Abstract # 462

Pre-Selection of patients who would respond to combination of chemotherapy and low dose immunotherapy using human histoculture platform

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Background

Only 1-3 % of patients with Head and Neck Squamous Cell Carcinoma (HNSCC) in low- and middle-income countries can afford Nivolumab treatment even though it is approved for recurrent and metastatic disease [1]. Low Dose Nivolumab (LDN) has shown similar efficacy compared to Standard Dose Nivolumab (SDN) in renal and lung cancer [2,3]. A recent study demonstrated that HNSCC patient sub-cohort treated with chemotherapy (CT) and LDN showed improved progression-free and overall survival compared to sub-cohort treated with CT alone [1]. To better understand the added benefit of the combination treatment, it is important to rule out the contribution of CT alone in the responding patients that might not result in a durable response. To address this, we employed the Farcast™ TruTumor, a near native human histoculture platform which can compare multiple treatment response simultaneously for the same patient sample.

Methods

Patient tissue samples: Fresh surgically resected or biopsy HNSCC samples (n=20) along with matched blood were collected from consented patients.

Histo-Culture workflow: The tumor sample was processed to generate thin explants, without enzymatic digestion, to retain the tumor microenvironment. Tumor explants were distributed into arms and cultured with media and autologous plasma. These arms were treated in culture with either LDN (7.3 µg/ml) or SDN (132 µg/ml) and CT (Methotrexate (220.8µg/ml)+erlotinib (2.5µg/ml)+celecoxib (0.7µg/ml) or Paclitaxel (2.7µg/ml)+Carboplatin (37.1µg/ml) alone or CT+LDN for 72 hours. The response was evaluated using histopathology, interferon-γ (IFN-g) cytokine release, flow cytometry and NanoString.

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 IFN-g, using Luminex Magpix instrument and data was analysed using MILLIPLEX™ Analyst software.

IHC: Cleaved Caspase 3 IHC was performed with 5µm sections obtained from the FFPE block using Ventana IHC automated staining system. Scoring was performed by certified pathologists

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.

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

Farcast TruTumor Histoculture Platform

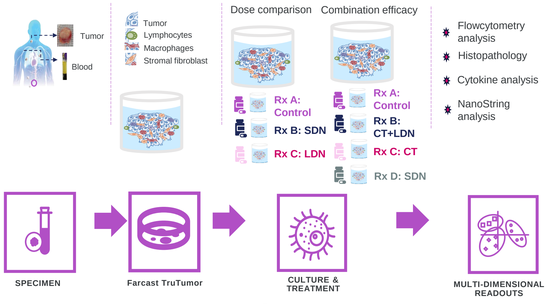


Fig. 1: Schematic representation of Farcast™ TruTumor Histoculture platform work-flow and downstream assays used for treatment response evaluation.

Patient Demography

Parameters	Categories	Values (%)
Age	<55	8 (40%)
	≥55	12 (60%)
Gender	Female	10 (50%)
	Male	10 (50%)
Grade	Grade 1	10 (50%)
	Grade 2	8 (40%)
	Grade 3	2 (10%)
Stage	II	4 (20%)
	III	3 (15%)
	IV	8 (40%)
	IVa	4 (20%)
	NA	1 (5%)
Primary/Recurrent	Primary	14 (70%)
	Recurrent	6 (30%)

Parameters	Categories	Values (%)
Collection procedure	Surgery	15 (75%)
	Biopsy	5 (25%)
Neo adjuvant treatment given	CT	1 (5%)
	RT	5 (25%)
	CT+RT	2 (10%)
	Naive	12 (60%)
Tumor Site	Tongue	3 (15%)
	Buccal mucosa	9 (45%)
	Others (Alveolus, Palate, Maxilla, Sub mandibular, GBS, neck skin nodule, Larynx/pharynx, Pyriform fossa)	8 (40%)

Table 1: Demography of patient (n=20) sample used for histoculture.

Complete receptor occupancy observed at Standard and Low dose Nivolumab treatment

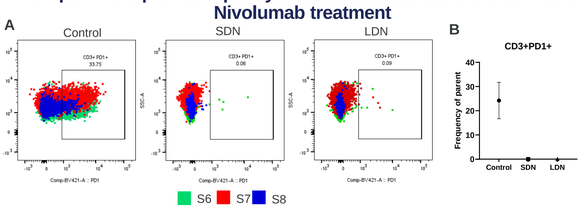


Fig. 2: A. Flowcytometry data showing masking of PD-1 receptor in standard dose Nivolumab (SDN) and Low Dose Nivolumab (LDN) treated arms. **B.** Graph showing CD3+ PD1+ cells in control and treated arms

Both doses exhibit similar T cell response

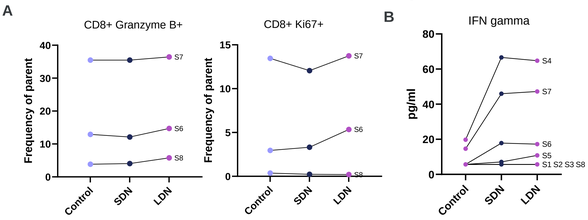


Fig. 3: A. Graph showing CD8+ Granzyme B+ and CD8+ Ki67+ population in control and SDN and LDN treated arms. **B.** Graph showing IFN gamma cytokine release upon treatment.

Comparable immune sub populations in response to SDN and LDN treatment

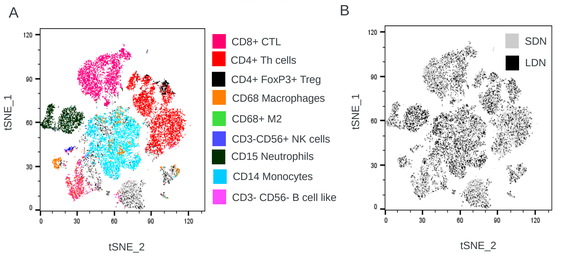


Fig 4: tSNE plot showing **A.** Various immune sub population in treated arms (n=3). **B**. Immune population in Standard dose Nivolumab (SDN) and Low dose Nivolumab (LDN) treated arms

SDN and LDN treatment exhibit similar tumor cytotoxicity

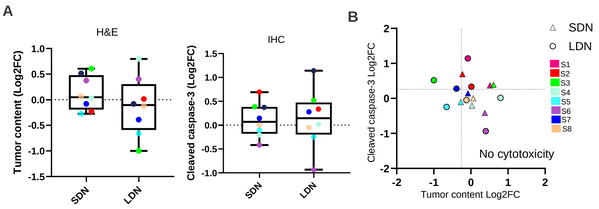


Fig. 5: A. Cohort level tumor cytotoxicity response in SDN and LDN treated arms. **B.** Dot plot showing Log2 fold change of tumor content against cleaved caspase 3 (n=8).

CT+LDN shows better response in 3 samples compared to CT & SDN

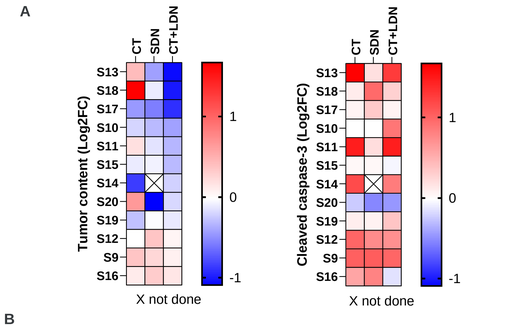
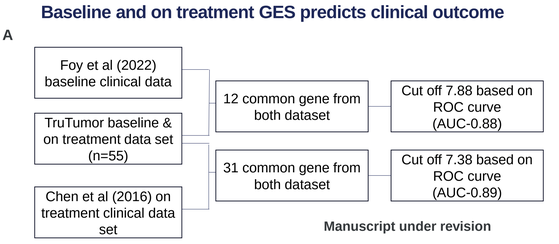


Fig. 6: A. Heatmap showing tumor content and cleaved caspase-3 expressed as log2FC (n=12). **B.** Graph representing percentage tumor content in control and treated arms.

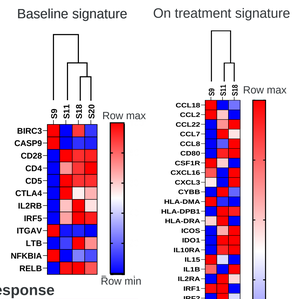
Baseline and on treatment GES predicts clinical outcome



B

ID	Baseline score	On treatment score
S9	7.00	6.84
S11	8.23	7.85
S18	8.68	7.99
S20	8.43	Not done

Responder
Non-Responder



C

TruTumor response

Clinical response	Responder	Non Responder
Responder	3	
Non-Responder		1

Clinical response is based on 5 treatment cycles

Fig. 7: A. Workflow for deriving gene set for prediction of response from TruTumor platform at baseline and on treatment. **B.** Heatmap showing the gene expression data for Baseline and on treatment samples (CT+LDN) represented as z-scores along with Euclidian distance based hierarchical clustering. **C.** TruTumor response prediction shows 100% concordance with clinical response.

Summary

The Farcast™ TruTumor platform thus provides the unique opportunity to identify patients who would truly benefit from treatment with CT+LDN combination. Furthermore, TruTumor response prediction showed 100% concordance with an observational trial where patients were treated with CT+LDN.

References

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