Abstract No: 4568

Evaluating response of Renal Cell Carcinoma to Tyrosine Kinase Inhibitor and Immune checkpoint inhibitor using a human histo-culture platform

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Introduction

Tyrosine Kinase Inhibitors (TKIs) and Immune checkpoint inhibitors (ICIs) are used in first line treatment of clear cell renal cell carcinoma (ccRCC). Identifying patients who truly benefit from these treatments remains a challenge. Developing newer and better therapy options that fail in the clinical phase is yet another unmet need. Both these limitations could be addressed by employing testing platforms that best capture the heterogeneity and complexity of the tumor within the patient. Farcast™ TruTumor is a near native human histoculture platform which retains the tumor and stroma along with the intra-tumoral immune compartment post culture that holds promise to improve treatment outcomes in patients.

Methods

Patient tissue samples: Fresh, surgically resected clear cell Renal Cell Carcinoma (ccRCC) 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. Explants were treated with Sunitinib (TKI: 27.7ng/ml) or Nivolumab (Nivo, anti-PD1: 132µg/ml) and cultured for 72 hrs. Media was replaced every 24 hours.

Flow cytometry analysis: 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 Analysis: The cultured supernatants at T0, T24, T48, T72 were tested for the presence of cytokines (IFN-g, Granzyme-B) using Luminex Magpix instrument and data was analysed using MILLIPLEX™ Analyst software.

IHC: Cleaved Caspase 3 & CD8 IHC was performed with 4µm sections obtained from the FFPE block using Ventana IHC automated staining system. Scoring was performed by certified pathologists.

mIHC was performed using T cell panel (CD8, CD4, Foxp3, panCK and nucleus counterstain) on 4µm FFPE sections of post treatment samples. Data was analyzed using QuPath analysis software.

NanoString Analysis: The RNA extracted arm-wise from the explant TMA (Tissue Micro Array) FFPE block was quantified using Tape Station and 50ng of RNA based on DV200 concentration was used for running on the NanoString IO360 panel. Data was normalized and analyzed using the nSolver™ Data Analysis software for post treatment RNA samples. Gene expression signatures (GES) were analyzed to evaluate response.

Statistical analysis: All data analysis and graphical representations were done using GraphPad Prism (Version 9). Mann-Whitney unpaired t-test was used to generate P-values. P value significance is represented as * (p<0.05) ** (p<0.01). Heat maps were generated on GraphPad.

Farcast™ TruTumor Histoculture Platform

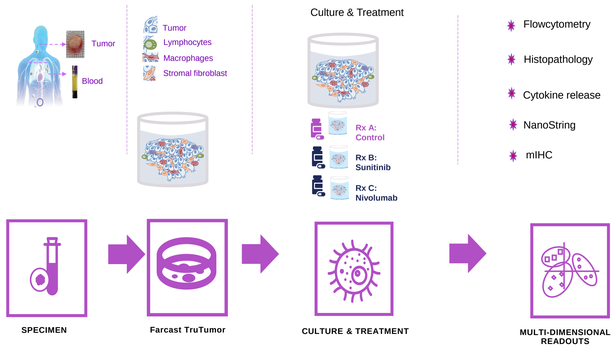

Fig. 1: Schematic representation of Farcast™ TruTumor Histoculture platform work-flow and downstream assays used for treatment response evaluation.

Mechanism of action of Nivolumab and Sunitinib

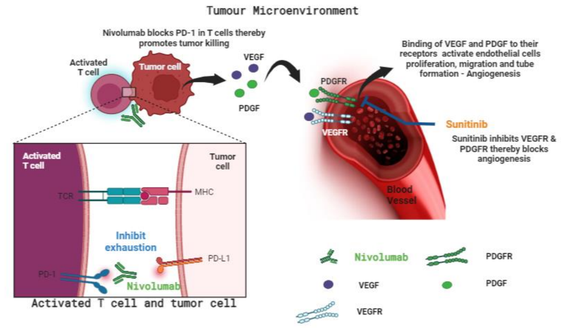

Fig. 2: Schematic representation depicting mechanism of action of Nivolumab and Sunitinib (created using BioRender.com)

Patient demography

n=4, HNSCC patient samples		
Parameters	**Categories**	**Values (%)**
Age	<54	2 (40%)
	>54	2 (50%)
Gender	Female	4 (100%)
Grade	Grade 1	3 (75%)
	Grade 2	1 (25%)
Stage	II	1 (25%)
	IV	3 (75%)
Primary/Recurrent	Primary	4 (100%)
Neo adjuvant treatment given	Yes	1 (25%)
	No	3 (75%)
Tumor Site	GBS	2 (50%)
	Tongue	1 (25%)
	Hard Palate	1 (25%)

n=13, RCC patient samples		
Parameters	**Categories**	**Values (%)**
Age	<60	6 (46.15%)
	≥60	7 (53.85%)
Gender	Female	3 (23.07%)
	Male	10 (76.93%)
Grade	Grade 1	6 (46.15%)
	Grade 2	7 (53.85%)
Stage	II	9 (69.24)
	III	1 (7.69%)
	IV	1 (7.69%)
	NA	2 (15.38%)
Primary/Recurrent	Primary	13 (100%)
Neo adjuvant treatment given	No	13 (100 %)
Tumor Site	Left Kidney	9 (69.23%)
	Right Kidney	4 (30.77%)

Difference in immune profiles of RCC and HNSCC

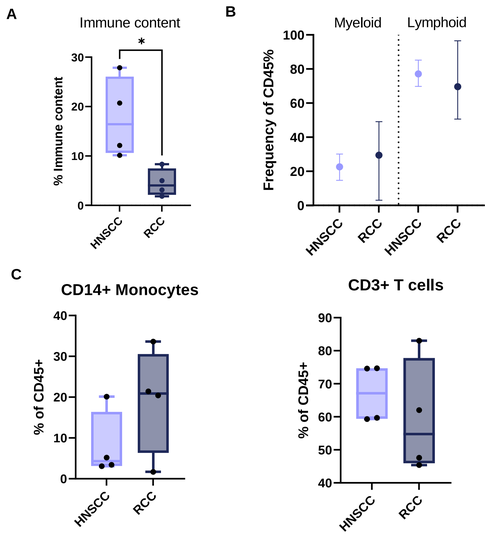

Fig. 3: A. Percentage immune content from H&E-stained slides in HNSCC and ccRCC samples at baseline (n=4). **B** Proportions of high SSC (Myeloid) and Low SSC (Lymphoid) sub-populations evaluated using flowcytometry. **C.** Percentage of predominant myeloid and Lymphoid cell types in HNSCC and RCC plotted as mean with range

Farcast™ RCC TruTumor Preserves tumor & immune cells post culture

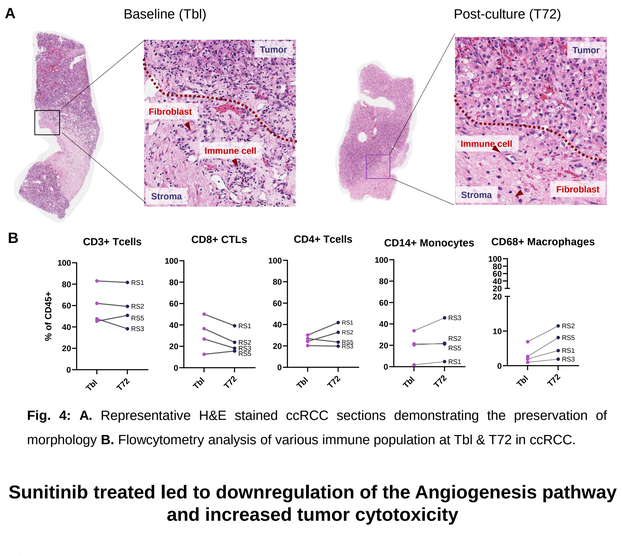

Fig. 4: A. Representative H&E stained ccRCC sections demonstrating the preservation of morphology **B.** Flowcytometry analysis of various immune population at Tbl & T72 in ccRCC.

Sunitinib treated led to downregulation of the Angiogenesis pathway and increased tumor cytotoxicity

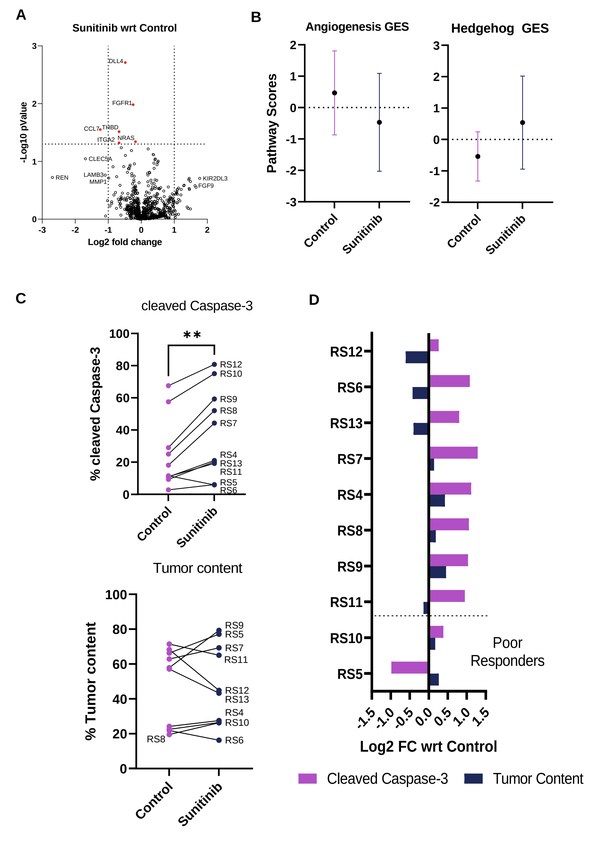

Fig. 5: A. Differential gene expression between control and sunitinib is represented as Volcano plot **B.** Plot showing GES pathway scores upon Sunitinib treatment. **C**. Cohort level tumor cytotoxicity response across samples. **D.** Waterfall graph showing sample level tumor cytotoxicity response. Poor responders (RS10 and RS5) have been labeled.

Sunitinib non-responder exhibited upregulated angiogenesis and tumor progression related pathways upon treatment

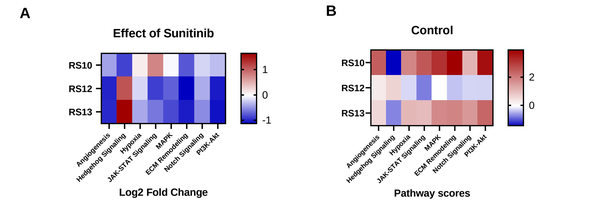

Fig. 6: A. Heatmap showing pathway scores on Sunitinib treatment expressed as Log2 Fold change with respect to control. **B.** Heatmap of pathway scores in control arm.

Sunitinib non-responders exhibited varying levels of response to Nivolumab treatment

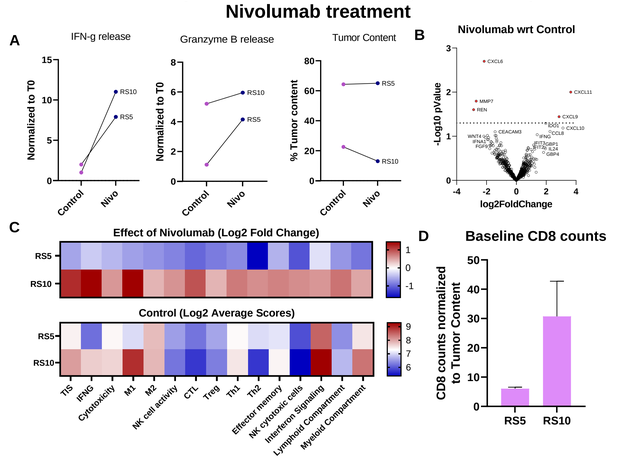

Fig. 7: A. Graphs showing IFN-g & Granzyme B cytokine release and tumour content variation upon treatment. **B.** Differential gene expression signature on nivolumab treatment wrt control is represented as volcano plot. **C.** Heatmap showing GES on nivolumab treatment as Log2 Fold change wrt control (top panel) and control arm as Log2 average GES (bottom panel) D. Graph showing baseline CD8 counts normalized to tumor content.

RS10 demonstrated classical Nivolumab response characteristics

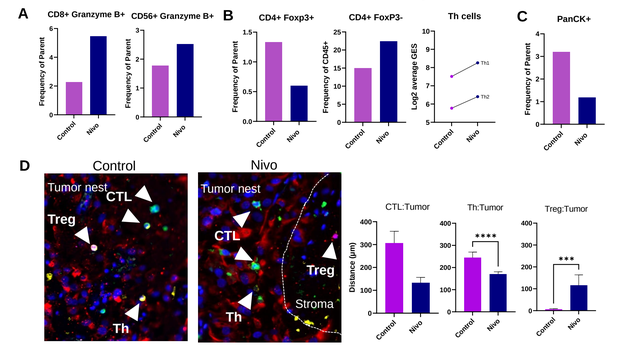

Fig. 8: A. Graph showing Granzyme B+ CD8+ and CD56+ NK cell variation on treatment. **B.** Graph showing variation in different CD4+ T cells on treatment. **C.** Graph showing variation in PanCK+ tumor cells on treatment. D. Representative images & graphs showing change in distance between CD4+FoxP3+ (Treg), CD8+ (CTL), CD4+FoxP3- (Th) and tumor cells.

Summary

Farcast™ TruTumor platform could provide powerful insights into the mechanisms of action for a wide range of therapy molecules in development to predict their efficacy in RCC. The platform also offers an opportunity to make personalized treatment choices for patients.