Dipeptidyl Peptidase Inhibitor BXCL701 synergizes with an OX40-agonist antibody resulting in synergistic anti-tumor response and survival in an animal model of colorectal cancer by bridging the innate and adaptive arms of the immune system

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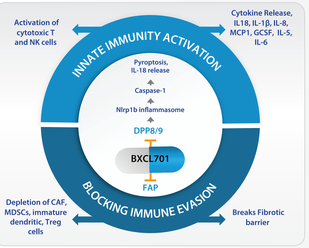
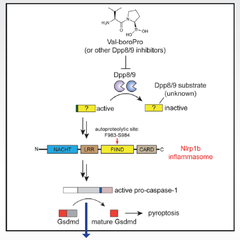
Abstract # LB-077

BACKGROUND

- BXCL701 (Talabostat; Val-boroPro) is a potent inhibitor of dipeptidyl peptidases, specifically DPP8, DPP9 and fibroblast activation protein (FAP).
- Utilizing Artificial Intelligence based approaches BXCL701 was uncovered as an agent that would potentially synergize with existing immunotherapies in novel combinations for cancer treatment [1]. BXCL701 in combination with an anti-PD-1 antibody (aPD-1) has demonstrated inhibition of tumor growth as well as up-regulation of immuno-stimulatory cytokines and tumor-infiltrating immune cells in animal models [1].
- In the present work cytokines/chemokines were evaluated in serum samples from animals in an acute PK/PD study revealing that BXCL701 stimulated several pro-inflammatory factors. The most stimulated cytokine was IL-18. This finding was consistent with recent literature which points out the presence of the BXCL701 targets, DPP8 and DPP9, in macrophages, and that inhibition of DPP8 and DPP9 activity in these cells activates the Nlrp1b inflammasome. Activation of this pathway leads to pyroptosis, a pro-inflammatory form of cell death [2] concomitant with the activation of caspase-1 and subsequent activation of pro-IL-1β and pro-IL-18.
- IL-18 bridges the innate and adaptive immune systems through induction of IFN-γ and the CD134 (OX40) pathway [3]. We conducted an *in vivo* study combining BXCL701, OX40 agonist (aOX40) and aPD-1 in a preclinical model (MC38) of colorectal cancer to test modifying the innate and adaptive immune systems.
- BXCL701 in combination with aOX40 or aOX40 and aPD-1 resulted in synergistic inhibition of tumor growth and a significant improvement in the median survival of animals.

BXCL701: MECHANISM OF ACTION

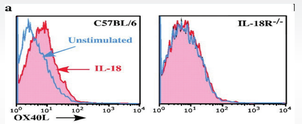




IL-1β, IL-18

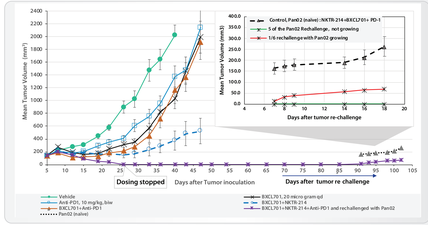
DPP8/9 inhibition stimulates the innate immune system. DPP8/9 inhibitors induce an inflammatory form of cell death called pyroptosis in monocytes and macrophages by activating the inflammosome sensor protein Nlrp1b which in turn activates pro-caspase -1 to mediate pyroptosis (Okondo et al. 2018, Cell Chem. Biol. 25, 1-6).

IL-18 induces OX40L expression on DCs that can boost T cell responses.



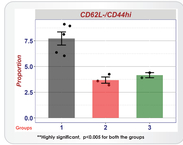
C57BL/6 (left plot) or IL-18-/- (right plot) mice adoptively transferred with SM1 cells were injected with medium alone (blue line) or IL-18 (red histogram). After 18 h, pooled PLN and MLN cells were enriched for DCs and CD11c+ cells were gated to examine OX40L expression. (J Immunol 2006; 177: 234-245)

IN VIVO ANTI-TUMOR IMMUNITY ESTABLISHED WITH TRIPLE COMBINATION



Earlier data demonstrated BXCL701 with PD-1 antagonist and NKTR-214 generated complete and durable responses (Rastelli L et al., 2018 ACSO Annual Meeting, Abstract #3085)

Combination with anti-PD1 and NKTR-214 stimulates immune system "durable complete response" mice and making them resistant to new tumors



Group 1: Pan02 immune re-re-challenge (n=5)
Group 2: Naïve mice receiving Pan02 cells (n=3)
Group 3: Naïve mice (n=2)

Tumor free and Pan02 immune mice were re-challenged for a second time with Pan02 cells. CD8+ Splenocytes were enumerated 4 days after the tumor inoculation by flow cytometry for the expression of CD62 and CD44. CD8+ effector memory cells are defined as CD62L-lo/CD44hi

METHODOLOGY - ACUTE PK/PD STUDY

Tumor model: Pan02

Tumor implant: Each mouse was inoculated subcutaneously at the front right flank region with an appropriate inoculum of cancer cells (approximately 3 x 10^6) in 0.1 ml of PBS for tumor development. The date of tumor cell inoculation is denoted as day 0. Approximately 22 days following tumor implant, mice with a mean tumor volume of ~ 345 mm^3 were randomized and the test articles were administered.

Treatments: Single dose of Control (Vehicle), and BXCL701 (20 µg qd) were dosed in mice bearing established (~345 mm^3) tumors.

Protocol: Tumors and plasma were harvested at the mentioned time points after the single dose of BXCL701. Plasma samples were analysed by Luminex and ELISA by Crown Biosciences.

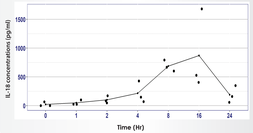
RESULTS - ACUTE PK/PD STUDY

Fold change in Cytokines/Chemokines with single dose of BXCL701 in a PK/PD study using Pan02 mouse (Female C57BL/6) model

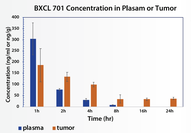
Cytokines	Time vs 0 hrs					
	1 hrs	2 hrs	4 hrs	8 hrs	16 hrs	24 hrs
IL-18	9.79	21.82	40.40	169.17	176.22	36.88
MCP-1	1.40	1.97	2.49	111.11	5.40	1.78
G-CSF	-2.12	1.26	-1.22	48.57	15.46	3.47
KC	1.06	1.37	2.35	22.23	1.34	-1.23
IL-5	-3.55	-2.21	2.82	17.34	17.50	7.17
IL-6	2.29	1.81	1.30	16.08	1.06	-1.71
MIP-1alpha	-1.93	-1.87	1.16	3.04	-1.93	-2.23
Eotaxin	1.28	1.44	1.85	2.89	1.79	1.49
IL-1alpha	1.02	-4.75	1.95	2.61	1.64	1.63
IL-17	1.04	1.42	-1.28	2.50	-1.01	-1.03
IP-10	-1.29	-1.14	-1.25	1.98	1.24	-1.10
IL-1beta	-1.29	1.25	-1.20	1.93	2.29	1.26
MIG	-1.38	-1.08	-1.23	1.44	1.89	1.41
MIP-2	-1.04	1.10	1.15	1.29	-1.03	-1.07
LIX	1.02	1.08	1.13	1.09	1.08	1.04
IL-15	-1.61	2.82	1.04	-1.16	-1.15	2.86
IL-9	-4.77	-1.30	-6.38	-4.13	-2.22	2.68

RESULTS - ACUTE PK/PD STUDY

IL-18 is the most stimulated cytokine by BXCL701



BXCL701 concentration in plasma and tumor of acute PK/PD study



Plasma and tumors were harvested from animals at different time points after the single dose of BXCL701 in Pan02 Murine Pancreatic Cancer Syngeneic model

METHODOLOGY - EFFICACY STUDY IN COLORECTAL CANCER MOUSE MODEL (MC38)

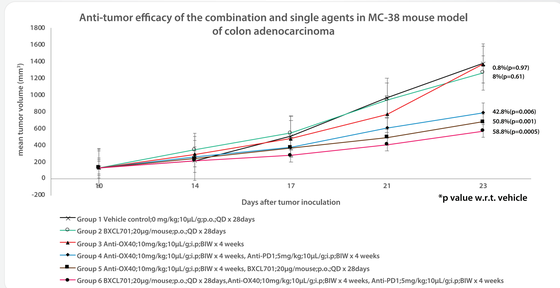
Tumor implant: Each mouse was inoculated subcutaneously at the front right flank region with an appropriate inoculum of cancer cells (approximately 1 x 10^6) in 0.1 ml of PBS for tumor development. The date of tumor cell inoculation is denoted as day 0. 11 days following tumor implant, mice with a mean tumor volume of ~ 120 mm^3 were randomized and the test articles were administered.

Treatments: Control (Vehicle), aOX40 (10 mpk, biw), BXCL701 (20 µg, qd) and aPD1 (5mpk, biw) were dosed in mice bearing established (~120mm^3) tumors.

Protocol: Tumor size and body weights were measured twice weekly. The median survival was calculated using Graph pad prism. Significance was calculated using Log Rank test.

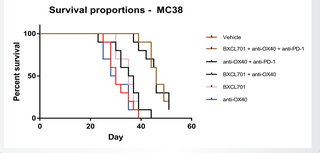
RESULTS

BXCL701 caused tumor growth inhibition with OX40-agonist, or with OX40-agonist and PD-1 antagonist



A significant tumor growth inhibition (TGI) was observed in animals when BXCL701 was added to aOX40 (p=0.005 v/s aOX40, p=0.001 v/s BXCL701) as well as a double combination of aPD1 and aOX40 (p=0.053 v/s aPD1+aOX40). Thus BXCL701 synergistically improves efficacy of these immunotherapy agents.

BXCL701 treatment increased median survival when combined with aOX40, or with aOX40 and aPD-1



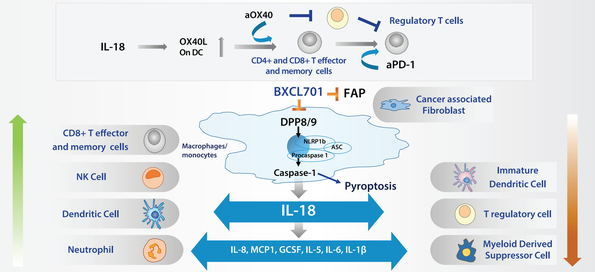
Combinations	Median Survival (days)	P value w.r.t. vehicle
Vehicle	30	
BXCL701	35	P > 0.05
aOX40	29	P > 0.05
BXCL701 + aOX40	46	P < 0.0001
aPD1 + aOX40	36	P > 0.05
BXCL701 +aPD1+ aOX40	46	P < 0.0001

BXCL701 demonstrated a significant improvement in the survival of animals when combined with aOX40. The median survival of animals treated with combination of BXCL701 and aOX40 was significantly increased to 46 days in comparison to BXCL701 alone (p < 0.0001), while combination of aPD1 and aOX40 treated animals had a median survival of 36 days (aPD-1 and aOX40 vs Vehicle P > 0.05). The triple combination of BXCL701, aOX40 and aPD1 resulted in the median survival of 46 days (p < 0.0001 vs Vehicle).

CONCLUSION AND FUTURE PERSPECTIVE

- **BXCL701 exhibited a synergistic anti-tumor activity and survival benefit in animals when combined with aOX40, or aOX40 and aPD1 combination.**
- **BXCL701 regulated cytokines are responsible for the observed synergism with aOX40 treatment.**
- **The present study recommends the clinical evaluation of BXCL701 and OX40-agonist combination in solid tumors.**

IL-18 secreted by macrophages in response to BXCL701 treatment bridges the activation of innate and adoptive immunity, and aids in overcoming immune-evasion leading to antitumor activity of BXCL701 and OX40-agonist combination



REFERENCES

[1] Rastelli et al., 2018 ACSO Annual Meeting, Abstract #3085
[2] Okondo et al. 2018, Cell Chem. Biol. 25, 1-6
[3] Maxwell et al. 2006, J of Immunol, 177: 234

ACKNOWLEDGEMENT

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