Poster No: A018

Characterization of macrophage population in Head and Neck Squamous Cell Carcinoma and Renal Cell Carcinoma and their role in modulating immune checkpoint blockade response

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Introduction

Tumor-associated macrophages (TAMs) and monocytes are an integral part of tumor microenvironment (TME) which modulates disease progression and drug response. M1 and M2 are the two well-defined subtypes of TAMs whose polarization influence response to immune checkpoint inhibitors (ICIs). Unavailability of data from complex models poses limitations to extensive characterization of these immune subpopulations. In this study, we have evaluated the role of monocytes and TAMs in modulating response to ICI, using the TM Farcast TruTumor histoculture platform.

Methods

Sample collection: Two different cancer indications, Head and Neck Squamous Cell Carcinoma (HNSCC) and Renal Cell Carcinoma (RCC) were used in the study. HNSCC (n = 30) and RCC (n = 20) tissue samples were collected along with matched blood from consented patients, post-surgery.

Histoculture: Tissue explants were generated and allotted to arms and cultured for 72 hours. Sixteen out of 30 HNSCC samples and 13 out of 20 RCC samples were treated with anti-PD1, Nivolumab, at a concentration of 132 µg/ml. Macrophage/monocyte sub-populations were characterized by performing flow cytometry and mIHC, T cell activity was assessed using interferon gamma (IFN-γ)) analysis, tumor cell cytotoxicity was assessed using cleaved Caspase 3 expression in tumor cells and reduction in tumor content by histopathological evaluation.

Flow Cytometry: The tumor explants were dissociated post culture with various treatments 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: Supernatants collected at T0, T24, T48, T72 were tested for the presence of cytokine (IFN-γ) using Luminex Magpix instrument and data was analysed using MILLIPLEX™ Analyst software.

Multiplex Immunohistochemistry (mIHC): Using 4µm FFPE sections, mIHC (comprising anti-CD68, anti-CD163/206, anti-CD8, anti-panCK and DAPI nuclear counterstain) was performed using Opal dyes (Akoya Biosciences or TissueGnostics TSA mIHC kits) for detection. Data was analyzed using QuPath analysis software.

Statistical Analysis: All data analysis and graphical representations were done using GraphPad Prism (Version 9). Mann-Whitney t-test was used to generate p-values. P value significance is represented as * (p<0.05) ** (p<0.01) *** (p<0.001). Correlation analysis was done by Spearman correlation.

Farcast™ TruTumor histoculture Platform

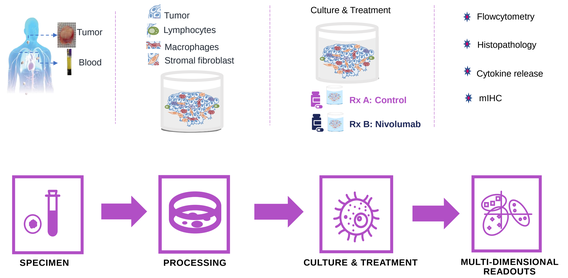

Fig. 1: Schematic representation of Farcast™ TruTumor histoculture platform work-flow and downstream assays used for treatment response evaluation.

Patient demography

N=30, HNSCC patient samples		
Parameters	**Categories**	**Values (%)**
Age	≤55.5	15 (50%)
	>55.5	15 (50%)
Gender	Female	17 (57%)
	Male	13 (43%)
Grade	Grade 1	7 (24%)
	Grade 2	19 (63%)
	Grade 3	3 (10%)
	Grade 4	1 (3%)
Stage	I	1 (3%)
	II	10 (33%)
	III	13 (43%)
	IV	6 (21%)
Primary/Recurrent	Primary	37 (100%)
Neo adjuvant treatment given	No	37 (100%)
Tumor Site	GBS	3 (10%)
	Tongue	4 (14%)
	Oral Cavity	1 (3%)
	Pharynx	1 (3%)
	Buccal Mucosa	16 (53%)
	Alveolus	4 (14%)
	Lip	1 (3%)

N=20, RCC patient samples		
Parameters	**Categories**	**Values (%)**
Age	≤53.5	10 (50%)
	>53.5	10 (50%)
Gender	Female	4 (20%)
	Male	16 (80%)
Grade	Grade 1	13 (65%)
	Grade 2	6 (30%)
	Grade 4	1 (5%)
Stage	I	7 (35%)
	II	7 (35%)
	III	2 (10%)
	IV	1 (5%)
	NA	3 (15%)
Primary/Recurrent	Primary	24 (100%)
Neo adjuvant treatment given	No	24 (100%)

RCC TME is enriched in M1 macrophages while HNSCC has higher proportion of Monocytes

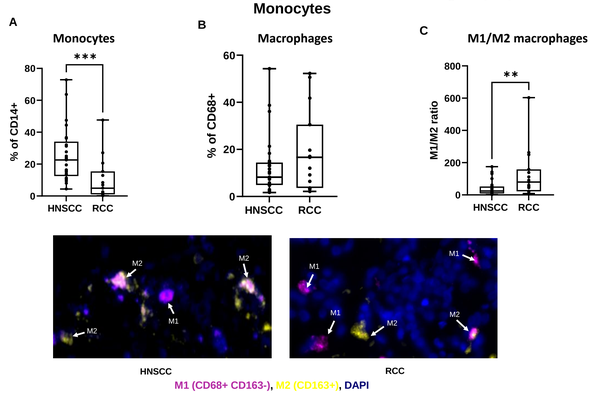

Fig. 2: Percentage of **A.** CD14+ cells **B.** CD68+ cells **C.** Ratio of M1/M2 cells in HNSCC (n=29) and RCC (n=15) samples determined by flow cytometry. **D.** Representative mIHC images showing M1 (CD68+ CD163-), M2 (CD163+) macrophages in the tumor microenvironment in an HNSCC and RCC sample.

Nivolumab treatment elicits response stronger IFNg release response in HNSCC compared to RCC

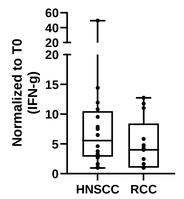

Fig. 4: Graph showing IFN-g release in HNSCC (n=16) and RCC (n=13) samples upon Nivolumab treatment.

RCC exhibits stronger correlation between IFNg release response and tumor cytotoxicity than HNSCC

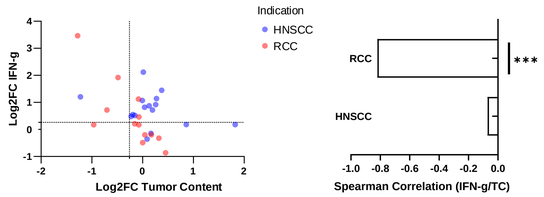

Fig.5. A. Two-dimensional plot showing change in IFNg secretion and decrease in tumor content for HNSCC and RCC samples **B.** Graph showing negative correlation between IFN-g release and tumor content (TC) in HNSCC (n=16) and RCC (n=13) samples post Nivolumab treatment.

Effect of Nivolumab treatment on CD8+GzmB+ and M1/M2 polarization is similar in both indications

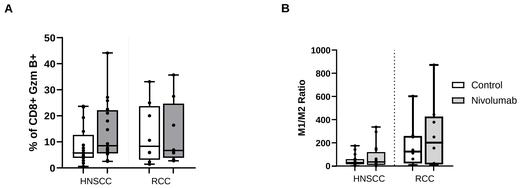

Fig. 6: Cohort level **A.** % of CD8+GzmB+ **B.** M1/M2 ratio upon Nivolumab treatment in HNSCC (n=16) and RCC (n=8) samples.

High IFNg release on Nivolumab treatment does not lead to improved efficacy in HNSCC

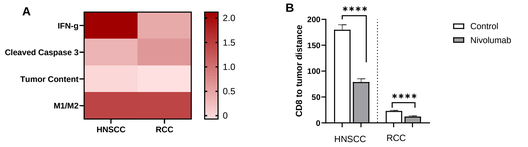

Fig. 7: A. Heatmap showing IFN-g release, caspase content, tumor content and M1/M2 profile

B. Graphical representation of CD8 distance from distance iin HNSCC (n=1) and RCC (n=1) samples.

RCC exhibits higher M1 proximity to tumor than HNSCC

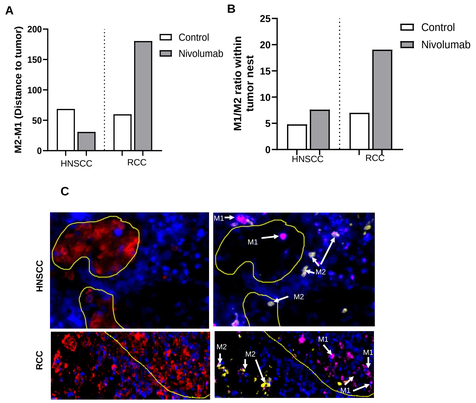

Fig. 8: A. Bar graph showing M1 & M2 distance to tumor nest. **B.** M1/M2 ratio within 5 um of tumor nest upon Nivolumab treatment. **C.** Representative mIHC images showing M1 (CD68+ CD163-), M2 (CD163+) macrophages in the TME of HNSCC and RCC sample post Nivolumab treatment. The tumor region is demarcated by yellow line.

Conclusion

- Monocyte and macrophage mediated immunosuppressive TME in HNSCC adversely affects Nivolumab efficacy. Combination with a myeloid reprograming agent might improve response of HNSCC to anti-PD1 therapy
- Farcast TruTumor, thus provides a unique platform that elucidates indication specific TME to test for optimal efficacy of combination therapies.

Acknowledgement

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