Dipeptidyl peptidase 4 (DPP4) inhibition is not solely responsible for the anti-tumor effects of BXCL701, an inhibitor of multiple DPPs, in a murine model of pancreatic ductal adenocarcinoma (PDAC)

Alexander Lekan[1], Rachael Maynard[1], Zoe X. Malchiodi[1], Annie Zuo[1], Sandra A. Jablonski[1], Veena Agarwal[2], Moses Donkor[2], Vincent O'Neill[2], and Louis M. Weiner[1]

[1] Georgetown University/Lombardi Comprehensive Cancer Center, Washington, DC; [2] BioXcel Therapeutics, New Haven, CT

Abstract

Immunotherapy has limited efficacy in PDAC. BXCL701, an inhibitor of DPPs 4, 8, 9, and Fibroblast Activation Protein [1], reverses the abilities of DPPs to block immune activation through truncation of chemokines, induction of fibrosis, and inhibition of inflammasome activation and IL-18 release. We previously demonstrated that BXCL701+αPD1 reduced tumor growth and increased T and Natural Killer (NK) cell infiltration in a subcutaneous, syngeneic, murine PDAC model [2]. However, the critical determinants of BXCL701's therapeutic benefit are unknown. Here, we examined the effects of an FDA approved DPP4 inhibitor (sitagliptin)+αPD1 in an orthotopic, syngeneic murine PDAC model. Analysis of mice at endpoint revealed no significant difference in tumor mass in mice treated with PBS control and sitagliptin+αPD1 (p=0.90). A significant improvement was seen with BXCL701+αPD1, as compared to PBS (p<0.0001) and sitagliptin+αPD1 treated cohorts (p<0.0001). Strikingly, 8/9 BXCL701+αPD1 treated mice showed no evidence of disease. No change in tumor fibrosis was observed between sitagliptin+αPD1 treated mice and control. Additionally, treatment with BXCL701+αPD1 was accompanied by dramatic increases in plasma cytokines related to inflammasome activation and Th1 response. Overall, these findings suggest that the anti-tumor effects of BXCL701+αPD1 therapy are not solely due to DPP4 inhibition and may require combined inhibition of multiple DPPs for therapeutic effect.

Methods

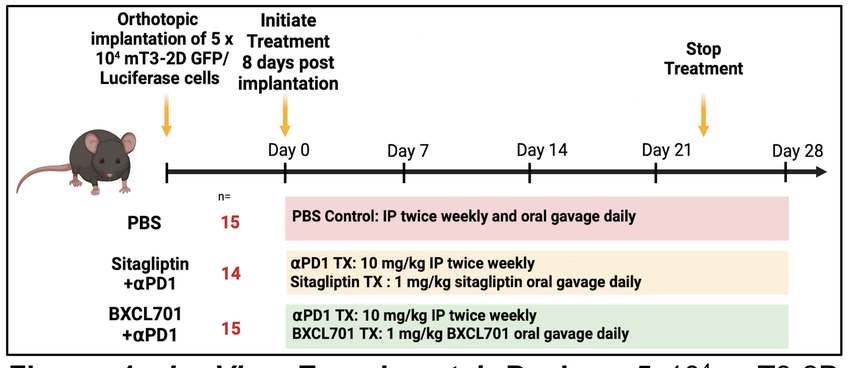

Figure 1. *In Vivo* Experimental Design: 5x10^4 mT3-2D *(Kras$^{+/G12D}$; p53$^{+/-R172H}$; Pdx-Cre)* GFP/luciferase-expressing cells were implanted orthotopically in the pancreas of C57BL/6J mice. Bioluminescence imaging was used to monitor tumor growth and mouse health.

- **Blood Collection and Chemokine Analysis:** Blood was collected, via submandibular draw, from 4-5 mice in each cohort and pooled. Analysis was done by Eve Biosciences.
- **Tumor Staining:** Tumors were excised, fixed, and stained with Masson's Trichrome to analyze for fibrosis.

Contact Information: Alexander Lekan (AAL75@georgetown.edu)

Results

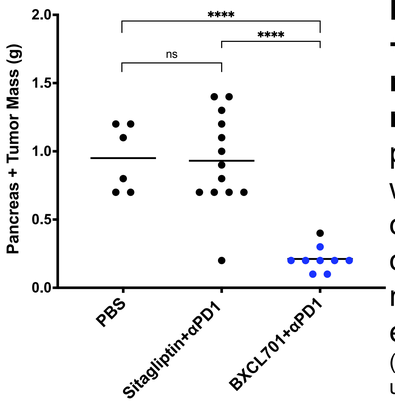

Figure 2. BXCL701 + αPD1 induces robust anti-tumor responses: Blue points indicate mice where no evidence of disease was observed, only normal pancreas at endpoint (Day 23). (****p<0.0001 determined by unpaired, two-tailed T-test).

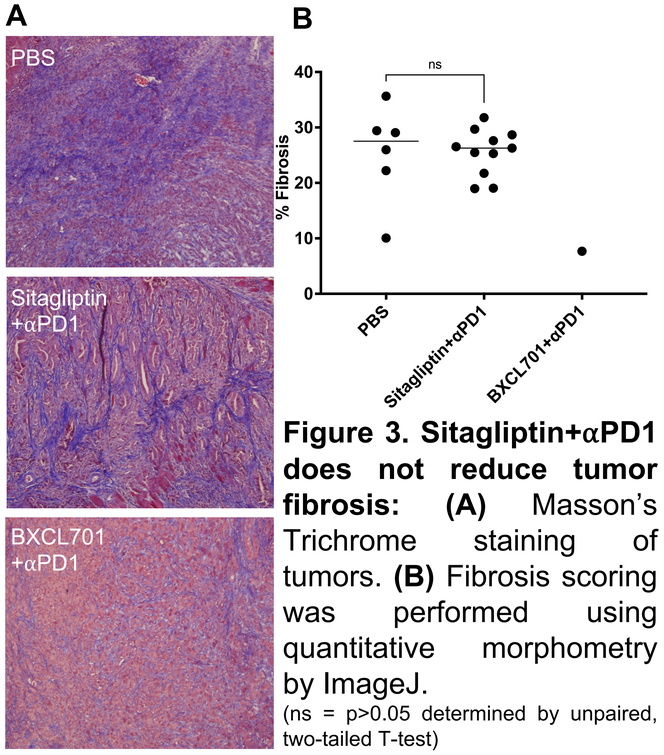

Figure 3. Sitagliptin+αPD1 does not reduce tumor fibrosis: (A) Masson's Trichrome staining of tumors. **(B)** Fibrosis scoring was performed using quantitative morphometry by ImageJ. (ns = p>0.05 determined by unpaired, two-tailed T-test)

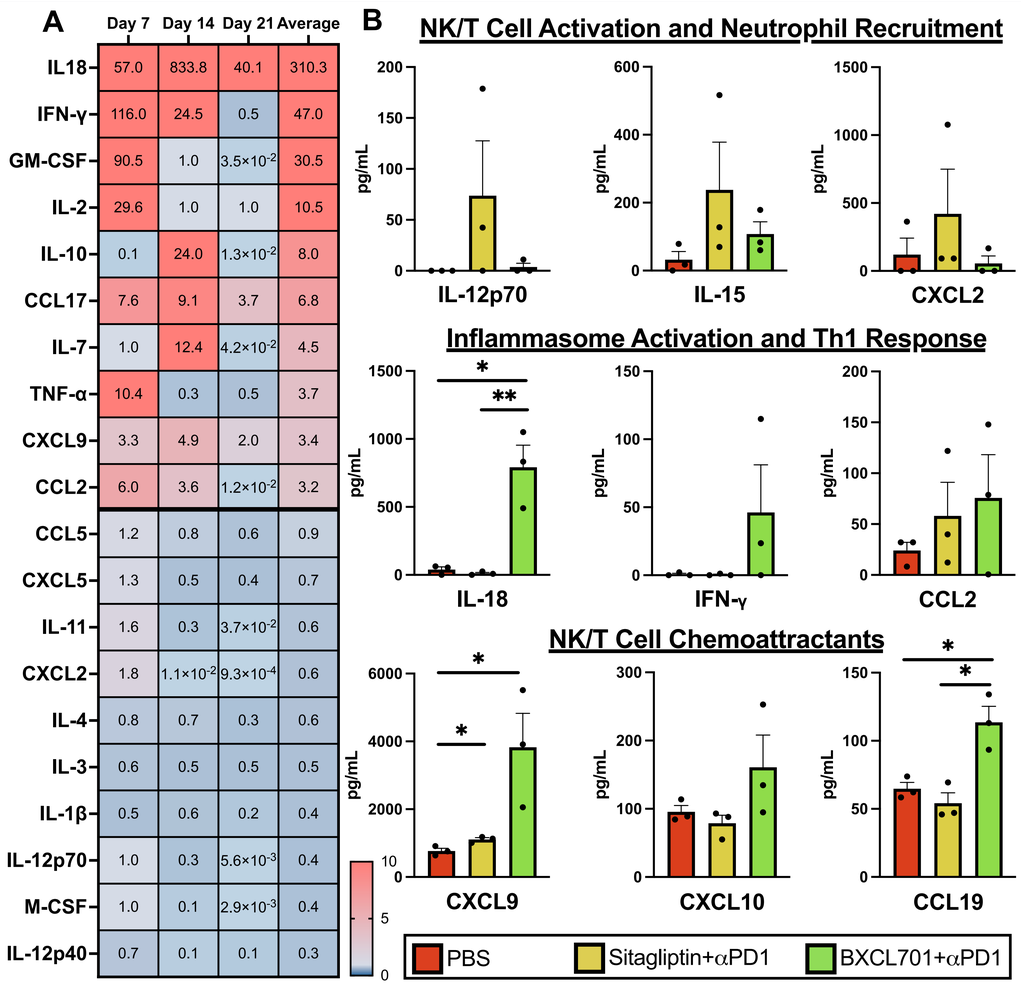

Figure 4. BXCL701 and Sitagliptin, each in combination with αPD1, increase expression of distinct cytokine subsets: (A) Heatmap demonstrating average fold-change of top 10 upregulated/downregulated plasma cytokine concentrations in mice bearing mT3-2D tumors treated with BXCL701+αPD1, as compared to sitagliptin+αPD1 treated mice. **(B)** Bar graphs demonstrating changes in select cytokines/chemokines. Each value is a single measurement of plasma pooled from 4-5 mice on Day 7, 14 or 21. Bars displayed with standard error of mean. (* = p<0.05, **p<0.01 determined by unpaired, two-tailed T-test).

Conclusions

- Sitagliptin+αPD1 therapy does not yield anti-tumor effects nor reduce intra-tumoral fibrosis.
- BXCL701+αPD1 treatment results in tumor clearance and increases in circulating cytokines associated with inflammasome activation, Th1 response, and NK/T cell Recruitment.
- **BXCL701+αPD1's anti-tumor effects appear dependent on inhibition of multiple DPPs, not only DPP4.**

References

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2.Fitzgerald A, Wang S, Agarwal V, et al. DPP inhibition alters the CXCR3 axis and enhances NK and CD8+ T cell infiltration to improve anti-PD1 efficacy in murine models of pancreatic ducta adenocarcinoma. J Immunother Cancer. 2021; 9: e002837.

Acknowledgements

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