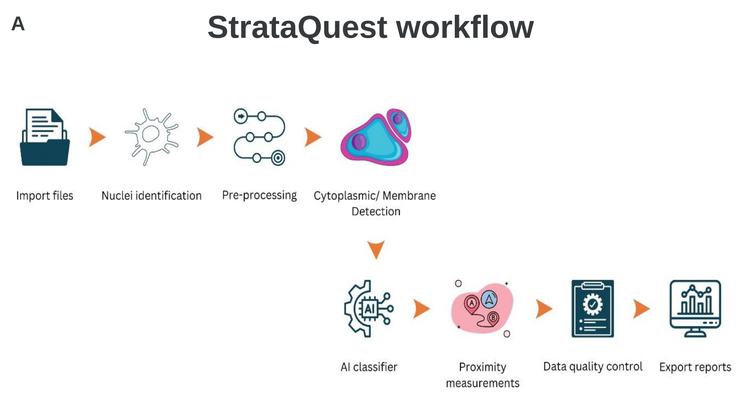

Fig. 1: (A) StrataQuest workflow (B) Representative mIHC images of different immune cell types

Cytokine: The culture supernatants were tested at T0, T24, T48, T72 time points for the presence of cytokines (perforin and granzyme-B) using Luminex MAGPIX instrument and data was analysed using MILLIPLEX™ Analyst software.

Graphical representations: All data analysis and graphical representations were done using GraphPad Prism (Version 9).

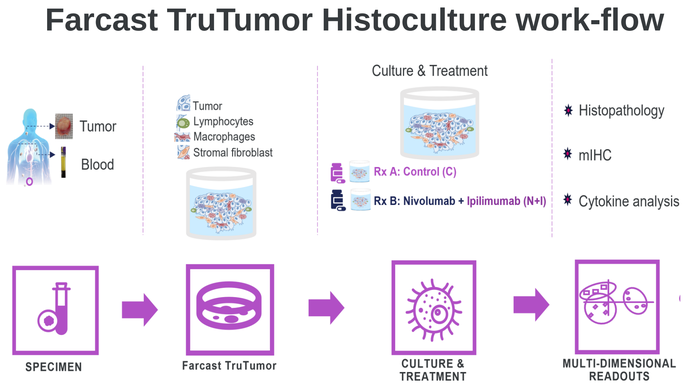

Fig. 2: Schematic representation of Farcast™ TruTumor Histoculture platform work-flow and downstream assays used for treatment response evaluation

Combination treatment with Nivolumab and Ipilimumab segregates the cohort

Parameter	Change with respect to control arm		
Cytolytic activity (Perforin/Granzyme B release)	>1.2 fold	>1.2 fold	<1.2 fold
Tumor cytotoxicity (cleaved caspase3 expression)	>1.2 fold		<1.2 fold
Categories	C1	C2	C3

Table 2: Definition of categories based on TruTumor platform cytolytic response and tumor cytotoxicity within tumor cells

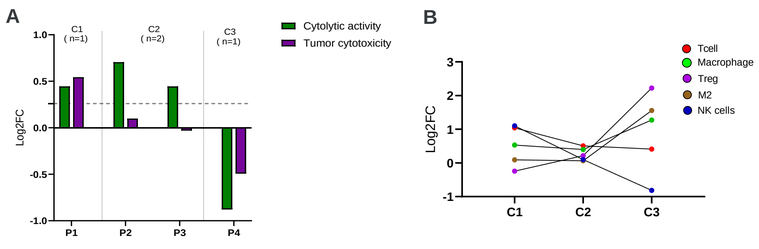

Fig. 3: (A) Bar graph representing tumor cytotoxicity and cytolytic activity on combinatorial treatment with Nivolumab and Ipilimumab (n=4). (B) Graphs representing cell types on treatment

C1 displays tumor cytotoxicity and cytolytic activity on treatment

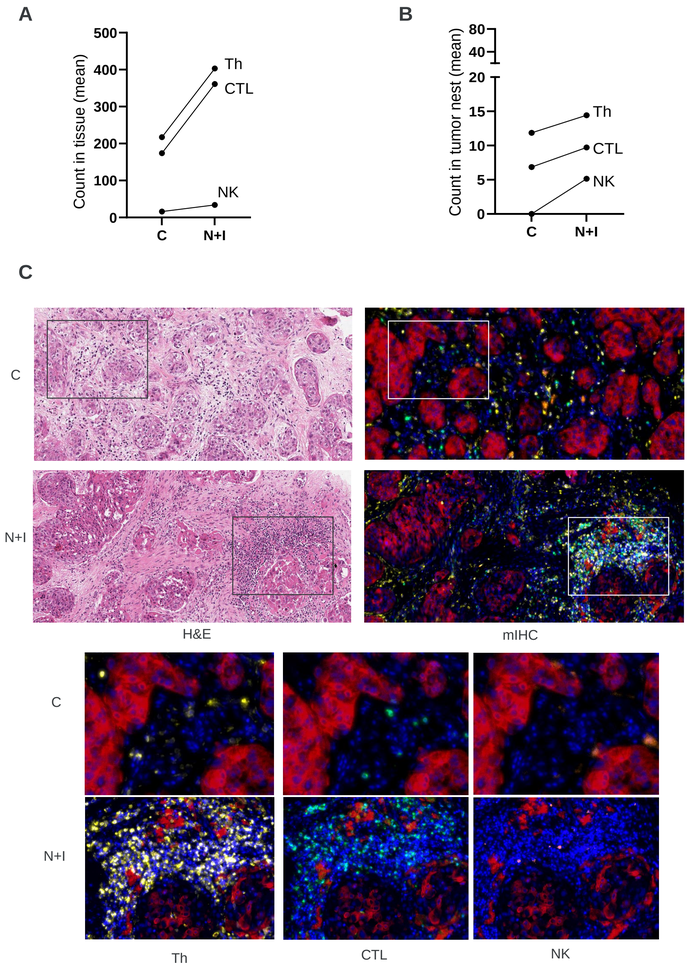

Fig. 4: Graphs representing CTL, Th and NK cell type in (A) tissue and (B) tumor (C) Representative images showing spatial contexture of the immune cell in control and on treatment

C2 shows infiltration of T cells and macrophages but not NK cells on treatment

Fig. 5: Graphs representing CTL, Th and NK cell type in (A) tissue and (B) tumor, (C) Representative mIHC images showing CD4 and CD8 cells in tumor area on treatment

C3 shows decrease in infiltration of cytolytic cells in tumor on treatment

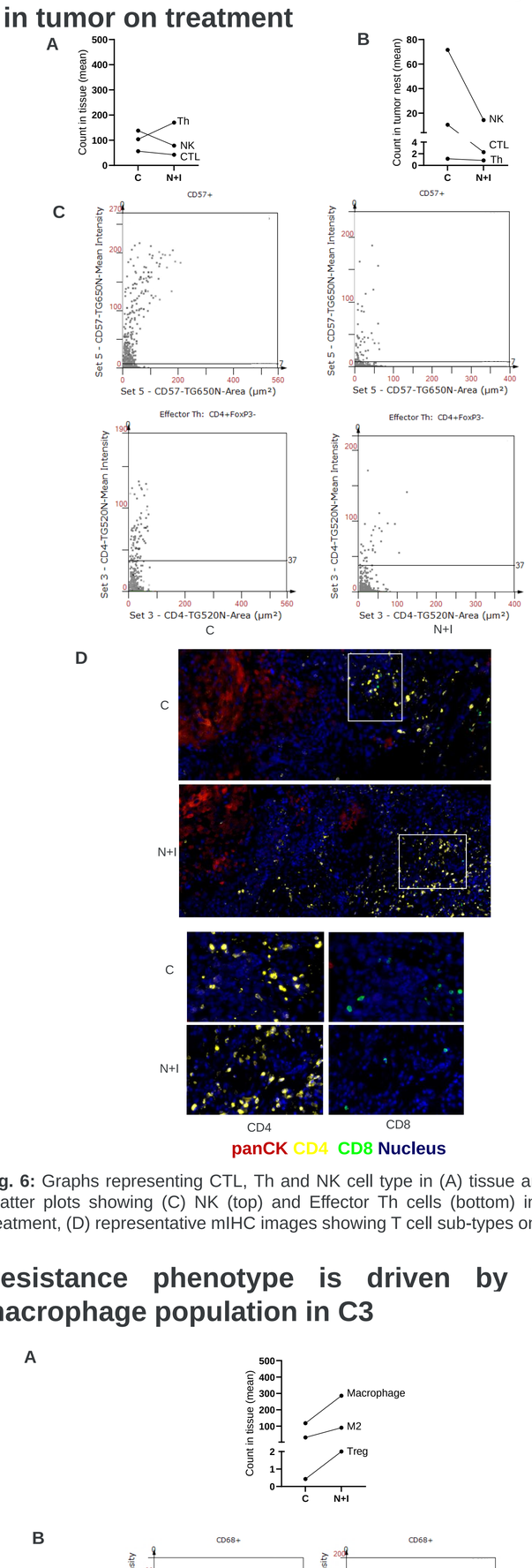

Fig. 6: Graphs representing CTL, Th and NK cell type in (A) tissue and (B) tumor, 2D scatter plots showing (C) NK (top) and Effector Th cells (bottom) in control and on treatment, (D) representative mIHC images showing T cell sub-types on treatment

Resistance phenotype is driven by Treg and macrophage population in C3

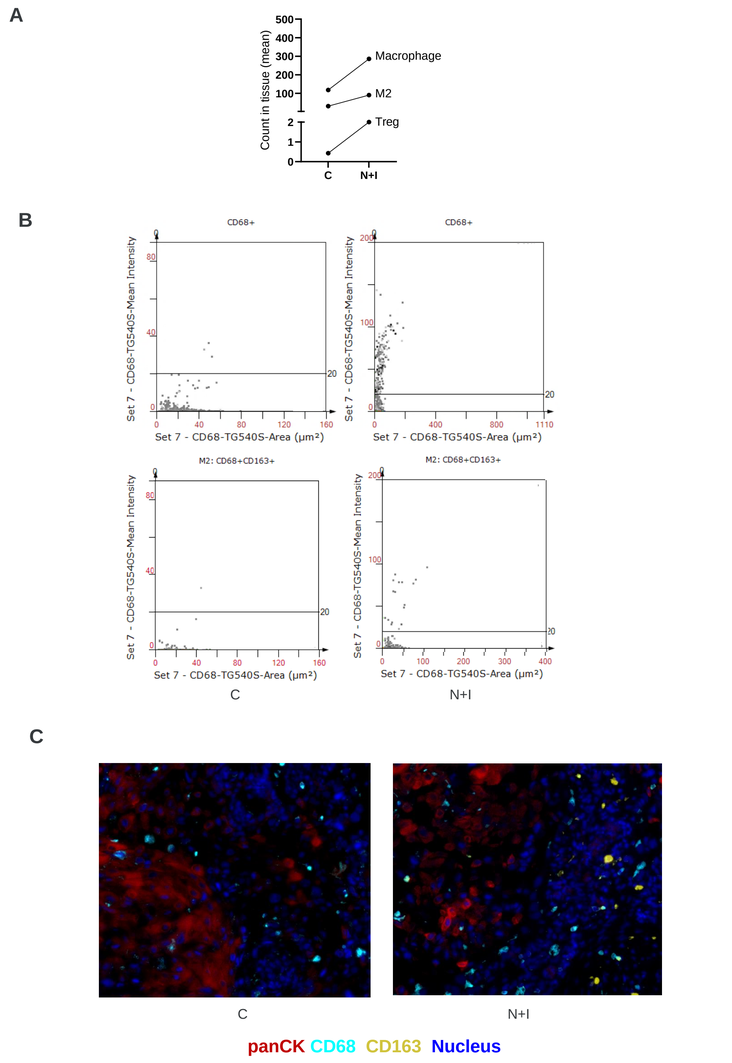

Fig. 7: Graphs representing (A) macrophage, M2 and Treg cell types in tissue, 2D scatter plots showing the (B) total macrophage (top) and M2 macrophage (bottom), (C) representative mIHC images showing macrophage infiltration in the tumor region on treatment

Conclusion

Treatment driven tumor cytotoxicity is dependent not only on the immune cell prevalence but also its spatial organization.