

PLN-101095 a selective inhibitor of integrins $\alpha v \beta 8$ and $\alpha v \beta 1$ augments the therapeutic benefits of immune checkpoint blockade therapy in human tumor histoculture model.



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Background

The involvement of transforming growth factor- β (TGF- β) in providing resistance to immune checkpoint inhibitor therapies is well-established across various cancer types. Targeting integrin $\alpha v \beta 8$ and $\alpha v \beta 1$ -mediated activation of TGF- β presents a promising strategy to counteract these immunosuppressive effects within specific tissues while minimizing systemic toxicities. In this study, we used the Farcast™ TruTumor platform, a human head and neck squamous cell carcinoma (HNSCC) histoculture model, to examine the impact of PLN-101095 (PLN) on T-cell reinvasion, spatial distribution, and tumor cytolytic functions to develop a biosignature for defining efficacy.

Study Design

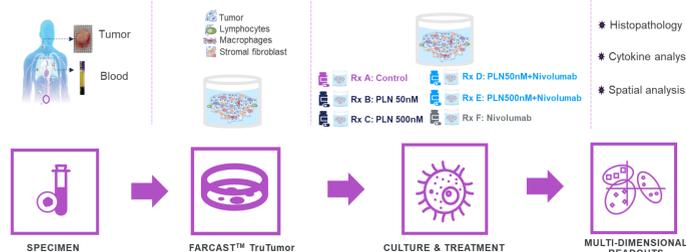


Figure 1: Schematic representation of Farcast™ TruTumor Histoculture platform work-flow and downstream assays used for treatment response evaluation.

Methods

Patient tissue samples: Fresh surgically resected Head and Neck Squamous Cell Carcinoma (HNSCC) tissue samples along with matched blood were collected from consented patients (n=10). All tumors used in the study were primary and treatment naive

No.	SAMPLE ID	AGE	GENDER	TUMOR GRADE	TUMOR STAGE	TUMOR SITE
1	111	47	Female	1	III	Buccal Mucosa
2	112	60	Female	1	III	Buccal Mucosa
3	127	49	Female	1	III	Buccal Mucosa
4	129	45	Male	1	II	Buccal Mucosa
5	141	27	Male	2	II	Tongue
6	147	45	Male	1	II	Buccal Mucosa
7	157	39	Male	1	IV	Tongue
8	160	55	Female	3	II	Buccal Mucosa
9	168	60	Female	1	II	GBS
10	169	44	Male	1	III	Buccal Mucosa

Table1: Demography of the patient sample used in this study

Histo-Culture workflow: The tumor sample was processed to generate thin explants without enzymatic digestion to retain the tumor microenvironment. Six arm with seven replicate tumor explants were cultured with media and autologous plasma. Explants in culture for 72 hours, were treated with either PLN monotherapy 50nM (P50) and 500nM (P500), 132ug/ml anti PD-1 antibody Nivolumab monotherapy (N), or Nivolumab combination with PLN at both concentrations (P50+N and P500+N) or untreated (Control, C).

Multiplex IHC (mIHC): mIHC was performed using a 3-marker (CD8, Granzyme-B and panCK) and nucleus counterstain (DAPI) panel on 4 μ m FFPE sections from post treatment samples. Data was acquired using Zeiss Axio observer and analysed using QuPath analysis software, adjudicated by certified Pathologist, in-house. Spatial analysis was performed within the demarcated tumor nest and necrosed segments (Fig. 2A) to score total and activated Cytotoxic T cells (CTLs) (Fig. 2B). CTL (both total and activated fractions) cluster parameters namely cluster size, cluster density etc., were computed using the QuPath software.

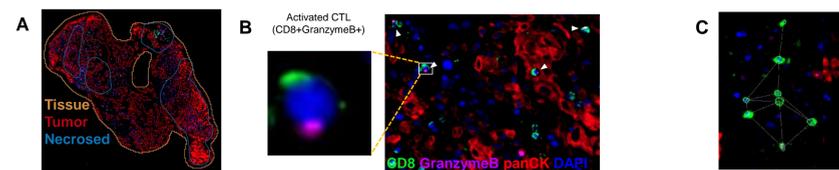


Figure 2: A. Representative mIHC image showing different regions annotation using Qupath. B. Representative image showing CTL (CD8+) and activated CTL (CD8+GranzymeB+) in TME. C. Image displaying a Delaunay's Cluster 2D for CTLs with distance threshold of 50µm

Cytokine Analysis: The supernatants collected at T0, T24, T48, T72 were used for analysis using Luminex Magpix instrument using a 6 plex (IFNg, IL10, TNFa, CXCL9, Perforin and Granzyme-B) Thermo Procata Plex kit and data was further analysed using Milliplex analyst software.

Histopathology & IHC: H&E staining and Cleaved Caspase 3 IHC was performed on 4 μ m sections obtained from the FFPE block using Leica automated multi-stainer system and Ventana IHC automated staining system, respectively. Scoring was performed by certified pathologists, in-house. Tumor content and Necrosis were evaluated from H&E-stained slides. Cleaved caspase 3 positivity was evaluated within the tumor compartment.

Putative mechanism of action of PLN-101095 (PLN)

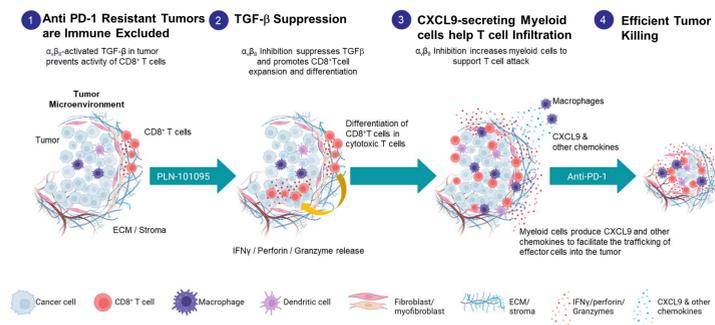


Figure 3: Pictorial representation of the putative mechanism of action of PLN-101095 (PLN)

Results

PLN treatment led to enhanced CTL proliferation and infiltration while the combination with Nivolumab exhibited higher levels of CTL activation within tumor nest

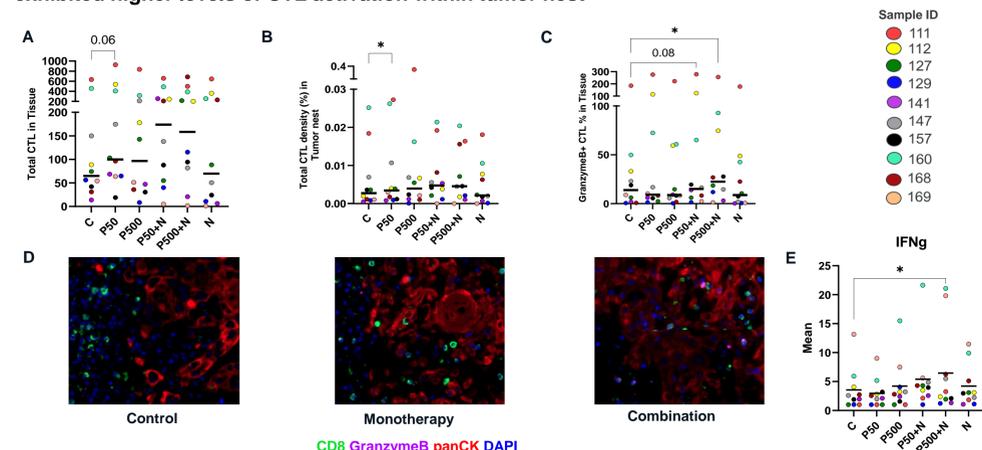


Figure 4: A. Total CTL (CD8 positive cells) within the whole tissue fragment across treatments. B. CTL density within tumor nest across treatments. C. Total GranzymeB+ CTL in the tissue across treatments. Data is represented as cell population or density at sample level with the median value of distribution demarcated. Paired non-parametric Wilcoxon signed rank test was used for significance assessment. * denotes significant p-value (<0.05). Near significance with 0.05<p-values \leq 0.08 is denoted by actual value. D. Representative images from sample 111 in control, monotherapy and combination demonstrating CTL proliferation, infiltration and activation compared to control. E. IFNg cytokine release normalized to T0 across arms in different samples. Wilcoxon matched-pairs signed rank test was performed to assess significance. * denotes significant p-value (<0.05).

PLN treatment led to CTL mediated necrosis

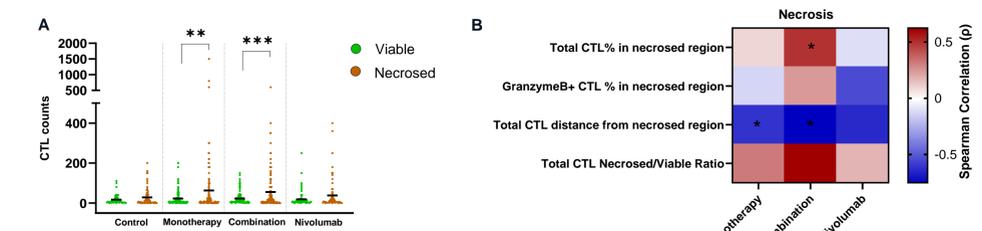


Figure 5: A. CTL count in viable and necrosed tumor. B. Spearman correlation analysis of change in prevalence and spatial distribution of CTL and GranzymeB+ (GzmB) CTL the change in Necrosis due to various treatments. * denotes significant correlation p-value (<0.05)

Combination therapy yielded improved efficacy compared to PLN and Nivolumab monotherapy

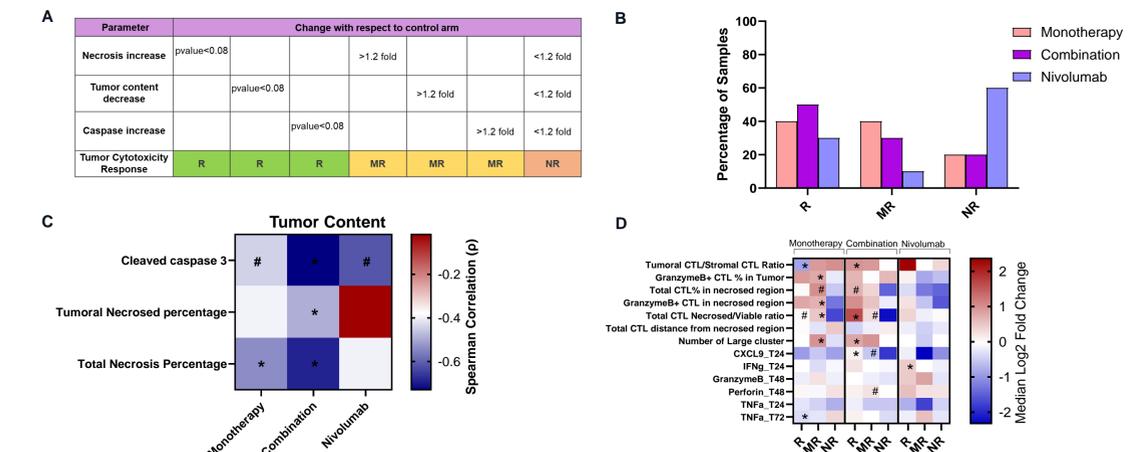


Figure 6: A. Definition of response based on TruTumor platform tumor cytotoxicity measured by necrosis increase, tumor content decrease and cleaved caspase3 expression within tumor cells. Responder (R), Moderate responder (MR) and non-responder (NR) defined based on the significance and level of change. B. Percentage of responders (R), moderate responders (MR) and non-responder (NR) in each treatment group. C. Spearman Correlation between tumor content and cleaved caspase3, Tumor necrosis and Total necrosis in the three treatment groups is represented. * denotes p-value <0.05, # denotes p-value <0.08. D. 13 parameters comprising CTL spatial and functional parameters were selected based on their correlation to tumor cytotoxicity across treatment groups. Data represent the mean fold change with respect to control for Responder (R), Moderate Responder (MR) and non-responder (NR). Mann Whitney unpaired t-test was performed between R or MR with NR to evaluate significance of correlation. * denotes p-value <0.05, # denotes p-value <0.08.

CTL spatial and functional parameters based biosignature segregates PLN treatment responders and non-responders better than for Nivolumab treatment

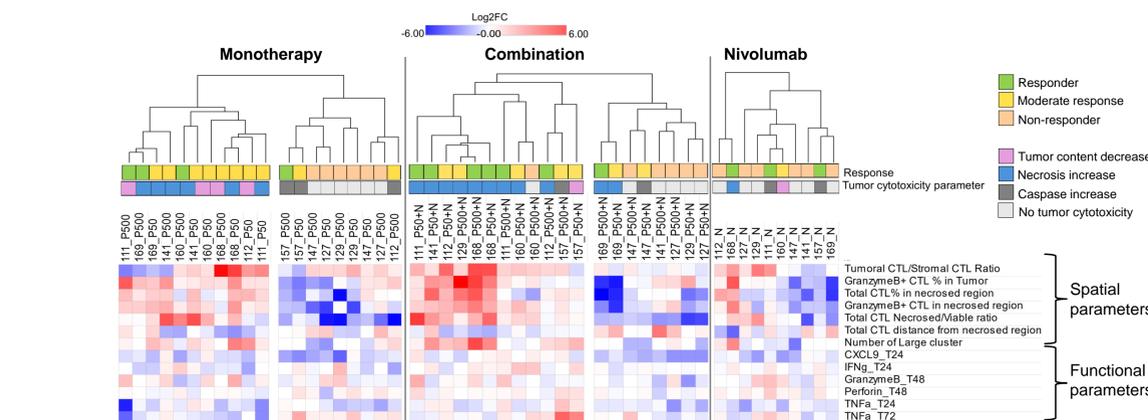


Figure 7: Spearman rank correlation based hierarchical clustering using the 13 selected parameters. The data represents Log2 Fold Change with respect to control for all treatment arms in each treatment group.

Conclusion

The TruTumor HNSCC histoculture platform effectively demonstrates PLN-101095 treatment response in the TME driven by proliferation, mobilization and activation of tumor resident CTLs, ultimately leading to tumor cytotoxicity. The augmentation of the efficacy on combining Nivolumab with PLN-101095 is also clearly illustrated.

A Phase 1 Study (NCT06270706) for PLN-101095 treatment in adults with advanced or metastatic solid tumors is currently under enrollment.