Integrin $\alpha_v\beta_1$ is Expressed in Multiple Solid Tumors and Drives the Adhesion of Cancer-Associated Fibroblasts to Latent TGF-β

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ABSTRACT

BACKGROUND: Inhibition of transforming growth factor beta (TGF-β) is an attractive strategy to augment the response of human tumors to immune checkpoint blockade (ICB) therapy. Given the toxicities associated with systemic targeting of TGF-β[1], inhibition of TGF-β-activating integrins $\alpha_v\beta_8$ and $\alpha_v\beta_1$ offers a promising strategy to block TGF-β in a tissue-restricted manner. Upregulation of integrin $\alpha_v\beta_8$ is associated with poor clinical outcomes[2,3], and therapeutic targeting of integrin $\alpha_v\beta_8$ is shown to elicit immune-inflamed tumor microenvironment (TME) in pre-clinical models[2, 3, 4]. However, due to the promiscuous binding of integrin subunit β_1 with multiple α subunits, determining the expression and functional role of integrin $\alpha_v\beta_1$ in solid tumors has remained challenging. Here, we evaluated $\alpha_v\beta_1$ protein expression across a diverse set of human tumor tissues and cancer-associated fibroblasts (CAFs) and investigated the functional role of integrin $\alpha_v\beta_1$ in cancer biology.

METHODS: $\alpha_v\beta_1$ protein expression in human tumor tissues and in primary human CAFs was evaluated via immunohistochemistry (IHC) and custom electroluminescence assay respectively. CAF adhesion to the TGF-β latency-associated peptide (LAP) was quantified in the presence or absence of PLN-101095 ($\alpha_v\beta_8$ and $\alpha_v\beta_1$ selective dual inhibitor) in vitro by high-content microscopy imaging. The activity of PLN-101095 in combination with anti-mPD-1 on fibrotic markers was evaluated in the EMT6 tumor model in vivo by gene expression analysis using the Nanostring® nCounter analysis system. PLN-101095-treated human breast tumor tissues were analyzed for fibrotic marker α-smooth muscle actin (αSMA) using immunofluorescence.

RESULTS: $\alpha_v\beta_1$ protein expression was detected by IHC analysis in both tumor regions and stromal-rich areas of human tumors. Compared to normal human lung fibroblasts, $\alpha_v\beta_1$ protein was elevated in primary human CAFs, with pancreatic stellate CAFs showing the highest protein expression (2.8-fold). Inhibition of $\alpha_v\beta_1$ with PLN-101095 blocked the binding of CAFs to LAP; the integrin RGD binding region of latent TGF-β, in a dose-dependent manner, demonstrating an $\alpha_v\beta_1$-specific interaction with latent TGF-β. EMT6 tumors treated with PLN-101095 and anti-mPD-1 showed a significant reduction in fibrotic markers compared to anti-$\alpha_v\beta_8$ and anti-mPD-1. Human breast tumor tissues treated with PLN-101095 ex vivo showed reduced expression of the fibroblast activation marker αSMA (2.9-fold) compared to vehicle-treated tissues, indicating a decreased TGF-β activity within the stromal regions of the TME.

CONCLUSIONS: Here, we demonstrate that the TGF-β activating integrin $\alpha_v\beta_1$ is expressed in multiple human tumors, is present on primary human CAFs, mediates CAF interaction with latent TGF-β, and has a functional role in mediating fibrotic gene expression in murine and human tumors. Combined, our previous[5] and current data demonstrate that inhibition of integrins $\alpha_v\beta_8$ and $\alpha_v\beta_1$ by PLN-101095 may have an advantage over targeting only $\alpha_v\beta_8$ by affecting both the tumor-immune and tumor-stromal compartments. These data provide the pre-clinical rationale to test PLN-101095 in multiple solid tumors representing a spectrum of immune and stromal composition. A first-in-human clinical trial investigating the combination of PLN-101095 with ICB in solid tumors is ongoing.

RESULTS

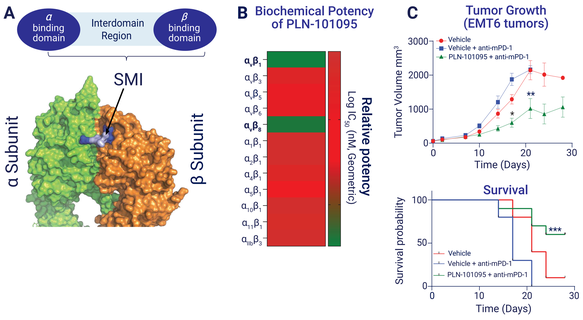

Figure 1: (A) Schematic representation of PLN-101095 small molecule integrin inhibitor (top), and a molecular rendering bound to integrin $\alpha_v\beta_8$ (bottom). (B) Heatmap showing the relative IC_{50} potencies of PLN-101095 versus indicated integrins. (C) PLN-101095 in combination with anti-mPD-1 significantly inhibits tumor growth (top) compared to anti-mPD-1 and increases survival of animals (bottom). *p=0.05, **p=0.01 calculated by two-way ANOVA. ***p=0.001 by log-rank test.

αvβ1 is expressed by both tumor and stromal cells

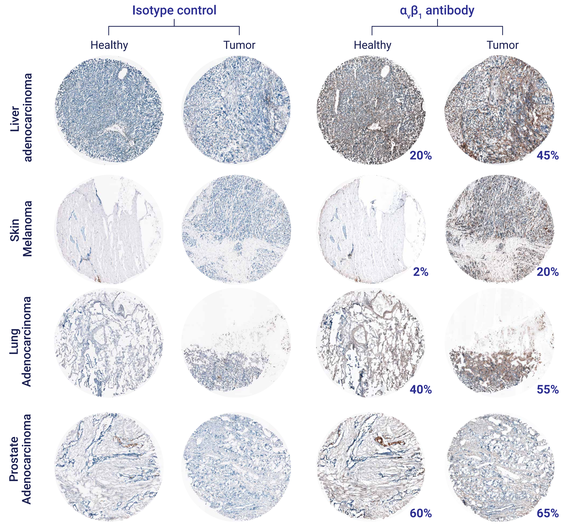

Figure 2: Representative images of OCT-embedded human tissue cores showing the expression of $\alpha_v\beta_1$ by IHC. The overall expression of integrin $\alpha_v\beta_1$ was found to be increased in tumor tissues compared to corresponding healthy tissues. IHC was performed using a specific $\alpha_v\beta_1$ antibody. Isotype control is shown to indicate the antibody specificity. Percent positive cells showing $\alpha_v\beta_1$ staining are indicated.

References: 1. Colak S. and ten Dijke P. (2017) Targeting TGF-β Signaling in Cancer. *Trends in Cancer* 3, 56–71. **2.** Takasaka N. et al. Integrin $\alpha_v\beta_8$–expressing tumor cells evade host immunity by regulating TGF-β activation in immune cells. JCI Insight. 2018;3(20):e122591. **3.** Lainé A, et al. Regulatory T cells promote cancer immune-escape through integrin $\alpha_v\beta_8$-mediated TGF-β activation. *Nat Commun.* 2021 Oct 28;12(1):6228. **4.** Dodagatta-Marri et al., 2021, Integrin $\alpha_v\beta_8$ on T cells suppresses anti-tumor immunity in multiple models and is a promising target for tumor immunotherapy. *Cell Reports* 36, 109309, July 6, 2021. **5.** SITC poster # 1352, 2022

Inhibition of $\alpha_v\beta_1$ prevents cell adhesion to LAP

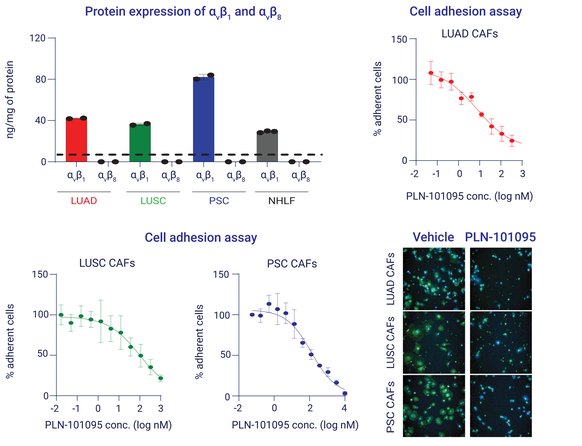

Figure 3: Bar graph (top left) shows the protein expression of integrin $\alpha_v\beta_1$ on CAFs isolated from indicated carcinomas compared to normal human lung fibroblasts (NHLF) determined by electroluminescence meso scale discovery assay. Integrin $\alpha_v\beta_8$ was undetected in CAFs and NHLFs. The black dotted line in the bar graph represents the lower limit of detection. Dose-response curves show that PLN-101095 inhibits the adhesion of lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), and pancreatic stellate (PSC) CAFs to TGF-β latency-associated peptide in a dose-dependent manner. Representative images show the adhesion of indicated CAFs on LAP-coated plates in the presence or absense of PLN-101095. Cell membranes were stained with Cell mask green dye and nuclei were stained with DAPI.

PLN-101095 reduces fibrotic markers in EMT6 tumors

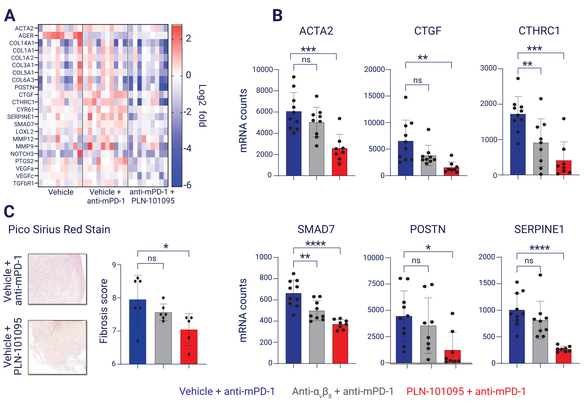

Figure 4: (A) PLN-101095 in combination with anti-mPD-1 reduces the expression of fibrotic markers in EMT6 tumors. (B, C) In a separate study, PLN-101095 in combination with anti-mPD-1 significantly reduced the expression of fibrotic markers, and tissue fibrosis in the tumor microenvironment compared to anti-$\alpha_v\beta_8$ in combination with anti-mPD-1. Error bars 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. ACTA2= Actin alpha 2. CTGF= Connective tissue growth factor. CTHRC1= Collagen triple helix repeat containing 1. SMAD7= Mothers against decapentaplegic homolog 7. POSTN= Periostin. SERPINE1= Plasminogen activator inhibitor-1. anti-mPD-1= anti mouse programmed death receptor-1 antibody.

PLN-101095 decreases fibroblast activation marker αSMA

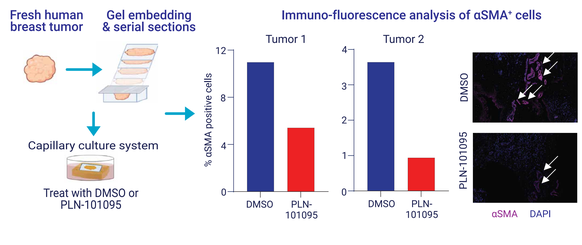

Figure 5: Freshly collected human breast tumor tissues treated with PLN-101095 ex-vivo for 48 hours show a decreased expression of αSMA compared to DMSO-treated tissue. Representative images of αSMA (white arrows) and DAPI-stained tissues are shown.

CONCLUSIONS

- PLN-101095 is a potent, dual inhibitor of integrins $\alpha_v\beta_8$ and $\alpha_v\beta_1$.
- Integrin $\alpha_v\beta_1$ is expressed both in the tumor and stromal compartments in human tumor tissues.
- PLN-101095 effectively blocks the adhesion of CAFs to TGF-β latency-associated peptide (LAP).
- PLN-101095 is superior to anti $\alpha_v\beta_8$ antibody in reducing fibrosis markers in the EMT6 tumor model.
- PLN-101095 effectively reduces the expression of the fibroblast activation marker αSMA in ex vivo-treated human breast tumor tissues.
- A first-in-human Phase I clinical trial of PLN-101095 with pembrolizumab in solid cancers is underway.

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