Identification of tumor microenvironment informed exosome biomarkers for predicting response to anti-PD1 therapy

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Background

On-treatment biomarkers for assessing the response to immune-oncology (I/O) therapy have a better predictive value than baseline markers, prior to treatment[1]. The Farcast TruTumor histoculture platform that preserves the near native tumor microenvironment (TME) has the potential to identify such biomarkers post treatment with I/O therapy[2]. Accessing fresh tumor sample is often challenging, limiting the clinical utility of such ex-vivo platforms. To address this limitation, we assessed exosomes from explant culture supernatants post-treatment, for identifying response biomarkers in the culture supernatant that mimic the TME response and will seek to validate these in exosomes from matched patient plasma.

Study design

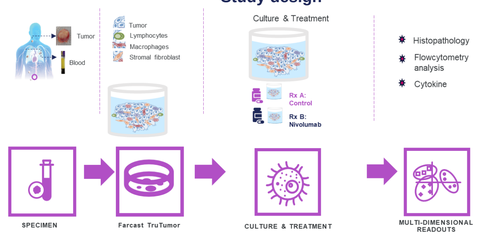

Fig. 1: Schematic representation of Farcast™ TruTumor Histoculture platform work-flow and downstream assays used for treatment response evaluation.

Methods

Patient tissue samples: Fresh, surgically resected Head and Neck Squamous Cell Carcinoma (HNSCC) tissue samples (n=4) 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 anti-PD1 (Nivolumab:132 µg/ml) and cultured for 72 hrs. Media was replaced every 24 hours. Response was evaluated using H&E, IHC, flow cytometry and cytokine release.

Exosome isolation and characterization: Exosomes were isolated from culture supernatant and plasma using a combination of precipitation and CD63/81/9 coated magnetic bead-based purification. Exosome size was verified using nano particle tracking analysis (NTA) and further characterized using multicolor flowcytometry.

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

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

Cytokine analysis: The cultured supernatants at T0, T24, T48, T72 were tested for the presence of cytokines (IFN gamma, Granzyme B, Perforin) using Luminex MAGPIX instrument and data was analysed using MILLIPLEX™ Analyst software.

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

Patient demography

S.No	SAMPLE ID	AGE	GENDER	TUMOR GRADE	TUMOR STAGE	HNSCC TUMOR SITE
1	S1	63	Female	2	IV	Buccal mucosa
2	S2	52	Male	2	IV	Lower alveolus
3	S3	71	Female	2	IVa	Buccal mucosa
4	S4	57	Female	1	IV	Posterior maxilla

Table 1: Donor demographics and clinical summary

Exosome characterization using NTA and flowcytometry

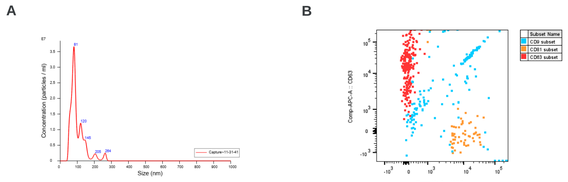

Fig. 2: A. Graph showing exosome size (nm) determined using NTA analysis. **B**. Flowcytometry data showing presence of CD9, CD63, CD81 positive exosomes after isolation from culture supernatant.

Exosome in culture supernatant and matched plasma

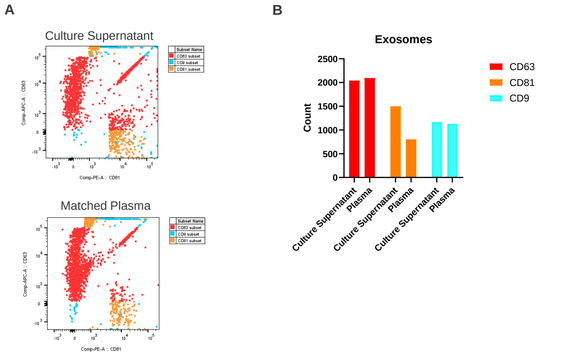

Fig. 3: A. Flowcytometry data showing presence of CD9, CD63, CD81 positive exosomes in culture supernatant and matched plasma. **B.** Graph showing presence of similar levels of CD63+ exosomes in culture supernatant and plasma.

T-cell reinvigoration and Tumor cytotoxicity in response to Nivolumab treatment

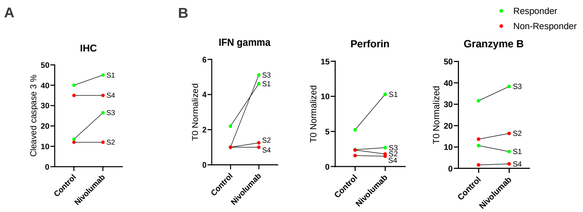

Fig. 4: A. Cleaved caspase-3 expression in tumor increased in responders on treatment with Nivolumab. **B.** IFN gamma, Perforin and Granzyme B release in control and Nivolumab treated arms.

T-cell derived exosome increase upon Nivolumab treatment

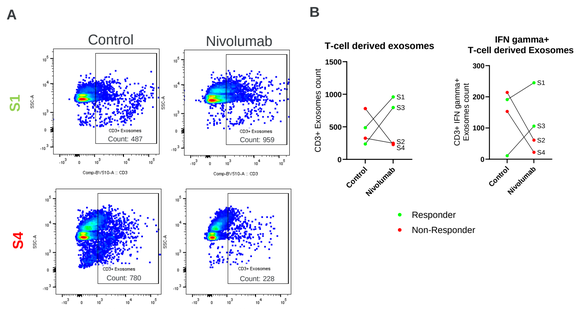

Fig. 5: A. Dot-plot showing CD3+ (T-cell derived exosome) in control and Nivolumab treated arms (Flowcytometry). **B.** Graphs showing CD3+ and CD3+IFN gamma+ exosomes.

Decrease in exhaustion upon Nivolumab treatment in responders

A

SAMPLE ID	PD1+ Occupancy in cells %	PD1+ Occupancy in exosomes %
S1	98	41
S2	98	35
S3	100	11
S4	99	0

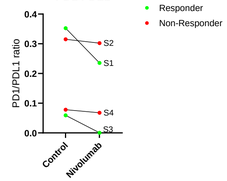

Fig. 6: A. Table showing PD1 occupancy (Nivolumab binding) in CD3+ T cells and CD3+ exosomes in Nivolumab treated arms. **B.** Plot showing PD1/PDL1 ratio in control and Nivolumab treated arms.

Increase in TCex/MPex ratio in responder

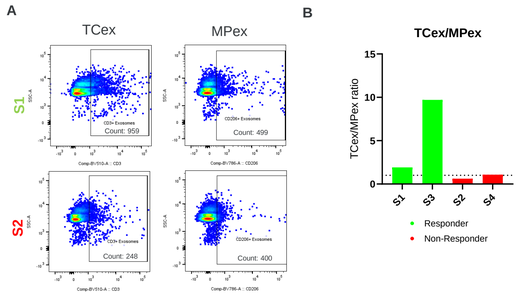

Fig. 7: A. Plot showing TCex (T-cell derived exosome) and MPex (M2 macrophage derived exosomes) in control and Nivolumab treated arms (Flowcytometry). **B.** Graphs showing ratio of TCex/MPex upon nivolumab treatment.

High levels of M2 macrophage in TME for non-responder

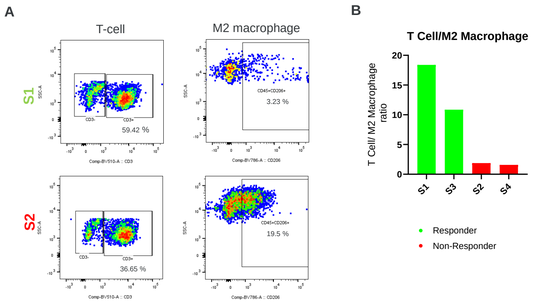

Fig. 8: A. Plot showing CD3+ T cells and CD206+ M2 macrophage in control and Nivolumab treated arms (Flowcytometry). **B.** Graphs showing T Cell / M2 macrophage ratio upon nivolumab treatment.

Summary

- Exosomal signals matched response phenotypes exhibited by the tumor microenvironment on treatment with anti-PD1 therapy.
- TruTumor platform presents a unique opportunity for identification of therapy response exosomal biomarkers that could be potentially tracked in periphery.

References

1. Chen, PL et al. Cancer Discov. 6, 827–837 (2016)
2. Basak, NP et al. Nat Commun. 15, 1585 (2024)

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