Elucidating the role of peripheral blood immune cell versus Intratumoral Immune Cells (IICs) in a tumor histo-culture model in response to Immune checkpoint inhibitors

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INTRODUCTION:

Immune cells in the Tumor immune Microenvironment (TiME) play an important role in mounting response to immunotherapy. The relative distribution and functional status of **Intratumoral Immune Cells** (**IICs**) are distinct from the peripheral blood immune cells from the same patient [1,2]. Model systems that co-culture **Peripheral Blood Nucleated Cells** (**PBNCs**) with tumor cells, spheroids or tumoroids have helped assess response to immunotherapeutic agents. However, these models are ineffective in capturing the complex immunobiology of native human tumors.

Farcast™ TiME is a human tumor histo-culture platform which retains the tumor, stroma and functional IICs post culture. In this study we used the Head and Neck Squamous Cell Carcinoma (HNSCC) TiME model to evaluate the added benefit of PBNC co-culture in modulating response to Immune Checkpoint Inhibitors (ICIs).

METHODS:

Patient tissue samples: Fresh, surgically resected HNSCC tissue samples were collected from consented patients. A matched blood sample from the patient was also collected.

Table 1: Donor demographics and clinical summary

Sample ID	Gender	Age	Indication	Site Details	Tumor Grade	Stage	Primary/Recurrent	Prior treatment
S1	Male	58	HNSCC	Lower Lip	II	IVA	Local Recurrent	6C-Cisplatin along with RT (2017), 4C- MTX+ 5FU (2020)
S2	Male	58	HNSCC	Supra Glottis	II	III	Primary	Naïve
S3	Female	60	HNSCC	Left lower Alveolus	II	II	Primary	Naïve
S4	Male	61	HNSCC	Left lateral border of Tongue	II	III	Primary	Naïve
S5	Female	73	HNSCC	Right Buccal Mucosa	I	I	Primary	Naïve

MTX- Methotrexate; 5FU- 5 Fluro Uracil

Flow cytometry analysis: The tumor explants were dissociated post culture with various treatments into single cells and stained with with Live/Dead dye, and cocktail of immune cell lineage markers (CD45, CD3, CD8, CD14, CD15, CD68). Data was acquired using BD LSR Fortessa Flow cytometer with appropriate compensation controls and analysed using FlowJo software.

Cytokine analysis: The cultured supernatants at T_0, T_{24}, T_{48}, T_{72} were tested for the presence of various cytokines (IFN-γ, Granzyme-B, Perforin, IL-10 and TNF-α) 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.

Statistical analysis: Data was organized using Excel (Microsoft Office 365) and statistical analysis was performed using Prism V9.0 (Graph Pad).

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Farcast™ TiME histo-culture platform:

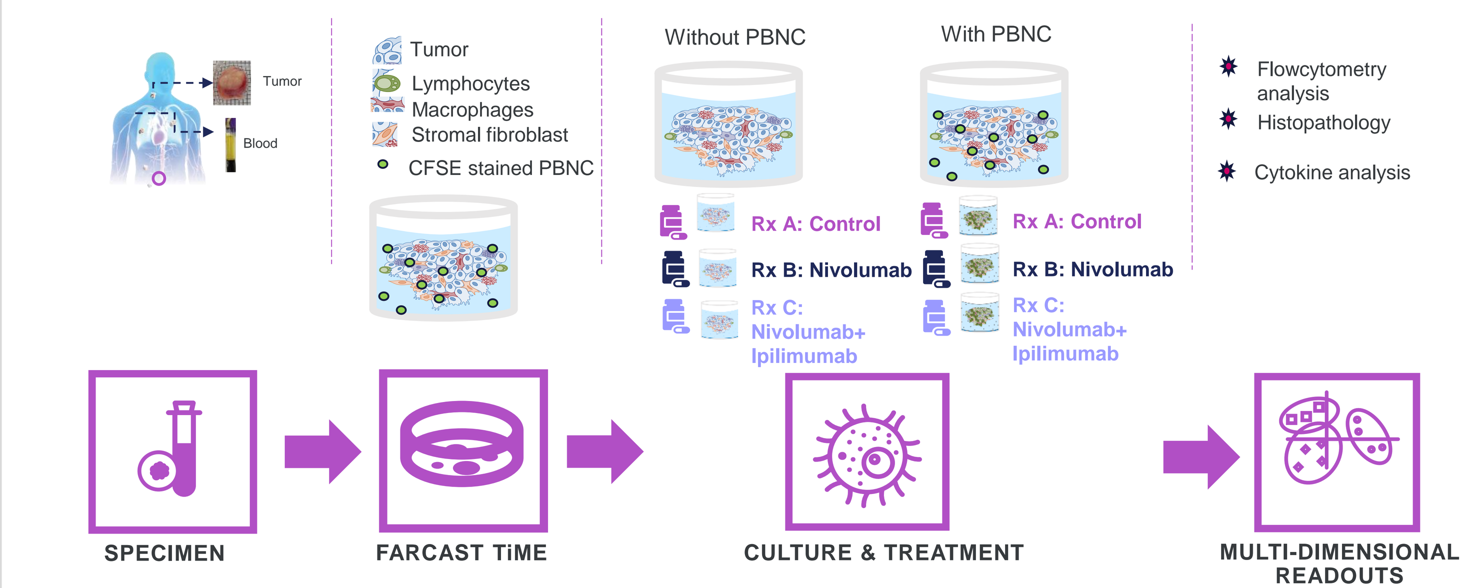

Fig. 1: Schematic representation of Farcast™ TiME Histo-culture platform work-flow and downstream assays used for treatment response evaluation.

Mechanism of Nivolumab and Ipilimumab

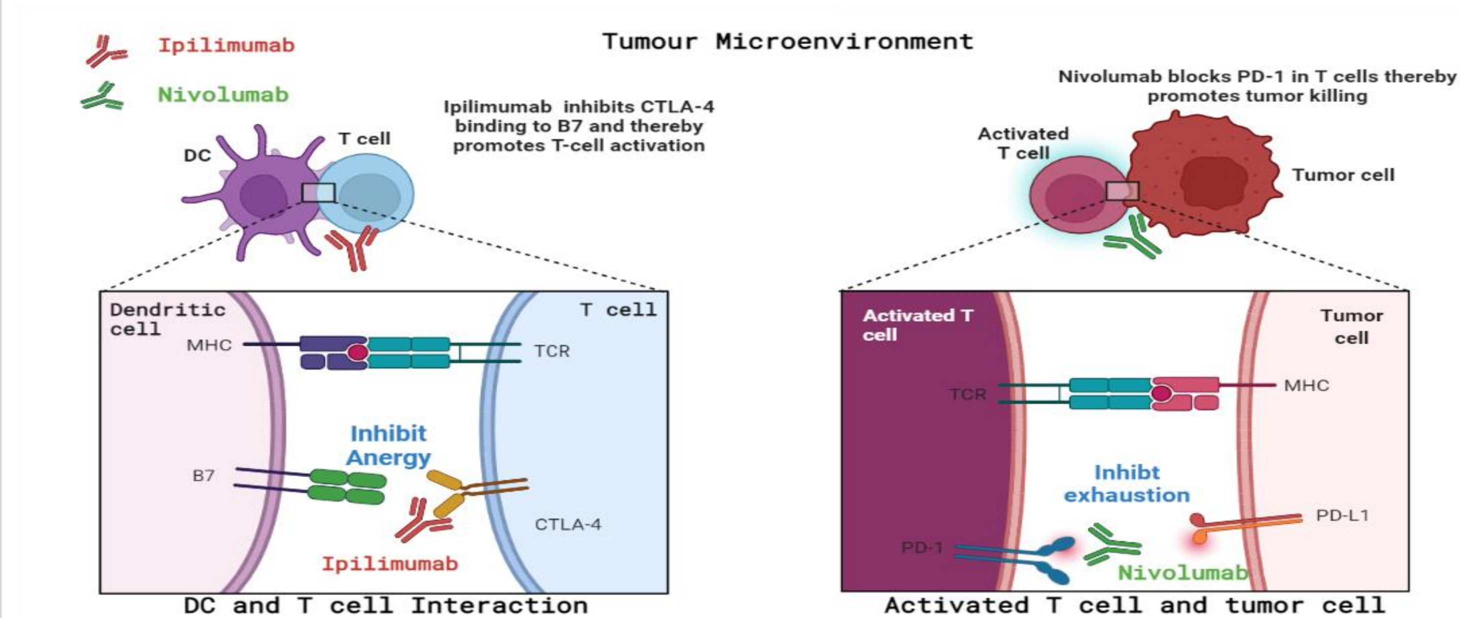

Fig. 2: Schematic representation depicting mechanism of action of Nivolumab and Ipilimumab (created using BioRender.com)

Results:

Tumor cytotoxicity was observed in S1 on drug treatment in absence of PBNCs co-culture

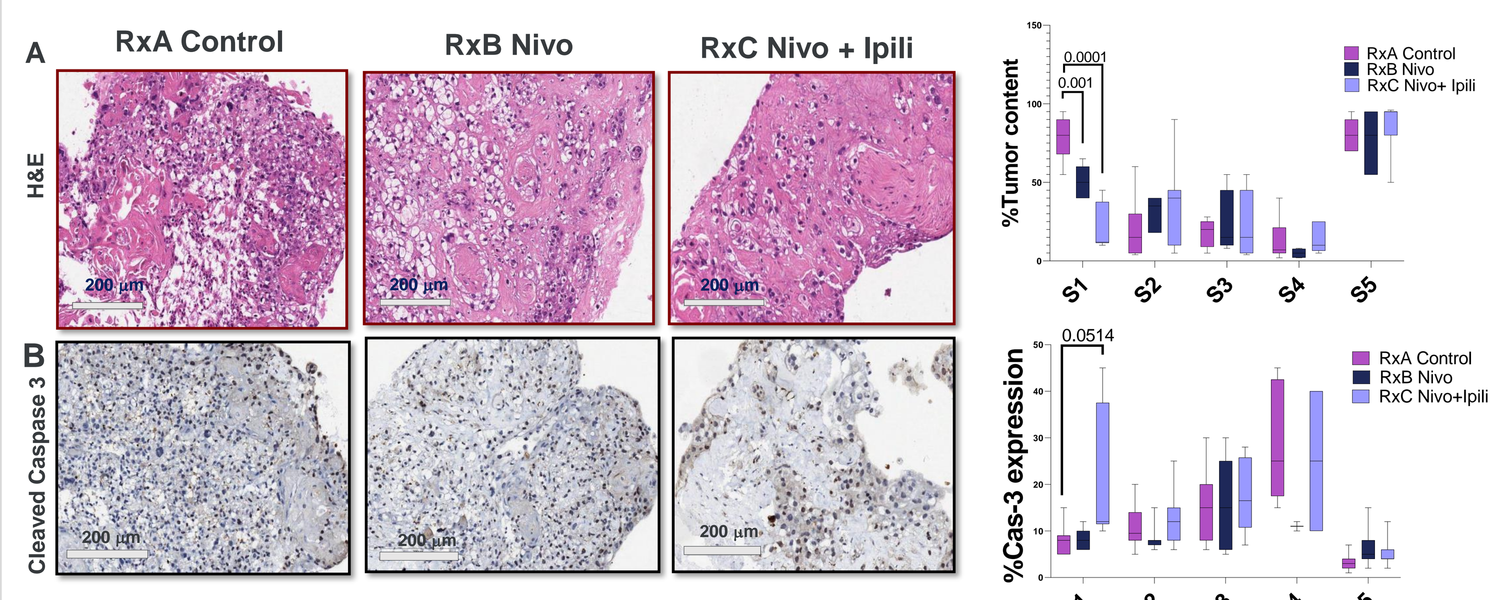

Fig.3: Evaluation of tumor content & Cleaved caspase 3 using H&E and IHC. A. Representative H &E images from sample 1 (S1) for control and treatment arms **B.** Representative Cleaved caspase 3 IHC images from sample 1. **C.** Percentage tumor content for control and treatment arms **D.** Percentage cleaved caspase in control and treatment arms

Level of PBNC infiltration varied across samples

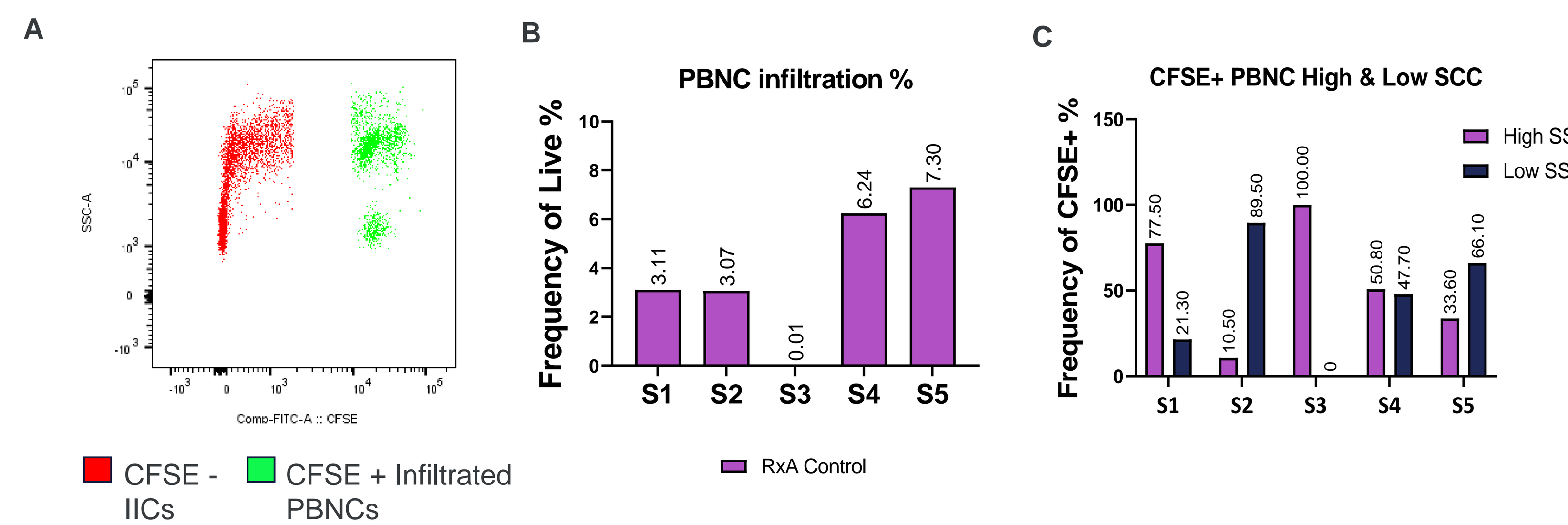

Fig.4: Evaluation of CFSE+ PBNC infiltration post 72 h of co-culture using flowcytometry analysis. **A.** Representative image showing CFSE+ infiltrated PBNC and IICs **B.** Percentage PBNC infiltration in RxA (Media control) arm of all samples **C.** Percentage High & Low SSC population of infiltrated PBNCs in control arm.

Treatment with drug led to enhanced PBNC infiltration in most samples

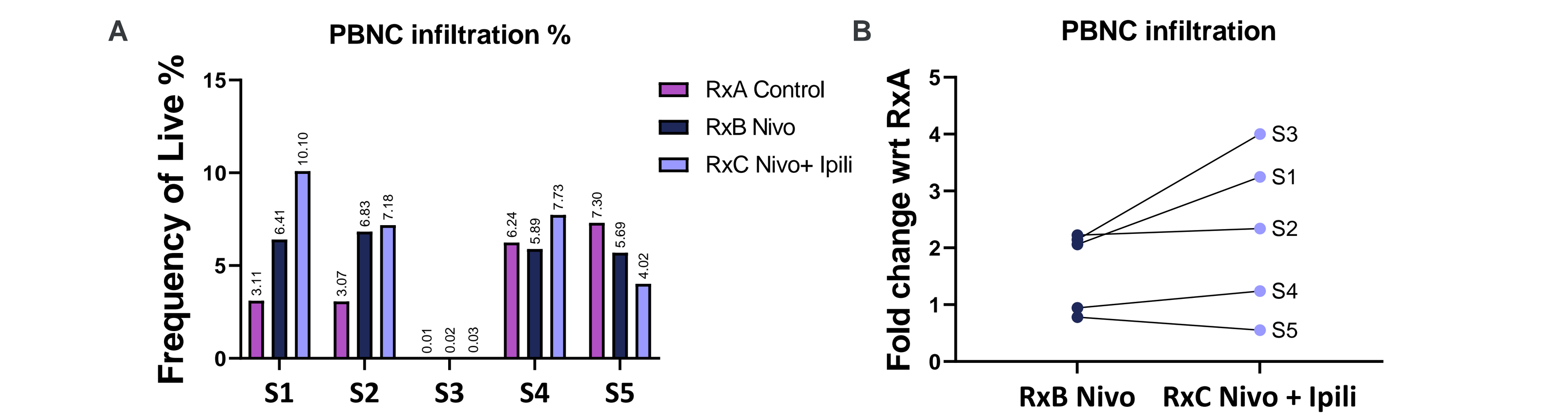

Fig.5: Evaluation of PBNC infiltration in response to treatment. **A.** Percentage PBNC infiltration in RxB (Nivolumab) and RxC (Nivolumab + Ipilimumab) arms post culture **B.** PBNC infiltration represented as fold change with respect to control arm (RxA).

Levels of Infiltration of High SSC sub populations varied across samples in response to treatment with high infiltration of neutrophils in sample S1

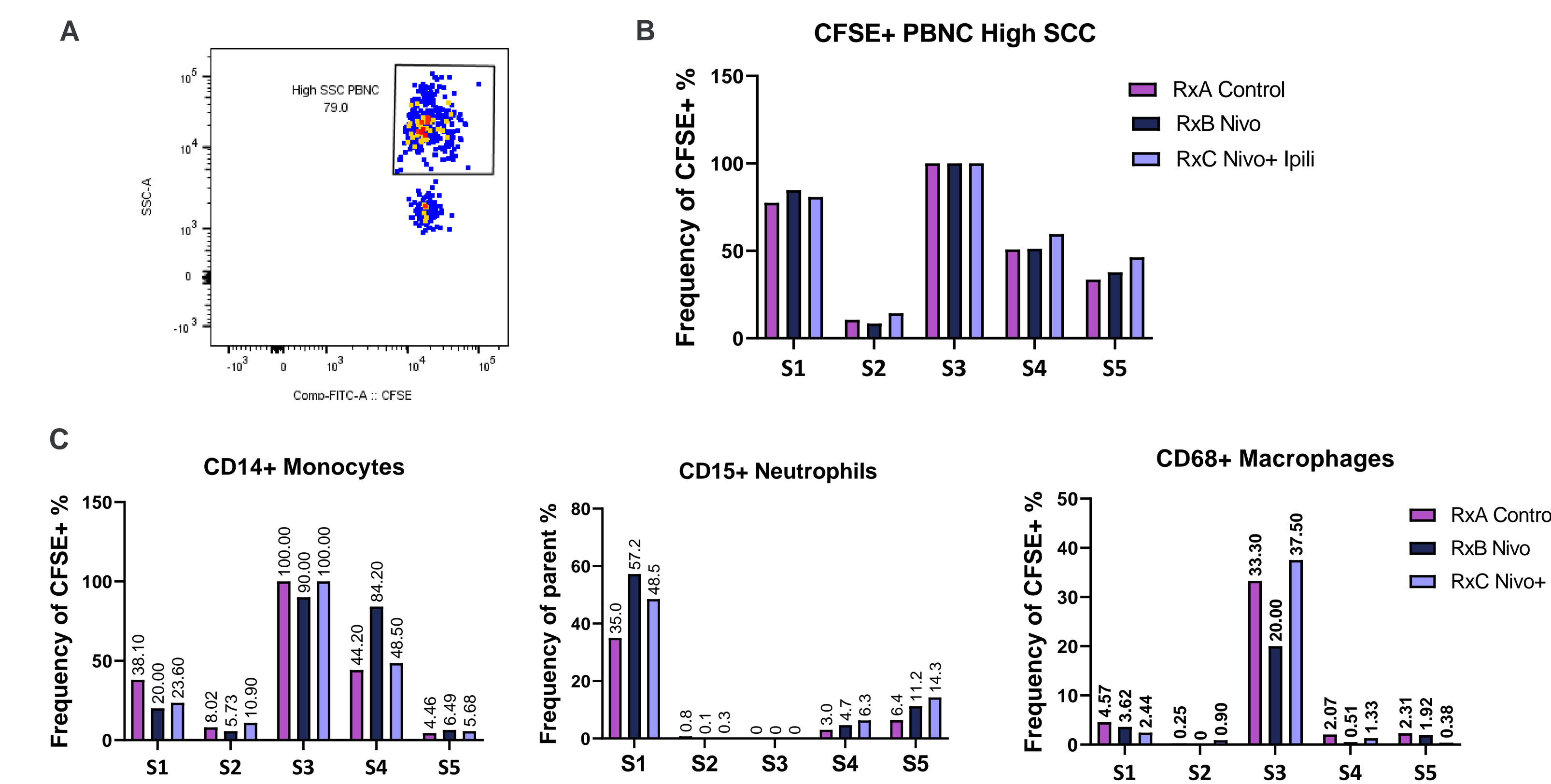

Fig.6: Evaluation of High SSC subpopulation in response to treatment by flowcytometry A. Representative flow gating to show High SSC population **b.** Percentage high SCC population infiltration in RxB and RxC arm **C.** Percentage infiltration of Monocytes, Neutrophils and Macrophages sub populations.

Reduction in CD8+ Infiltration was observed in response to treatment

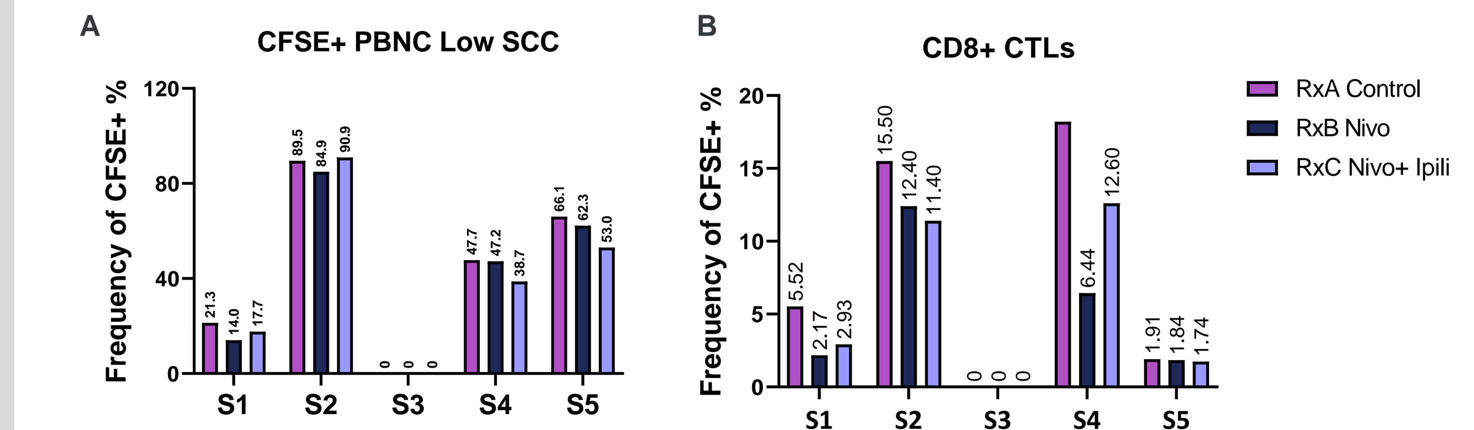

Fig.7: Evaluation of Low SSC subpopulation in response to treatment**. A.** Percentage low SCC population infiltration in control and response to treatment. **B.** Percentage infiltration of CD8+ population in control and response to treatment

Reversal of drug response in S1 on co-culture with PBNCs evident with loss of T cell cytokine release

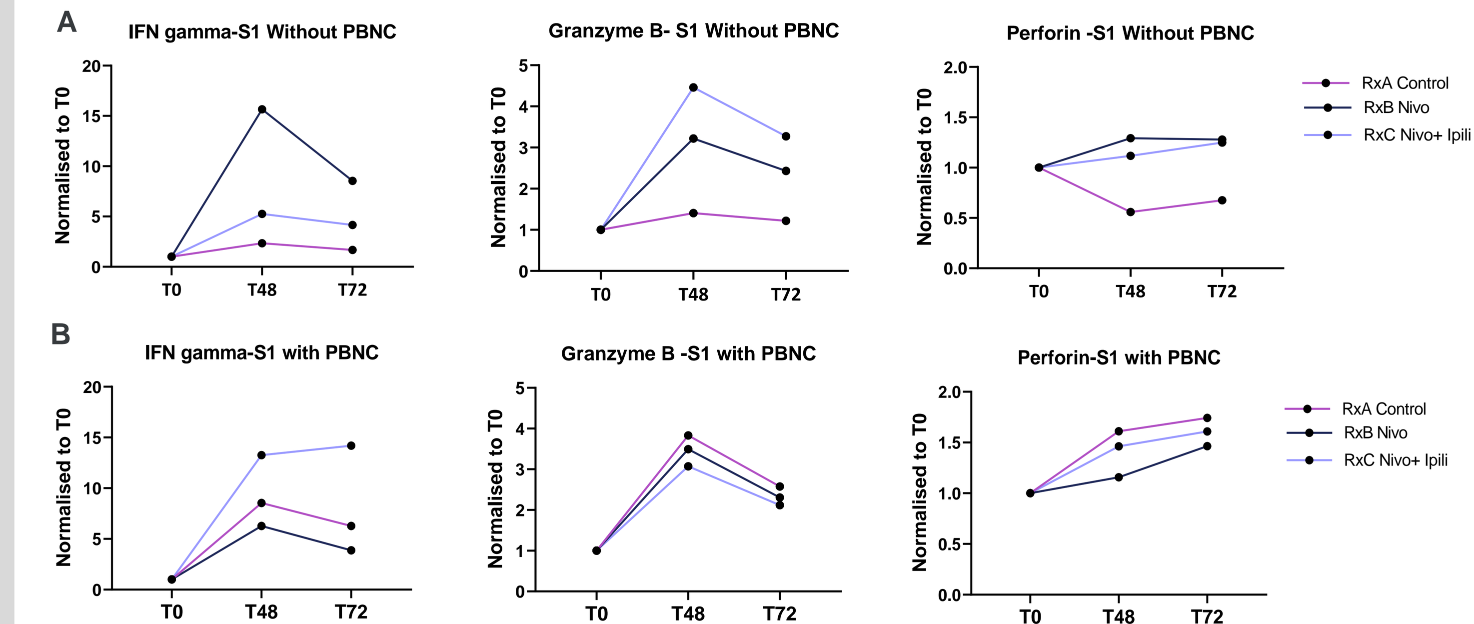

Fig.8: Evaluation of cytokine release over 72h culture period in sample 1. **A.** Level of IFN-γ, Granzyme-B and Perforin in the absence of PBNC coculture **B.** Level of IFN-γ, Granzyme-B and Perforin in the presence of PBNC coculture

SUMMARY & CONCLUSIONS

1. For ICI therapy, IICs are sufficient to elicit response.
2. Co-culture with PBNCs did not enhance efficacy of immune check point inhibitor drugs like Nivolumab and Ipilimumab.
3. Infiltrating PBNCs, in fact, modified the immune environment of the tumor leading to a response that differed from IIC driven response.
4. Inclusion of PBNCs is not universally beneficial to elicit response across different drug classes.
5. The choice of platform should be driven by the class of agent to be tested and Farcast™ TiME provides this unique and flexible option.

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