

TGF β Blockade Augments PD-1 Inhibition to Promote T-Cell-Mediated Regression of Pancreatic Cancer

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) remains remarkably lethal with a 5-year survival rate of 8%. This is mainly attributed to the late stage of presentation, as well as widespread resistance to conventional therapy. In addition, PDAC tumors are largely nonimmunogenic, and most patients have displayed incomplete responses to cancer immunotherapies. Our group has previously identified TGF β as a crucial repressor of antitumor immune function in PDAC, particularly with respect to cytotoxic T lymphocytes. However, pharmacologic inhibition of TGF β signaling has had limited efficacy in clinical trials, failing to promote a significant antitumor immune response. Hence, in this work, we extend our analysis to identify and circumvent the mechanisms of resistance to TGF β signal inhibition in PDAC. Consistent with our previous

observations, adoptive transfer of TGF β -insensitive CD8⁺ T cells led to the near complete regression of neoplastic disease *in vivo*. However, we demonstrate that this cannot be recapitulated via global reduction in TGF β signaling, through either genetic ablation or pharmacologic inhibition of TGFBR1. In fact, tumors with TGF β signal inhibition displayed increased PD-L1 expression and had no observable change in antitumor immunity. Using genetic models of advanced PDAC, we then determined that concomitant inhibition of both TGF β and PD-L1 receptors led to a reduction in the neoplastic phenotype, improving survival and reducing disease-associated morbidity *in vivo*. Combined, these data strongly suggest that TGF β and PD-L1 pathway inhibitors may synergize in PDAC, and this approach warrants clinical consideration.

Introduction

TGF β is a well-established modifier of the pancreatic tumor microenvironment (TME), though its overall contributions to carcinogenesis are poorly understood. Previous *in vivo* studies have found that, upon epithelial loss of TGF β signaling, the P48-Cre/LSL-Kras (KC) mouse model of PanIN disease developed advanced pancreatic cancer with increased incidence and severity of developing adenocarcinoma from neoplastic lesions (1–3). In contrast, systemic deficiency of *Tgfb1* in the Elastase-Kras^{G12D} model of cystic papillary neoplastic (CPN) disease (4) led to a reduction in the neoplastic phenotype (5). Combined, these data underscore the need for increased understanding of the multiple roles of TGF β in pancreatic ductal adenocarcinoma (PDAC) pathobiology. This is especially

important prior to the introduction of TGF β inhibitors to the management of PDAC patients.

In our previous work, we determined that TGF β represses the activation of cytotoxic T lymphocytes (CTL) in early PDAC development. Indeed, adoptive transfer of *Tgfb1* haplo-insufficient (*Tgfb1*^{+/-}) T cells into KC mice leads to a cytotoxic response toward developing lesions and a significant reduction in tumor burden (6). Clinically, TGF β expression was inversely associated with that of GranzymeB (GrzB), a surrogate marker of cytotoxicity (6). These findings appear to support therapeutic targeting of TGFBRs in pancreatic cancers to promote T-cell-mediated cytotoxicity. In this work, we extend our analysis to determine the potential efficacy of systemic TGF β signal inhibition, particularly with respect to antitumor immunity. We found that, although selective TGF β inhibition in CD8⁺ T cells leads to regression of neoplastic disease, systemic blockade of TGF β signaling fails to promote cytotoxicity due to compensatory upregulation of PD-L1 on the cancer epithelium. By targeting both TGF β and PD-L1 receptors, we were able to promote the T-cell-mediated clearance of PDAC, and this approach warrants further exploration in the management of pancreatic cancer patients.

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Materials and Methods

Chemicals and reagents

Galunisertib [LY2157299 (7), Supplementary Fig. S1A] was purchased from Selleck Chem, reconstituted as per the manufacturer's instructions, and used at 75 mg/kg *in vivo*. The anti-PD-1 antibody RMP1-14 was purchased from BioXCell and used at fixed dose of 200 μ g per injection.

Flow cytometry

Mesenteric lymph nodes or spleens were isolated, ruptured, washed in PBS, and contents filtered. Two million cells were seeded into a round-bottom 96-well plate, washed in PBS, incubated with a golgi plug/protein transport inhibitor (BD biosciences), and stained with anti-CD4-APC-Cy7, CD25-PE (BD biosciences), CD69-APC, CD8-PerCP-Cy5.5 (Biolegend), and an Alive/Dead kit (Invitrogen) at 1:200 to 1:1,500 in PBS at room temperature for 20 minutes. Cells were then fixed with 1% PFA in PBS for 10 minutes at room temperature and stained with either anti-FoxP3-FITC (eBioSci) or anti-Perforin-FITC (Biolegend) at 1:150 in perm/stain buffer (BD biosciences) for 30 minutes over ice and washed 3 times with perm/wash buffer (BD biosciences). Cells were analyzed with a BD Fortessa Cytometer. All flow plots correspond to live, single cells and are representative of 100,000 events unless otherwise stated.

CD8 isolation and adoptive transfer

Cells were isolated from the spleen, and CD8⁺ cells isolated by a dynabead CD8-positive isolation kit were used as per the manufacturer's specification (Life Technologies). After isolation, 2 million CD8 cells were resuspended in PBS and injected into the anesthetized recipient animals via the retro-orbital vein. Recipient animals were euthanized after 1 month and tissues subject to pathologic analysis.

Mice

P48-Cre x LSL-KRAS^{G12D} (KC), *Tgfb1*^{+/-}, *KC/Tgfb1*^{+/-}, and Pdx1-Cre x LSL-KRAS^{G12D} x LSL-TP53^{R172H} (KPC) mice were generated as described. KC mice were administered adoptive transfer of either naïve or *Tgfb1*^{+/-} CD8⁺ T cells and euthanized at 3 months. Similarly, 6-week KPC mice were administered intraperitoneal injection of either a PBS vehicle, Galunisertib, anti-PD-1, or Galunisertib/anti-PD-1 once every other day. This cohort was euthanized either after 2.5 months or when moribund. For euthanasia, animals were anesthetized with xylazine/ketamine until unresponsive to toe tap and/or agonal breathing. Thoracotomy served as the primary method of euthanasia and exsanguination the secondary method.

Histology, IHC, immunofluorescence, and slide scoring

Age-matched mice were euthanized, and the pancreas, colon, small bowel, liver, and spleen were subjected to pathologic examination. Tissues were fixed in 10% formalin, paraffin-embedded, and sections at 4 mm interval were cut from each tissue and stained with hematoxylin and eosin (H&E), trichrome (Sigma Aldrich), or via IHC or immunofluorescence (IF).

For IHC, slides were deparaffinized by xylenes and rehydrated by ethanol gradient, then heated in a pressure cooker using DAKO retrieval buffer. Endogenous peroxidases were quenched in 3% hydrogen peroxide in methanol for 30 minutes. Tissues were blocked with 0.5% BSA in PBS for 30 minutes and incubated with primary antibodies against pSMAD2 or PD-L1 (Cell Signaling Technology) at 1:50–1:200 overnight at 4°C. Slides were developed using either Streptavidin or secondary antibodies, followed by DAB substrate/buffer (DAKO).

For IF, slides were heated via pressure cooker in DAKO retrieval buffer and tissues blocked with 0.5% BSA in PBS for 1 hour at room temperature. Sections were exposed to primary antibodies against CK19 (University of Iowa Hybridoma Bank), PCNA, CD3 (Santa Cruz Biotechnology), or GrzB (Abcam) at 1:50–1:200

overnight at 4°C. Slides were developed using AlexaFluor 488- or 594-conjugated secondary antibodies (1:200–1:1,000, Abcam), mounted in DAPI-containing media (Santa Cruz Biotechnology), exposed to DAPI, FITC, and Texas Red filters, and images superimposed. All counts and scores were performed by two blinded investigators. For any contradicting scores, a third investigator was consulted.

Authentication of key resources

KC, *Tgfb1*^{+/-}, *KC/Tgfb1*^{+/-}, and KPC mice were bred in house and genotypes verified by PCR for all relevant transgenes (Pdx1-Cre, P48-Cre, *Tgfb1*^{+/-}, LSL-KRAS^{G12D}, LSL-TP53^{R172H}). Galunisertib is routinely verified in our lab by the ability to neutralize TGFβ1-induced pSMAD2 phosphorylation *in vitro* by Western blot as seen in our previous publication (6). The effectiveness of Galunisertib *in vivo* is similarly validated by the absence or reduction of pSMAD2 by IHC. In addition, the anti-PD-1 antibody RMP1-14 is verified by the manufacturer for the ability to recognize purified mouse PD-1 by Western blot and has been successfully used to neutralize PD-1 signaling in murine PDAC (8).

Statistical analysis

Data were analyzed by two-way ANOVA and fit to a general linear model in Minitab16, the validity of which was tested by adherence to the normality assumption and the fitted plot of the residuals. Results were arranged by the Tukey method and were considered significant at $P < 0.05$. Results are presented as mean ± SEM unless otherwise noted.

Study approval

All experiments involving the use of mice were performed following protocols approved by the Institutional Animal Care and Use Committee at the University of Illinois at Chicago. Slides were obtained from patients who provided written informed consent. Tissues were deidentified and provided by the Northwestern University Pathcore following local Institutional Review Board approval.

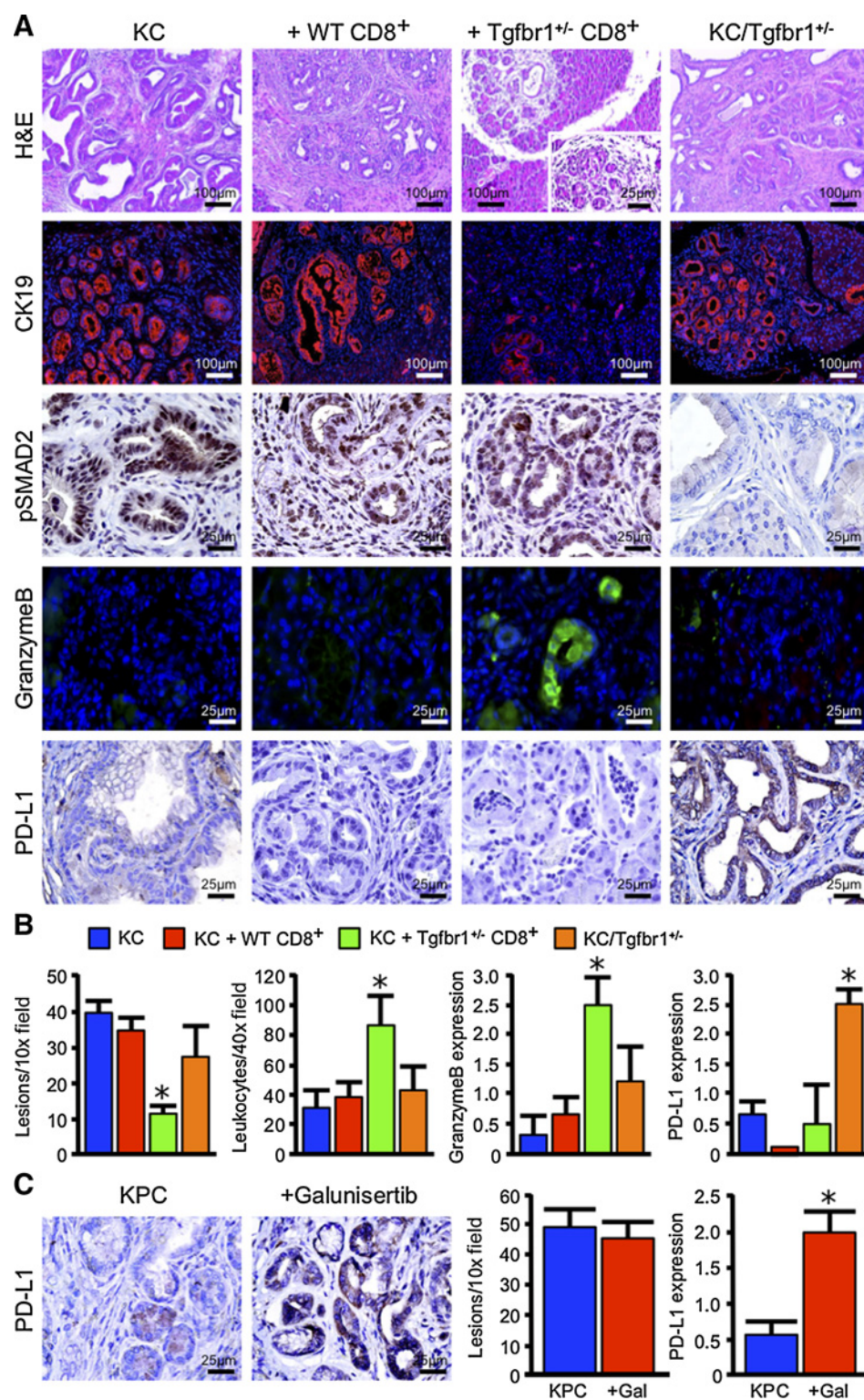
Results

TGFBR1-deficient CTLs reduce tumor burden, but systemic reduction of TGFβ signals fails to promote cytotoxicity

To determine the clinical utility of strategies targeting the TGFβ pathway, we first generated KC mice and conducted adoptive transfer experiments as described in our previous study (6), but this time comparing them to animals with systemic ablation of TGFβ signaling akin to that seen using a TGFβ pathway inhibitor. In brief, KC mice were allowed to reach 2.5 months of age, at which time they develop significant neoplastic disease. We then administered retro-orbital injection of either 2×10^6 wild-type (WT) CD8⁺ T cells, or CD8⁺ T cells from *Tgfb1*^{+/-} mice, and euthanized the mice after 30 days. Consistent with our previous findings (6), WT CD8⁺ T cells failed to significantly alter lesion development, yet *Tgfb1*^{+/-} CD8⁺ T cells led to rapid clearance of the neoplastic phenotype and an increase in both GrzB producing T cells (Supplementary Fig. S1B and S1C), as well as GrzB deposition in the remaining neoplastic tissues (Fig. 1A and B, $N = 3$ /group).

Although these findings indicate that TGFβ signal depletion enhances the cytotoxic immune response, they do not

Figure 1. TGFBR1-deficient CTLs reduce tumor burden, but systemic reduction of TGFβ signals fails to promote cytotoxicity. **A**, P48-Cre x LSL-KRAS^{G12D} (KC) mice were bred to generate a model of conditional expression of oncogenic KRAS^{G12D}. Mice were then administered retro-orbital injection of either 2 × 10⁶ WT CD8⁺ T cells (WT CD8⁺), or T cells from Tgfb1 haplo-insufficient mice (Tgfb1^{+/-} CD8⁺). Tissues were collected 30 days after adoptive transfer. In addition, KC mice were crossed to the Tgfb1 haplo-insufficient animals, from which the protective Tgfb1^{+/-} T cells were derived, to generate KC/Tgfb1^{+/-}. Tissues were then collected and stained with H&E, or via IHC for the ductal marker CK19, TGFβ effector pSMAD2, the cytotoxic surrogate GrzB, or for the immune checkpoint PD-L1. **B**, Tissue sections were quantified by two independent investigators and averages displayed ± SEM (N = 3–6/group). **C**, Pdx1-Cre x LSL-KRAS^{G12D} x LSL-TP53^{R172H} (KPC) mice were used to concomitantly target expression of oncogenic KRAS^{G12D} and P53^{R172H} mutations to the pancreas to generate a model of aggressive PDAC. At 6 weeks of age, KPC mice were treated every other day with intraperitoneal injection of 75 mg/kg of the TGFBR1 inhibitor Galunisertib, and tissues were collected after 2.5 months. Tissue sections were then stained by H&E or for PD-L1 by IHC, quantified by two independent investigators, and averages displayed ± SEM (*, P < 0.05, N = 4/group).



necessarily predict responsiveness to broad spectrum or globally administered TGFβ inhibitors. Therefore, we crossed KC mice into the Tgfb1^{+/-} background to generate KC/Tgfb1^{+/-}. Despite KC/Tgfb1^{+/-} mice harboring the protective Tgfb1^{+/-} transgene in T cells, these animals had no significant alteration

in disease course, nor any observable increase in cell-mediated cytotoxicity or GrzB deposition (Fig. 1A and B, N = 3–6/group).

In addition to TGFβ, immune checkpoints such as PD-L1/PD-1 have been identified as a primary mediator of immune tolerance in the pancreas during islet cell transplantation (9). Although

TGF β signal inhibition has enhanced immune checkpoint inhibition in immunogenic cancer types (10), this combination has yet to be explored in the management of PDAC. We therefore first assessed mice for expression of PD-L1, which is often upregulated in the pancreatic TME (Supplementary Fig. S2). Interestingly, only KC/*Tgfb1*^{+/-} mice displayed an increase in PD-L1 expression on the surface of the neoplastic epithelium (Fig. 1A and B, *N* = 3–6/group). To determine whether pharmacologic inhibition of TGF β signals would similarly cause the upregulation of PD-L1, we next used the Pdx1-Cre x LSL-KRAS^{G12D} x LSL-TP53^{R172H} (KPC) model of advanced PDAC and administered the most widely used TGF β pathway inhibitor Galunisertib (LY2157299). In accordance with our previous results, when 6-week-old KPC mice were treated with 75 mg/kg of the TGFBR1 inhibitor Galunisertib, mice also displayed increased PD-L1 expression and had no significant alteration in lesion development (Fig. 1C, *N* = 4/group).

Concomitant TGFBR1 and PD-1 inhibition significantly reduces tumor burden *in vivo*

In order to determine the physiologic importance of PD-L1 upregulation in response to Galunisertib, we generated KPC mice and after allowing PDAC to develop, administered an IP injection every other day of either a saline vehicle (*N* = 9), 75 mg/kg Galunisertib (*N* = 4), a fixed 200 μ g dose of the PD-1–neutralizing antibody RMP1-14 (anti-PD-1, *N* = 4), or a combination of Galunisertib and anti-PD-1 (Fig. 2A, *N* = 7). Both Galunisertib and PD-1–neutralizing antibodies (e.g., Nivolumab or Pembrolizumab) have been used in clinical trials for PDAC (11, 12). However, as human and murine PD-1 proteins differ significantly in the extracellular domain (13), it was necessary to utilize a mouse-specific PD-1–neutralizing antibody for this study.

Although Galunisertib was highly effective at neutralizing SMAD2 phosphorylation (Fig. 2B and C), once again there was no significant effect on tumor development apart from the development of regions of cystic papillary neoplasms. Similarly, anti-PD-1 failed to significantly alter disease course as a monotherapy as previously reported (8). However, when the two agents were combined, the KPC mice displayed a significant reduction in the neoplastic phenotype despite the increased PD-L1 expression (Fig. 2A–C; Supplementary Figs. S3–S5). This was also associated with a modest survival benefit in dual-treated mice compared with untreated mice, which was not seen in single-agent controls (Fig. 2C; Supplementary Fig. S6, *P* = 0.0221). This was also accompanied by improvement in weight gain (Supplementary Fig. S3B), as well as reduction in both fibrosis and proliferation in remaining areas of disease (Fig. 2B and C). Further, although there was no observable change in pancreas length (Supplementary Fig. S4B), mice treated with both Galunisertib and anti-PD-1 had reductions in pancreas weight consistent with a reduction in the tumor stroma (Supplementary Fig. S4C). Despite the reduced tumor burden, there were no obvious signs of autoimmunity or drug toxicity in mice treated with the combination regimen (Supplementary Fig. S5).

Galunisertib cooperates with PD-1 inhibition to promote T-cell–mediated regression of advanced PDAC

Consistent with the observed reduction in tumor burden, on histology mice treated with the combination regimen displayed an overwhelming lymphocyte infiltrate, which localized strongly to remaining areas of disease (Fig. 3A and B). These infiltrating cells were uniformly positive for CD3, affirming their T-cell

lineage. In addition, the T-cell infiltrate of Galunisertib and anti-PD-1 dual-treated mice had robust expression of GrzB, which also localized to any remaining areas of neoplastic disease (Fig. 3A and B; Supplementary Fig. S7, *N* = 3/group). These local changes in immune function were further verified by flow cytometry of the mesenteric lymph nodes, which revealed similar increase in T-helper activation (CD4⁺CD69⁺) cells exclusively in mice treated with the combination regimen (Fig. 3C and G; Supplementary Fig. S8). As macrophages are a primary target of Th1 cells and may have an important role in the response to immune checkpoint inhibitors (14), we also determined the presence of CD11b/GR1⁺ granulocytes. Although these cells were largely absent from the lymph nodes, we found a significant increase in CD11b/GR1⁺ cells in the spleen. Further, these CD11b/GR1⁺ cells were largely positive for the mouse macrophage marker F4/80 (Fig. 3D and G; Supplementary Fig. S8). Most importantly, mice treated with the combination regimen also had increased CTL activation in the mesenteric lymph nodes (CD8⁺CD69⁺ cells), as well as enhanced IFN γ , GrzB, and Perforin production (Fig. 3D–G; Supplementary Fig. S8), further affirming an increase in cell-mediated cytotoxicity.

Discussion

To date there are no curative strategies in the management of advanced pancreatic cancer patients. As PDAC often presents in late stages, most efforts focus on extending life when possible and providing supportive/palliative care. Immune checkpoint inhibition has been a major therapeutic breakthrough in many cancers. The PD-1–neutralizing antibody Pembrolizumab is now approved for non–small cell lung cancers, melanoma, some squamous cell carcinomas, and Hodgkin's lymphoma. Similarly, the anti-PD-1 antibody Nivolumab is first line in metastatic melanoma and has been approved for progressive squamous cell carcinomas of the head and neck, renal cell carcinoma, and non–small cell lung cancer (15).

As a result, investigators have been eager to determine whether similar strategies targeting PD-1/PD-L1 are applicable in the management of PDAC. Importantly, PD-L1 is often overexpressed in PDAC and is associated with poor outcomes (16). Though ineffective as a monotherapy, the combination of Nivolumab with Abraxane is showing early promise, as is the combination of Nivolumab with GVAX-based immunotherapy (17). Though encouraging, these trials appear to only modestly extend survival, and overall mortality remains high.

The mechanisms underlying the reduced efficacy of immune checkpoint inhibition in pancreatic cancer remain unclear. Therefore, it is essential to dissect alternate means through which tumor cells promote peripheral immune tolerance in order to combine with immune checkpoint inhibitors to improve their efficacy and further extend survival. As discussed and demonstrated, TGF β is an important immunomodulator that plays a role both in physiologic and pathologic tolerance. In the absence of TGF β signaling, T lymphocytes rapidly acquire high levels of FasL and GrzB, two main functional components for cytotoxicity. Similarly, T cells expressing a constitutively active form of TGFBR1 also remain refractory to full activation (18).

However, much like anti-PD-1 antibodies, drugs targeting the TGF β pathway have also only shown partial efficacy in the clinic. In an ongoing phase II clinical trial, Galunisertib was added to the first-line agent Gemcitabine, which extended overall survival from

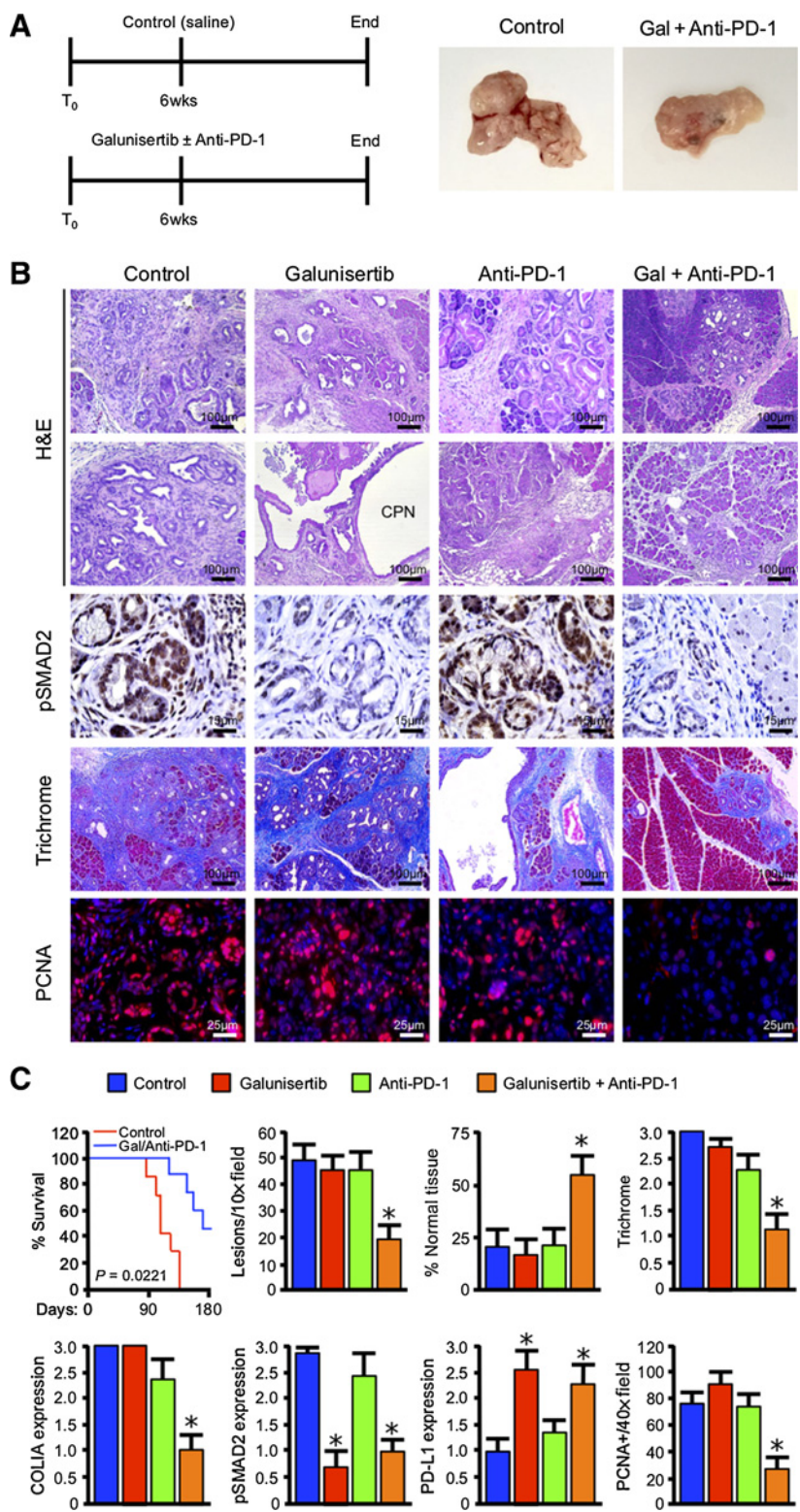
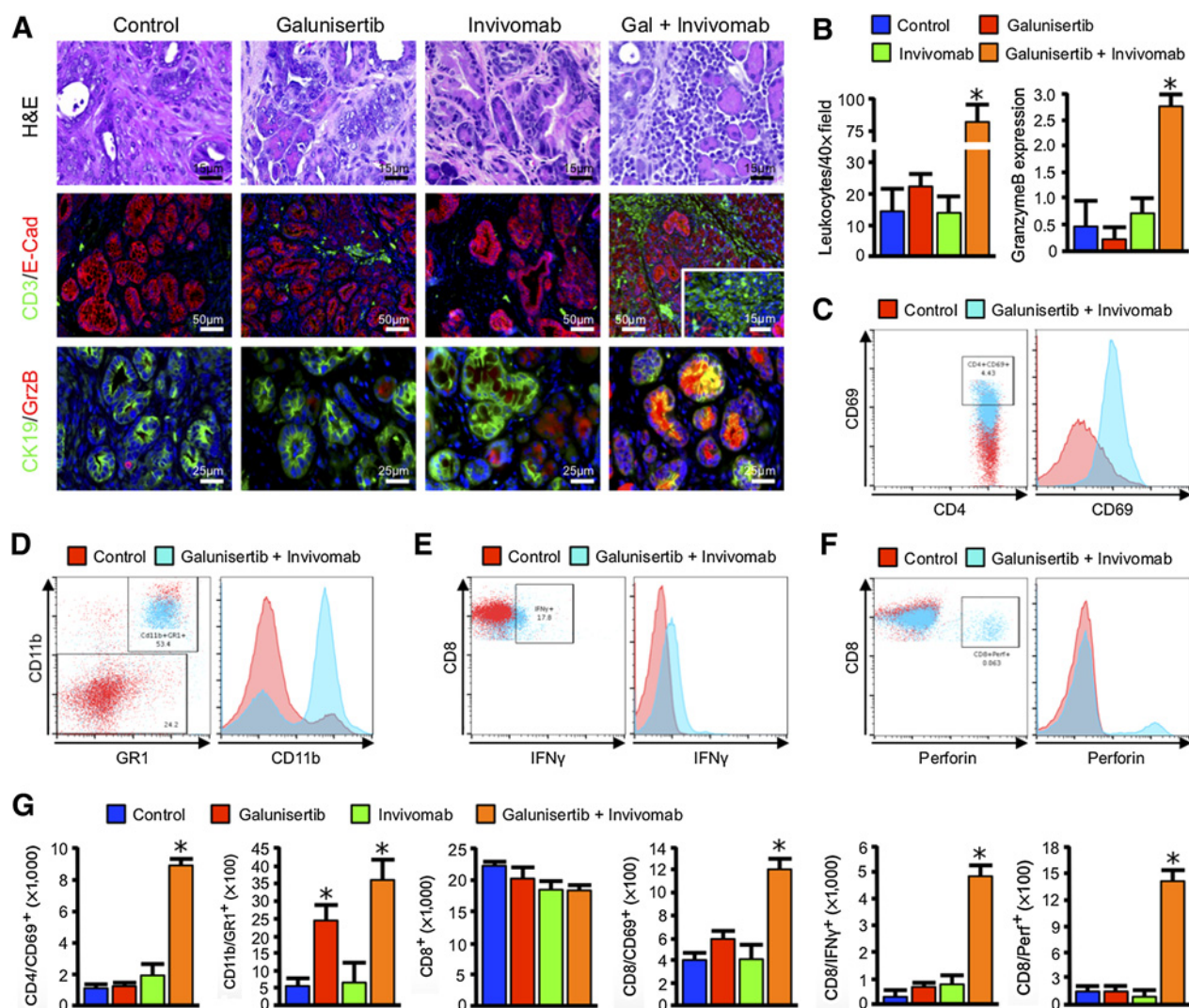


Figure 2. Concomitant TGFβRI and PD-1 inhibition significantly reduces tumor burden *in vivo*. **A**, Pdx1-Cre x LSL-KRAS^{G12D} x LSL-TP53^{R172H} (KPC) mice were regenerated as a model of aggressive PDAC. At 6 weeks of age, mice were administered intraperitoneal injection every other day either a PBS vehicle (Control), 75 mg/kg of the TGFβRI inhibitor Galunisertib, 200 µg of an anti-PD-1-neutralizing antibody (anti-PD-1), or a combination of Galunisertib and anti-PD-1. The pancreas was then collected either when the animals were moribund or at the conclusion of the study (180 days after enrollment), and gross changes in pancreas gland structure evaluated. **B**, The pancreas from control- and drug-treated mice was then stained with either H&E or Masson's Trichrome. Sections were also stained via IHC for the TGFβ effector pSMAD2 or the proliferation surrogate PCNA. **C**, Kaplan-Meier curve indicating survival for Control and Galunisertib/Anti-PD-1-treated mice measured in days after treatment began ($N = 7-9$ /group). In addition, tissue sections were quantified by two investigators and averages displayed \pm SEM. (*, $P < 0.05$, $N = 3-4$ /group).

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**Figure 3.**

Galunisertib cooperates with PD-1 inhibition to promote T-cell-mediated regression of advanced PDAC. **A**, $Pdx1-Cre \times LSL-KRAS^{G12D} \times LSL-TP53^{R172H}$ (KPC) mice were used as a model of advanced PDAC, and animals were treated with either a PBS vehicle (Control), 75 mg/kg of the TGFBR1 inhibitor Galunisertib, 200 μ g of an anti-PD-1-neutralizing antibody (anti-PD-1), or a combination of Galunisertib and anti-PD-1. The pancreas from control and drug-treated mice was stained with H&E and evaluated under high power, or dual-stained for the T-cell marker CD3 and the epithelial surrogate E-Cadherin (E-Cad), or for the duct cell marker CK19 and the cytotoxic surrogate GrzB. **B**, Tissue sections were subsequently quantified and averages displayed \pm SEM (*, $P < 0.05$, $N = 3-6$ /group). Lymphoid tissues were harvested and evaluated by flow cytometry for **(C)** $CD4^+CD69^+$ dual-positive populations in the mesenteric lymph nodes, **(D)** $CD11b^+Gr1^+$ granulocytes in the spleen, and **(E, F)** $CD8^+IFN\gamma^+$ or $CD8^+Perforin^+$ dual-positive cells in the mesenteric lymph nodes. **G**, Populations identified by cytometry across all four groups were quantified and averages displayed \pm SEM (*, $P < 0.05$).

7.2 to 10.9 months, and substantially reduced serum levels of TGF β 1 (19). However, should therapies targeting the TGF β pathway induce PD-L1 upregulation, this may substantially limit their therapeutic efficacy. Similarly, as TGF β directly suppresses effector T-cell function independent of PD-L1, its near ubiquitous overexpression in pancreatic cancer patients may also explain the limited efficacy of PD-1/PD-L1 inhibitors in pancreatic cancer patients (Fig. 4).

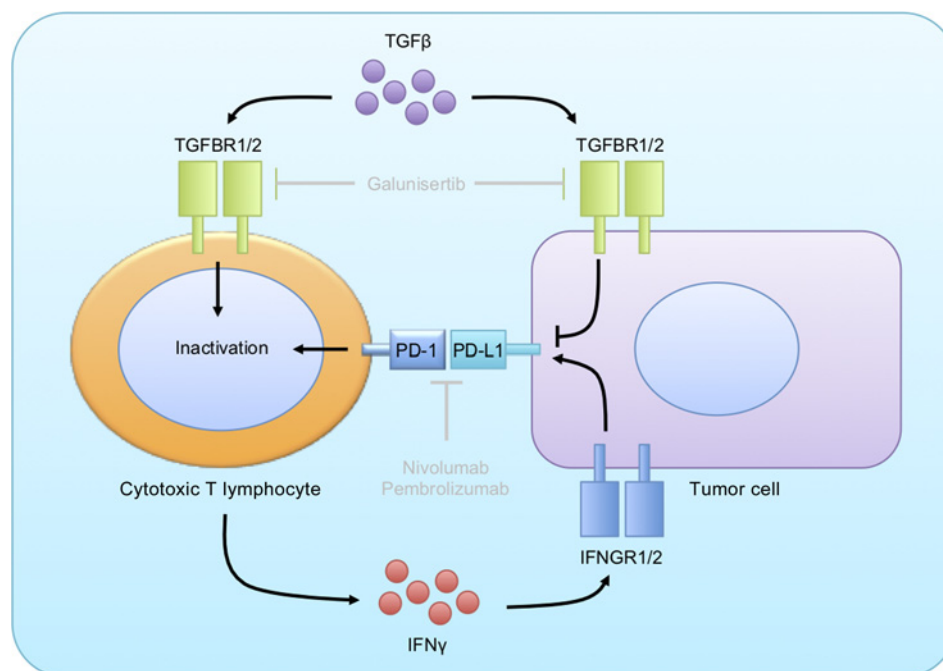
The link between TGF β signaling and immune checkpoint inhibition is now becoming clear, and their combination is showing early efficacy in other cancers types (10). For instance, in metastatic urothelial cancer patients, a poor response to the anti-PD-L1 antibody Atezolizumab was associated with a TGF β

signaling signature in fibroblasts. Similarly, the combination of TGF β and PD-L1 inhibition greatly enhanced antitumor immune function (20). In addition, concomitant TGF β /PD-L1 pathway inhibition greatly enhanced T-cell responses in esophageal squamous cell carcinoma (21), and blockade of genetically reconstituted models of colon cancer metastasis (22). Further, TGF β pathway inhibitors enhanced responsiveness to PD-1/PD-L1-neutralizing antibodies in mice bearing poorly immunogenic 4T1-LP breast tumors (23).

Despite this increasing body of evidence, this combination has not been fully explored in PDAC. As it is well established that TGF β is almost ubiquitously overexpressed in the pancreatic TME and is itself a primary means for immune evasion, it is essential to

Figure 4.

Model for mechanism of action for combined TGFβ and PD-1 pathway inhibition. Both TGFβ and PD-1 are known to repress the function of CD8⁺ cytotoxic T cells in PDAC. However, our data appear to suggest that TGFβ negatively regulates the expression of PD-L1 on the neoplastic epithelium, either directly (as shown) or indirectly, potentially through IFN γ as shown. Hence, pharmacologic ablation of TGFβ signals via the drug Galunisertib leads to the accumulation of PD-L1 in the cancer epithelium, failing to promote a substantial antitumor immune response. Hence, combination therapy with an anti-PD-1 antibody and a TGFβ pathway inhibitor is necessary to have therapeutic efficacy, and such a combination regimen warrants clinical consideration.



further study the ability of TGFβ inhibitors to promote immune cell clearance of pancreatic tumors, as well as overcome the innate resistance of pancreatic cancers to immune checkpoint inhibitors and improve patient outcomes.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: D.R. Principe, H.G. Munshi, P.J. Grippo

Development of methodology: D.R. Principe, P.J. Grippo

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): D.R. Principe, A. Park, M.J. Dorman, S. Kumar, N. Viswakarma, J. Rubin, C. Torres, P.J. Grippo, A. Rana

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): D.R. Principe, A. Park, M.J. Dorman, S. Kumar, N. Viswakarma, H.G. Munshi, P.J. Grippo, A. Rana

Writing, review, and/or revision of the manuscript: D.R. Principe, H.G. Munshi, P.J. Grippo, A. Rana

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): D.R. Principe, A. Park, R. McKinney, A. Rana

Study supervision: D.R. Principe, P.J. Grippo, A. Rana
Other (partial funding of this study): P.J. Grippo

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