Therapy with BXCL701, a DPP8, DPP9, DPPIV and FAP inhibitor, in combination with anti-PD1 antibody in a syngeneic murine pancreatic ductal adenocarcinoma (PDAC) model improves treatment outcomes, induces intratumoral NK cell infiltrates and induces a marked reduction in tumor stromal fibrosis

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Abstract #3373; Poster #6636

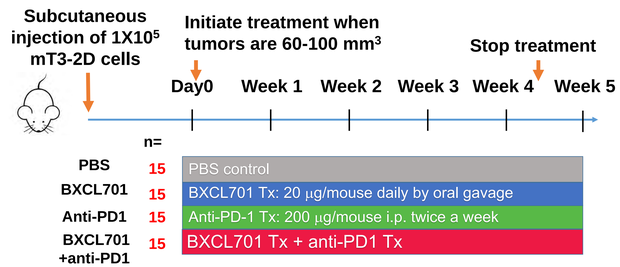
Abstract

PDAC is typically resistant to chemotherapy and immunotherapy; therefore, novel strategies are needed to enhance therapeutic response. BXCL701 is a well-studied inhibitor of dipeptidyl peptidases 4, 8, 9, and Fibroblast Activation Protein, and has been postulated to work through cytokine induction and macrophage pyroptosis [1, 2]. We examined the effects of BXCL701 and/or anti-PD1 antibody therapy in murine models of PDAC. In the mT3-2D PDAC model, combination therapy of established (~75mm^3) sc tumors reduced tumor growth to a greater extent (PBS: 1349±230 mm^3 on day 42 vs BXCL701+ anti-PD1 Ab: 355±161mm^3, p<0.0001) following a 28-day treatment program of PBS, BXCL701 (1mg/kg by daily oral gavage), anti-PD1 Ab (10mg/kg ip twice weekly) or the combination of BXCL701+ anti-PD1 Ab. Treatment with either BXCL701 or anti-PD1 Ab alone had minimal anti-tumor effects. BXCL701+ anti-PD1 Ab therapy was accompanied by significant tumor infiltration of macrophages and NK cells, by flow cytometry analysis. A dramatic reduction of tumor stromal fibrosis by Masson's trichrome stain was found in tumors treated with BXCL701 alone or BXCL701+anti-PD1 Ab. These findings suggest that the combination of BXCL701 with anti-PD1 antibody therapy can exert anti-tumor effects associated with increased intratumoral NK cells and macrophages content and the loss of fibrosis that may facilitate immunotherapy efficacy.

Introduction

- Pancreatic cancer is resistant to standard chemotherapy and immune-based regimens in part due to the lack of tumor infiltrating lymphocytes and the dense fibrotic microenvironment.
- BXCL701 (Talabostat; Val-boroPro) is a potent inhibitor of dipeptidyl peptidases 4, 8, 9, and fibroblast activation protein (FAP).
- It has been demonstrated that BXCL701 in combination with an anti-PD1 antibody inhibited tumor growth with upregulation of tumor-infiltrating immune cells as well as immuno-stimulatory cytokines in the MC38 syngeneic mouse model [1] .
- In this study, we examine the therapeutic effect of BXCL701 and/or anti-PD1 antibody in a murine PDAC tumor model.

Methods



Tumor model: mT3-2D pancreatic tumor model

1X10^5 mT3-2D murine pancreatic cancer cells [3] (*Kras*$^{+/G12D}$, *p53*$^{+/-R172H}$) were injected subcutaneously in the right flank of syngeneic C57BL/6 mice. When establish tumor volume reached 60-100 mm^3, mice were treated with PBS, BXCL701, anti-PD1 antibody, or combination of BXCL701 and anti-PD1 antibody. Tumor volumes were measured twice weekly. Tumors were dissected and processed for flow cytometry analysis and Masson's trichrome stain.

Results

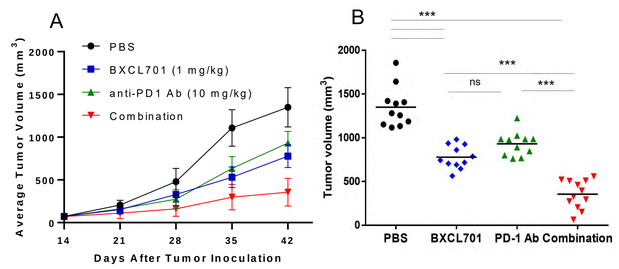


Fig 1. BXCL701 treatment improves the therapeutic effect of anti-PD1 antibody in mT3-2D tumor model.
(A) Tumor growth curve depicting the average tumor volume in C56BL/6 mice (n=15) treated with PBS, BXCL701, anti-PD1 Ab, combination of BXCL701 and anti-PD1 Ab. (B) Tumor volume for PBS, BXCL701, anti-PD1 Ab, or combination therapy treated groups at the end of treatment. (***p<0.001 as determined by ANOVA followed by Tukey's multiple comparison test).

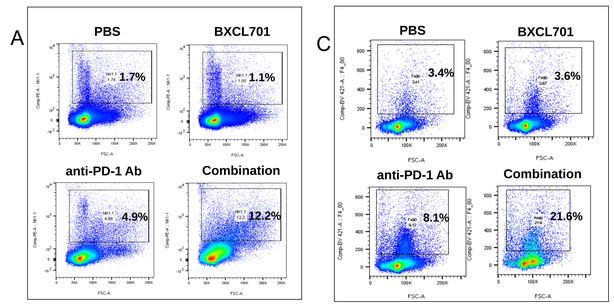


Fig 2. BXCL701 treatment with anti-PD1 antibody therapy significantly increases the infiltrations of NK cells (left panel) and macrophages (right panel) in the PDAC tumor microenvironment.
(A, C) Representative graphs of flow cytometry analysis of NK cell and macrophage infiltration as percent of CD45+ cells in the collected tumors from PBS, BXCL701, anti-PD1 Ab and combination treatment groups.
(B, D) Flow data representing NK cell and macrophage infiltration as percent of live cells in the collected tumors from PBS, BXCL701, anti-PD1 Ab, combination therapy treatment group. (n=12, ***p<0.001 as determined by ANOVA followed by Tukey's multiple comparison test).

Results

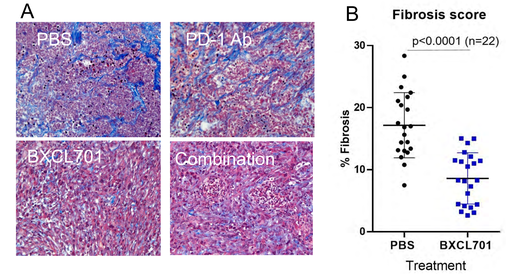


Fig 3. BXCL701 treatment reduces fibrosis in the tumor micro-environment. (A) Masson's Trichrome stain demonstrating intense fibrosis formation in a representative mT3-2D pancreatic tumor from a PBS treated mouse, while BXCL701 or combined Rx markedly decreased fibrosis. (B) Fibrosis scoring was performed using quantitative morphometry by Image J. BXCL701 treated mouse tumors showed significantly decreased fibrosis compared to PBS treatment (n=22 pooling results from two separate experiments).

Conclusions

- BXCL701treatment improves the therapeutic effect of anti-PD1 antibody therapy.
- BXCL701 decreases fibrosis of the tumor microenvironment, which may facilitate the influx of tumor infiltrating immune cells
- These findings suggest that the combination of BXCL701 therapy can exert anti-tumor effects associated with increased intratumoral NK cells and macrophages content by remodeling the tumor microenvironment and transforming cold, non-inflamed tumors into hot immune-sensitive tumors.

References

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Acknowledgements

The authors would like to thank the Flow Cytometry and Cell Sorting Shared Resource, the Histopathology & Tissue Shared Resource at Lombardi Comprehensive Cancer Center for their support in this project. This work is supported by NCI Grant NCI Grant CA50633.

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