Combination of a Dipeptidyl Peptidase Inhibitor BXCL701 and Biased CD122 Agonist NKTR-214 with Anti-PD1 Provides Functional Immunological Memory through Inflammatory Cell Death

Abstract # P368

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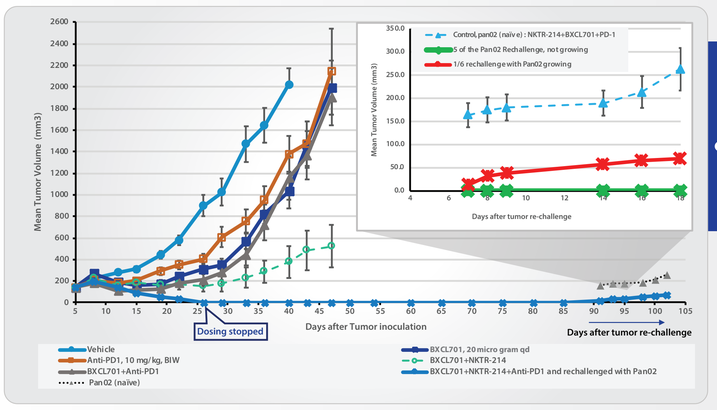
BACKGROUND

- 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].
- NKTR-214 is a clinical-stage biologic that comprises interleukin-2 (IL2) protein bound by multiple releasable polyethylene glycol (PEG) chains. NKTR-214 was invented to harness the potent immune stimulatory benefits of the IL2 pathway to maximize anti-tumor responses and minimize unwanted biological side effects [2,3].
- In a murine syngeneic model of pancreatic cancer (Pan02), BXCL701 in combination with an anti-PD-1 antibody and NKTR-214 (a CD122-biased agonist) has demonstrated the generation of complete and durable responses accompanied by the establishment of immunologic memory [1]. The immune activation generated by the triple combination implies that these three agents are acting non-redundantly and complementary.
- In the present work previous observations were extended to other syngeneic mouse models wherein similar complete and durable responses with functional immunologic memory were noted (MC-38, WEHI-164) but not in all models tested (RM-1, B16F10).
- Based on IHC and flow cytometry based immunophenotyping data there appears to be a correlation between the presence of a high-density of tumor associated macrophages in models responsive to the triple combination.
- The recent literature points out the presence of 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 proinflammatory form of cell death [4,5] concomitant with the activation of caspase-1 and subsequent activation of pro-IL-1β and pro-IL-18.
- It is proposed that BXCL701 stimulated macrophages rapidly "inflame" the tumor microenvironment thereby priming the cancer for other immune effector cells that have been activated through the combination of checkpoint inhibition and NKTR-214 stimulation.

[1] Rastelli L et al.,.2018 ACSO Annual Meeting, Abstract #3085
[2] Charych D etal., PLoS One. 2017 Jul 5;12(7):e0179431.
[3] Adi Diab etal., Presented at SITC 2017, Maryland, Poster #O20
[4] Okondo MC et al., Nat Chem Biol. 2017 Jan;13(1):46-53.
[5] Okondo MC et al., Cell Chem Biol. 2018 Mar 15;25(3):262-267



Earlier data demonstrated BXCL701 with PD-1 antagonist and NKTR-214 generated complete and durable response in Pan02 mouse model of pancreatic adenocarcinoma [1]

METHODOLOGY

Tumor model: : Pan02, MC38, WEHI-164, RM1, B16F10

Tumor implant: Each mouse was inoculated subcutaneously at the front right flank region with an appropriate inoculum of cancer cells (approximately 1-3 x 10^6) in 0.1 ml of PBS for tumor development. The date of tumor cell inoculation is denoted as day 0. Approximately 5 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), NKTR-214 (0.8mg/kg q9d), BXCL701 (20 µg qd) and anti-PD1 (200 µg, biw) were dosed in mice bearing established (~120mm^3) tumors .

Tumor-free animals exhibiting complete response to combined immunotherapy were enrolled to receive re-challenge of the same cells with which they were first inoculated. For this portion of the study, naïve animals also received a tumor cell inoculation.

Protocol: Tumor size and body weights were measured twice weekly. The untreated syngeneic mouse tumor models were profiled using IHC of the tumors with anti-mouse Ab (rabbit anti-mouse F4/80, monoclonal antibody, cat. no.:70076,Cell signaling) to analyse the macrophage density within the tumors.

KEY FINDINGS

1. Tumor regression for BXCL701 and NKTR-214 with anti-PD1 in MC38, WEHI 164 mouse model

The mice treated with BXCL701 20 µg qd, in combination with NKTR-214 (0.8 mg/kg; q9d) and PD-1 antagonist (200 µg; biw) exhibited significant tumor reduction as noted from day 10 (Figure 1 & 2) in the syngeneic mouse models of colorectal adenocarcinoma (MC38) and fibrosarcoma (WEHI-164). Of the mice treated with the triplet combination, 100% became tumor-free by day 35. These animals remained tumor free for more than 100 days.

2. BXCL701, NKTR-214 and anti-PD1 induces anti-tumor immunity

The treatment of established tumors (~ 120 mm^3) with the triple combination resulted in 100% tumor-free mice (6/6) in MC38 model by day 35. All the animals remained tumor free for more than 100 days until they were re-challenged.
It was found that 6/6 re-challenged mice (only 1 mouse showed slight increase in tumor volume) rejected tumor growth unlike naive mice, demonstrating the generation of long-term tumor-specific memory response in the MC38 mouse model (Figure 1).
In case of the WEHI model, out of the 6 mice that received treatment after the establishment of tumors (~ 110 mm^3), 3 mice completed dosing while 3 animals had to be removed from the study due to dosing complications. However, the 3 mice that completed the triple treatment, showed complete tumor regression from day 35 onwards. These 3 mice remained tumor free for more than 100 days until they were re-challenged. Further, no tumor growth was observed in these mice upon re-challenge, as compared to the naïve mice, demonstrating the generation of long term tumor-specific memory response (Figure 2).

3. Macrophage density profiling of responsive and non-responsive tumor models

IHC of Pan02, MC38 and WEHI-164 tumors from untreated animals with anti-mouse Ab (rabbit anti-mouse F4/80, monoclonal antibody, cat. no.:70076; Cell Signaling), as per the manufacturer's protocol revealed high macrophage densities in the responsive models. The IHC of the RM-1, B16 F10 tumor models on the other hand showed low macrophage density (Figure 4). The IHC of the respective models corroborate their responses to the triple combination treatment and anti-tumor activity (Figure 3). Flow cytometry profiling of TIL populations of syngeneic animal models reveal that responsive models have a generally higher proportion of macrophages relative to CD45+ cells (Table 1).

Figure 1. MC38 mouse model shows complete tumor regression as well as anti-tumor immunity in all the mice treated with triple combination of BXCL701, NKTR214 and PD-1 antagonist

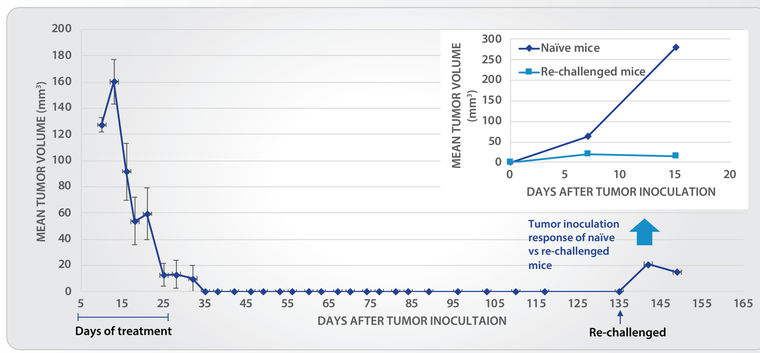


Figure 2. WEHI-164 mouse model for fibrosarcoma shows complete tumor regression and anti-tumor immunity in 3/6 mice with triple combination treatment of BXCL701, NKTR214 and PD-1 antagonist

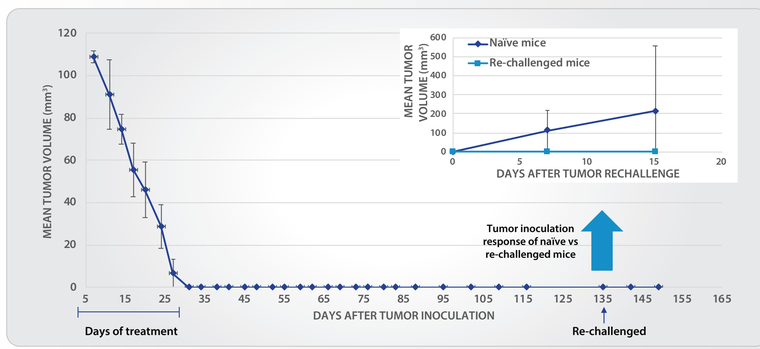


Figure 3. Triple Combination Responses across various Syngeneic Mouse Model

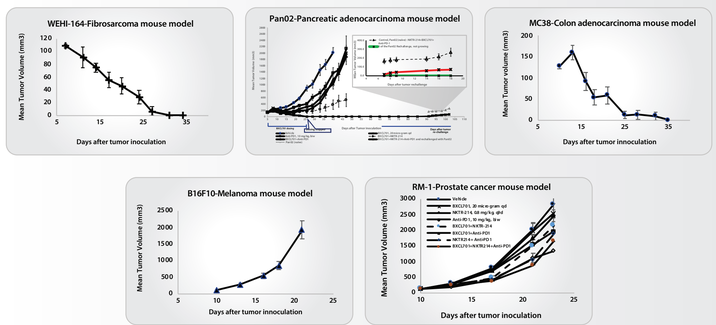


Figure 4. Macrophage Density In Tumors Correlates With Triple Combination Responses (IHC Data)

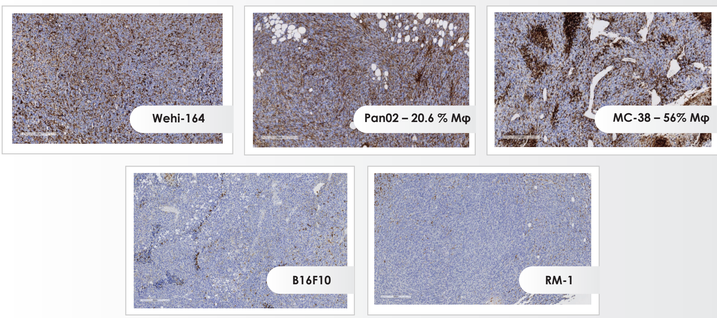


Table 1. Macrophage Density In Tumors Correlates With Triple Combination Responses (FACS Data)

	CD45+	CD3+	CD4+ CD8-	CD4- CD8+	Effector CD4+ Tcells	T-reg	m-MDSC	NK	NKT	g-MDSC	Macrophages	M1	M2	CD4+ PD-1+	CD8+ PD-1+	CD45- PD-L1+	M2/M1
MC38	40.70	16.60	7.30	6.90	6.70	0.60	4.60	4.00	2.20	0.40	56.10	41.90	13.90	11.30	17.20	93.30	0.33
Renca	66.80	15.20	10.20	3.10	5.30	4.70	0.80	2.10	1.10	0.80	40.00	28.50	11.00	9.80	17.10	81.80	0.39
Pan02	56.20	6.50	2.70	2.40	1.30	1.40	7.60	0.30	0.30	3.20	20.60	2.50	16.60	2.70	1.40	1.00	6.64
EMT-6	89.80	29.50	8.20	4.00	7.50	0.60	12.50	1.10	0.30	15.00	10.70	1.50	9.10	0.10	0.10	0.90	6.07
B16F10	18.50	16.60	4.70	4.50	3.80	0.90	13.50	3.90	0.80	2.90	9.90	3.70	5.10	27.30	17.80	11.00	1.38
RM-1	47.70	17.20	7.40	5.60	5.70	1.50	30.10	7.30	0.60	0.60	9.50	3.80	5.40	0.40	0.90	0.60	1.42
A20	-	48.20	34.20	9.00	28.00	5.80	2.50	4.60	5.10	1.80	4.70	0.40	4.20	49.10	40.50	48.10	10.50
CT26	65.90	31.00	17.00	5.90	12.60	4.40	25.20	9.00	3.20	2.10	3.30	0.80	2.40	19.20	31.90	81.30	3.00
MBT-2	69.20	8.50	3.30	2.50	1.50	1.60	31.70	5.10	0.20	16.20	2.60	0.30	2.30	0.50	0.80	42.70	7.67
H22	28.10	6.70	4.10	0.80	2.00	2.10	32.80	3.10	0.30	13.10	2.20	0.20	1.90	4.40	5.30	1.60	9.50

Baseline tumor infiltrating leukocyte (TIL) populations were assessed by flow cytometry. CD45+ cell populations were gated from tumor derived single cell suspensions and then subsequently analyzed for leukocyte sub-populations. Cancer models responsive to the combination of BXCL701 + NKTR-214 + anti-PD-1 (green shaded) tended to have higher density of macrophages than those non-responsive (red shaded).

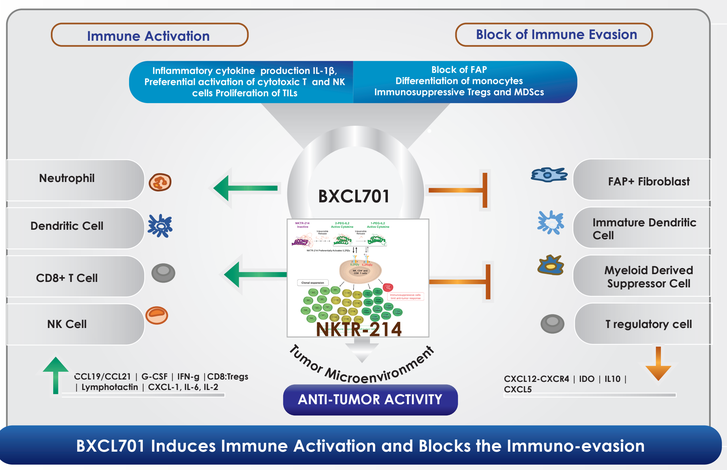
HYPOTHESIS

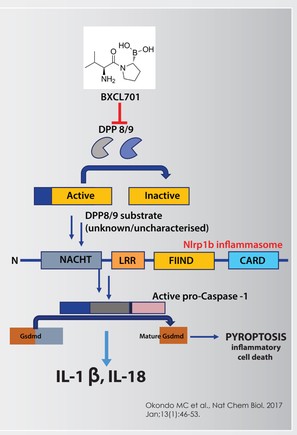
BXCL701 combination Impacting the tumor microenvironment

- Impact on cytokine profile
- Immuno-phenotype Impact
- Pre-clinical evidence of synergy with ICI
- Clinical Evidence of single agent efficacy

Innate	Adaptive
Impacts the pro-inflammatory cytokine release Caspase mediated IL-1 beta release via DPP8/9 inhibition and immunogenic death of monocytic cell	Agents that activated the effector T cell population; Leading to expansion of effector T & memory T cells (Eg: NKTR214)
Enhances Neutrophils and NK cells Impacts macrophages in the tumor milieu	Increases the ratio of tumor killing CD8+ T cells to T reg cells in tumors
Oral administrative regimen Combination with ICI in murine tumor model demonstrated synergy in tumor inhibition and increase in innate immune cells	Antibody-like dosing regimen Complementary mechanism of action to checkpoint inhibition demonstrated in murine tumor models
Clinically demonstrated stimulation of Effector T cells Natural Killer cells Neutrophils & Macrophages	Clinically assessment in patients with locally advanced or metastatic solid tumors: increase CD8+T and NK cells in the tumor microenvironment, Minimal impact on T regs; (Eg: NKTR214).

MoAs Inhibit DPP 8/9 & FAP and activates macrophages while suppressing Tregs, converting tumors from Cold to Hot





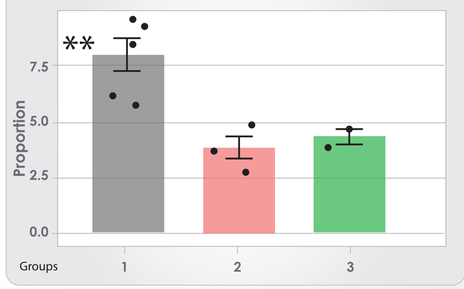
Okondo MC et al., Nat Chem Biol. 2017 Jan;13(1):46-53.

BXCL701 Induces Immune Activation and Blocks the Immuno-evasion

Immuno-phenotype for memory response

Pan02 study re-re-challenge analysis shows development of effector memory T cells

Effector memory CD8+ T cells: CD67L-CD44hi



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

** Highly significant, p<0.005 for both the groups

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 CD62L and CD44. CD8+ effector memory cells are defined as CD62L-lo/CD44hi

These data confirm the development of a CD8+ effector memory T cell response in animals immune to Pan02 cells through the triple combination of BXCL701+anti-PD1+ NKTR-214

CONCLUSION AND FUTURE PERSPECTIVE

**This data supports the role of macrophages in executing the anti-tumor impact of BXCL701 and its combinations.
It provides the rationale for a mechanistically based predictive biomarker that can potentially be used in the clinical application of this triple combination therapy.**

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