Selective Small Molecule Inhibitor of Integrin αvβ8 and αvβ1 Has Single Agent Activity and Potentiates Immune Checkpoint Blockade Therapy

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ABSTRACT

Background: Activated transforming growth factor-β (TGFβ) fosters an immuno-suppressive tumor microenvironment (TME) that contributes to immune-checkpoint inhibitor (ICI) resistance and treatment failure in cancer.[1] Alpha V-integrins, specifically αvβ8 and αvβ1, are effective activators of TGFβ, and both are present in the TME. αvβ8 is found on tumor and immune cells,[2,3] and αvβ1 is expressed on fibroblasts in the stromal compartment.[4] We describe the development of PLN-101095, a small molecule inhibitor of αvβ8 and αvβ1, and characterize its anti-tumor MoA in two immune-excluded, anti-programmed death receptor-1 antibody (anti-mPD-1)-resistant mouse tumor models.

Methods: PLN-101095 potency and selectivity was determined using specific assays, including a cellular assay[5] that measures TGFβ release by three integrins. The activity of PLN-101095 alone was assessed on EMT6 murine tumors and on ex-vivo cultured human breast cancer tissues. In vivo efficacy of PLN-101095 in combination with anti-mPD-1 was evaluated in EMT6 and Pan02 syngeneic mouse models by monitoring animal survival, tumor growth, tumor infiltration of $CD8^+$ T cells, and expression analysis of pro-inflammatory and anti-fibrotic genes.

Results: PLN-101095 showed selective inhibition of integrins αvβ8 and αvβ1 at nanomolar potency and >100-fold selectivity against all other integrins tested. Treatment of mice bearing EMT6 tumors with PLN-101095 alone significantly impaired the tumor growth, and increased cytotoxic $CD8^+$ T cells and their infiltration from the periphery into the tumor. PLN-101095 reduced pSMAD-TGFβ signaling and fostered an immune-activated TME. Notably, PLN-101095 exposure caused an elevated PD-L1 expression in the TME. In addition, the immune-activated TME was marked by an increase in M1 macrophages and activated NK cells. Human breast cancer tissues exposed to PLN-101095 ex vivo showed a significant increase in granzyme B^+ $CD8^+$ T cells. PLN-101095 in combination with anti PD-1 significantly reduced tumor growth, reduced fibrosis, and improved animal survival. Similar anti-tumor responses were observed in Pan02 tumors.

Conclusion: PLN-101095 effectively inhibited the immunosuppressive effects of TGFβ and promoted cytotoxic $CD8^+$ T cell infiltration in EMT6 and Pan02 tumors. PLN-101095 potentiated anti PD-1 therapy in EMT6 and Pan02 tumors. Based on these observations, a first-in-human clinical trial investigating the effect of PLN-101095 in combination with ICIs is planned.

RESULTS

Figure 1.

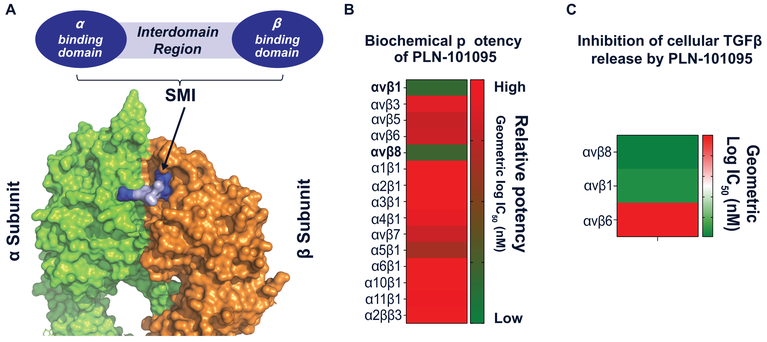

Figure 1: (A) Schematic representation of a small molecule inhibitor and the regions of modifications (top), and a model of a small molecule inhibitor bound to αvβ8 (bottom). (B) Heatmap showing the relative potency of PLN-101095 against indicated integrins. (C) PLN-101095 potently inhibits the TGFβ release from αvβ8 and αvβ1.

Figure 2: Mechanism of action of PLN-101095.

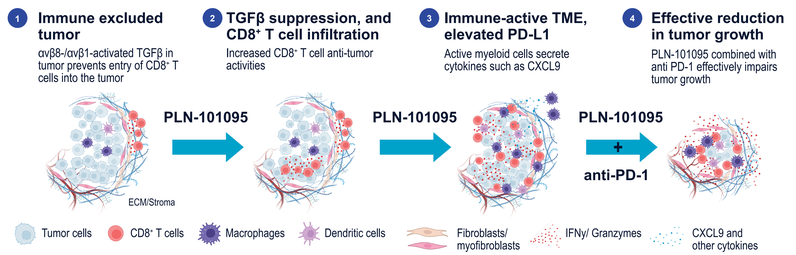

Figure 2: Integrin αvβ8- and αvβ1-activated TGFβ leads to an immunosuppressive TME and exclusion of $CD8^+$ T cells from the tumor. Inhibition of integrins αvβ8 and αvβ1 by PLN-101095 releases this immunosuppression, facilitates tumor infiltration of cytotoxic $CD8^+$ T cells, and activates myeloid cells that secrete pro-inflammatory cytokines, resulting in an immune-activated TME. PLN-101095 increases the expression of PD-L1 on tumor cells, and combining PLN-101095 with anti-PD-1 therapy leads to an effective reduction in tumor growth.

Figure 3: PLN-101095 promotes $CD8^+$ T cell infiltration in EMT6 tumors.

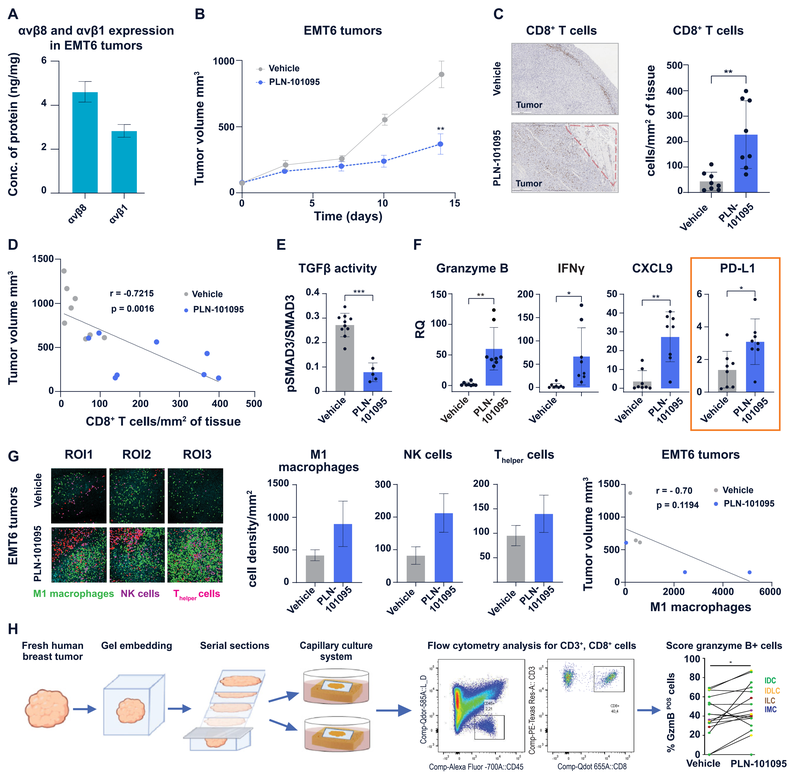

Figure 3: (A) Protein levels of αvβ8 and αvβ1 in EMT6 tumors measured on Meso Scale Discovery (MSD™) platform. (B, C) PLN-101095 treatment significantly impairs EMT6 tumor growth and facilitates tumor infiltration of peripherally restricted $CD8^+$ T cells. (D) $CD8^+$ T cell infiltration and tumor volumes are negatively correlated. (E) PLN-101095 reduces TGFβ activity in tumors, assessed by pSMAD3/SMAD ratio on MSD™. (F) PLN-101095 treatment elevates transcripts indicative of immune-activated TME. (G) Multiplexed Ion Beam Imaging (MIBI) of additional infiltrating immune cell types following PLN-101095 treatment. M1 macrophage numbers negatively correlated with tumor volumes. (H) Freshly collected human breast cancer tissues treated with PLN-101095 ex vivo show increased tumor infiltration of granzyme B^+ $CD8^+$ cells. Error bars in the growth curves and bar graphs in (G) show ± SEM, and the in the remaining bar graphs ± S.D. *p = 0.05, **p = 0.01, *** p = 0.001 calculated by student's t test. IDC, invasive ductal carcinoma; IDLC, invasive ductal lobular carcinoma; ILC, invasive lobular carcinoma; IMC, internal mammary node chain; n.s, not significant; r, Pearson correlation; ROI, region of interest.

Figure 4: PLN-101095 potentiates anti-PD-1 therapy in EMT6 tumors.

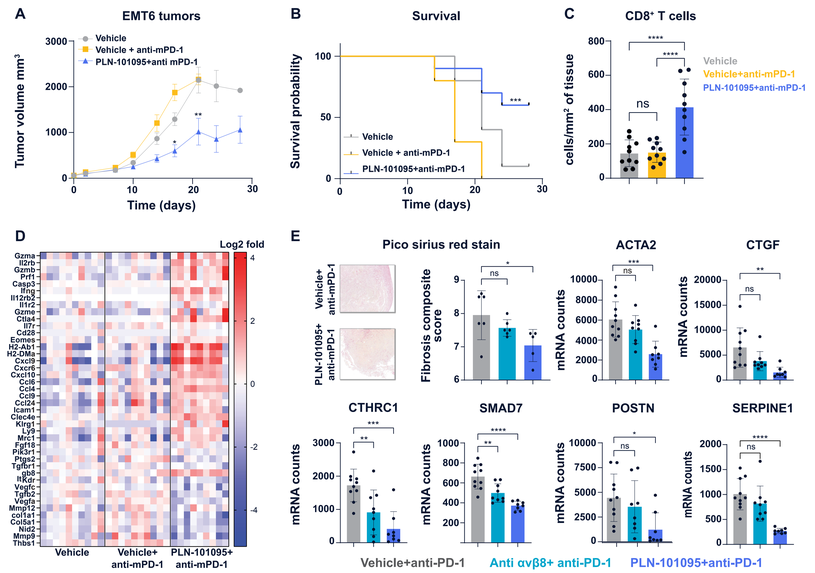

Figure 4: (A-D) PLN-101095 in combination with anti-PD-1 significantly impairs tumor growth, improves animal survival, facilitates tumor infiltration of $CD8^+$ T cells, and causes immune-activated TME. (E) PLN-101095+anti-PD-1 is superior to anti αvβ8+anti-PD-1 in reducing fibrosis. Error bars in the growth curves show ± SEM, and in the bar graphs show ± S.D. *p = 0.05, **p = 0.01, ***p = 0.001, and ****p = 0.0001 calculated by one-way ANOVA.

Figure 5: PLN-101095 potentiates anti-PD-1 therapy in Pan02 tumors.

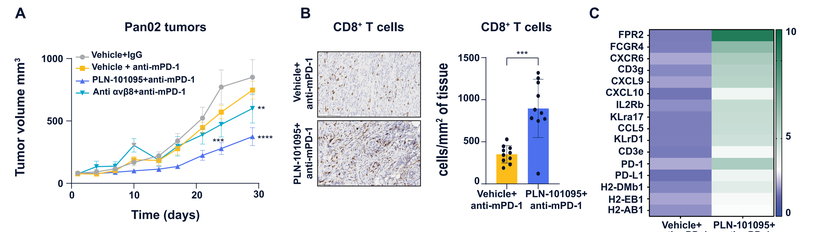

Figure 5: (A-C) PLN-101095 in combination with anti-PD-1 significantly impairs tumor growth, facilitates tumor infiltration of $CD8^+$ T cells, and causes immune-activated TME. Error bars in the growth curves show ± SEM, and in the bar graph ± S.D., **p = 0.01, ***p = 0.001, and ****p = 0.0001 calculated by one-way ANOVA.

SUMMARY

- **PLN-101095 is a potent, selective inhibitor of integrins αvβ8 and αvβ1.**
- **Single-agent PLN-101095 treatment suppresses activated TGFβ, impairs tumor growth, and facilitates $CD8^+$ T cell infiltration into murine EMT6 tumors.**
- **PLN-101095 in combination with anti-PD-1 therapy impairs tumor growth and fibrosis in the murine EMT6 model, and reduces tumor growth in the Pan02 model.**
- **A first-in-human clinical trial investigating the effect of PLN-101095 in combination with ICIs is planned.**

References: 1. Pickup M, Novitskiy S, Moses HL. *Nat Rev Cancer.* 2013;13(11):788-799. **2.** Dodagatta-Marri E, Ma HY, Liang B, et al. *Cell Rep.* 2021;36(1):109309. **3.**Takasaka N, Seed RI, Cormier A, et al. *JCI Insight.* 2018;3(20):e122591. **4.** Tabula Sapience Consortium, Jones RC, Karkanias J, et al. *Science.* 2022;376(6594). **5.** Abe M, Harpel JG, Metz CN, Nunes I, Loskutoff DJ, Rifkin DB. *Anal Biochem.* 1994;216(2):276-284. **Acknowledgements:** We acknowledge Dr. Julien Marie and his team at Cancer Research Center of Lyon INSERM, for conducting the study with human breast cancer tissues.