DIPEPTIDYL PEPTIDASE-9 (DPP9) OVEREXPRESSION IS A POTENTIAL RESPONSE-PREDICTIVE BIOMARKER OF BXCL701 AND PEMBROLIZUMAB COMBINATION TREATMENT IN mCRPC PATIENTS WITH SCNC PHENOTYPE

M Donkor, V Agarwal, S Kupchick, R Deshpande, D Newmin, and V O'Neill
BioXcel Therapeutics, 555 Long Wharf Drive, New Haven, CT, 06511

Abstract #661

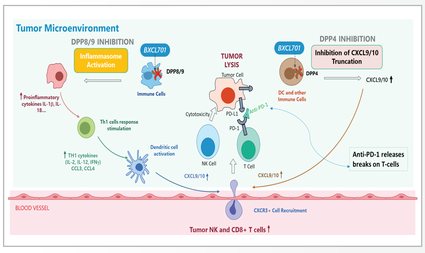
ABSTRACT

BACKGROUND: BXCL701 (talabostat), an oral innate immune activator, is currently in a Phase 2a/b trial in combination with pembrolizumab in patients with metastatic castration-resistant prostate cancer (mCRPC) of small cell neuroendocrine (SCNC) phenotype. Preclinical data suggest that BXCL701 inhibition of DPP8/9 and DPP4 is a pharmacologic strategy that can increase immune cell content and function in the tumor microenvironment (TME) to enhance the efficacy of immunotherapy[1]. Here, we report an exploratory biomarker analysis of samples from the Phase 2a study

METHODS: To characterize the cellular and molecular landscape of the TME, the Neogenomics MultiOmyx immunofluorescence platform was applied to pre-treatment FFPE tissues of 4 responders (Rs) and 8 non-responders (NRs). Similarly, mutational profiling of circulating tumor DNA was assessed to determine the predictive value of mutation burden or specific gene mutations. Additional mechanism-based pharmacodynamic endpoints were evaluated by comparing pre-treatment and post-treatment immune activation by serum cytokine analysis using the MSD platform

RESULTS: In this exploratory analyses, baseline DPP9 expression was higher in tumors of Rs, with median number of positive cells per mm^2 of 23.3 (range 0-341) in the stroma and 0 (range 0-419) in the tumor bed vs. 0.0 (range 0-812) and 0.0 (range 0-162) (p= 6.47e-6 and 0.112) respectively) in NRs. Programmed-death ligand-1 (PD-L1) expression and immune cell infiltration were low with innate immune cells as the predominant cell type. CD16-positive cells, CD68-positive macrophages, and T cells but not PD-L1 expression in the TME predicted the responder population. This phenotype along with a low mutation burden and low microsatellite instability (MSI) in these tumors suggest a pharmacologic mechanism where BXCL701 modulates an otherwise cold ICI-refractory TME into a permissive phenotype that enhances responsiveness to PD-1 blockade. Analysis of cytokine levels demonstrated general increases with a notable 15.1- vs. 7.4-fold induction of IFN-gamma in the Rs vs. NRs respectively although this did not reach statistical significance in this small sample set. Increased production of the inflammasome-dependent cytokines IL-1 beta and IL-18 was also observed, rising maximally to 42-fold. Other effector cytokines including IP-10 (range 0.6-14.0-fold), CXCL9 (range 1.3-55.6-fold) and TNF-alpha (range 1.3-4.3-fold) were also remarkably induced post-dose

MECHANISM OF ACTION OF BXCL701 AND PEMBROLIZUMAB COMBINATION

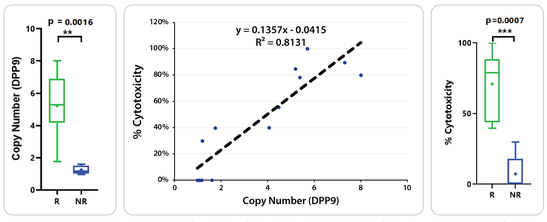


Adapted from Journal for ImmunoTherapy of Cancer 2021; 9:e002837. doi:10.1136/jitc-2021-002837

Broad DPP inhibition enhances antitumor immune response via two mechanisms: (1) DPP8/9 inhibition by BXCL701 activates inflammasome leading to production of proinflammatory and Th1 cytokines, and CXCL9/10 increase that enhances CXCR3+ NK and CD8+ T cell infiltration leading to cancer cell death. (2) DPP4 inhibition increases CXCL9/10 concentration and enhances CXCR3+ NK and CD8+ T cell infiltration into tumors

DPP9 COPY NUMBER CORRELATES WITH BXCL701 CYTOTOXICITY IN LEUKEMIC CELL LINES AND IS A POTENTIAL PREDICTIVE BIOMARKER IN LEUKEMIAS

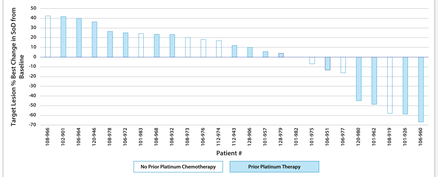
Out of total 13 cell lines 10 were AML cell lines



*** p-value calculated by non-parametric Mann-Whitney Test, **R** – Responder Cell Lines, **NR** – Non-Responder Cell Lines

Agarwal et al. SITC 2022, Abstract #25

For human leukemic cell lines, cell death in response to BXCL701 treatment was found to be directly correlated to DPP9 copy number with a correlation coefficient of 0.81. A statistically significant difference was observed between responder cell lines (cell lines that exhibited >30% cytotoxicity when treated with BXCL701) as compared to non-responder cell lines (cell lines showing <30% cytotoxicity when treated with BXCL701). This data was previously presented at SITC 2022

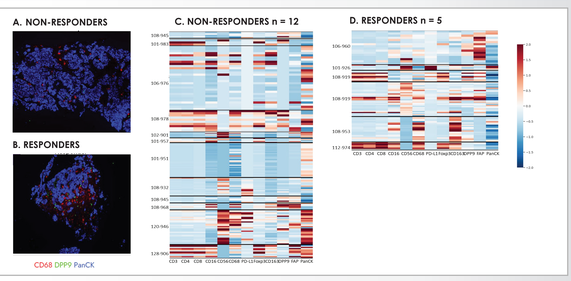


BEST RESPONSE

n = 25 RECIST 1.1 Evaluable Patients-Data as of 19-DEC-22

All evaluable patients are MSS and/or TMB low

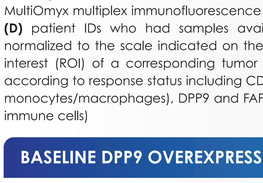
REPRESENTATIVE COLOR OVERLAY IMAGES AND HEATMAPS OF FFPE TISSUE EXPRESSION OF BIOMARKERS IN NON-RESPONDER AND RESPONDER PATIENT POPULATIONS



Representative color overlay images of the baseline expression of PanCK (blue), CD68 (red) and DPP9 (green) single markers are shown for a non-responder **(A)** and a responder **(B)** Heatmap showing the baseline expression density of the 12 biomarkers analyzed by the Neogenomics MultiOmyx multiplex immunofluorescence platform for 12 non-responders **(C)** and 5 responders **(D)** patient IDs who had samples available. Biomarker heatmap expression density was normalized to the scale indicated on the right of the graph. Each row represents a region of interest (ROI) of a corresponding tumor sample. There was separation for some biomarkers according to response status including CD68 (a marker for macrophages), CD163 (a marker for monocytes/macrophages), DPP9 and FAP (targets of BXCL701) and CD16 (a marker for innate immune cells)

BASELINE DPP9 OVEREXPRESSION IS PREDICTIVE OF RESPONSE

A)

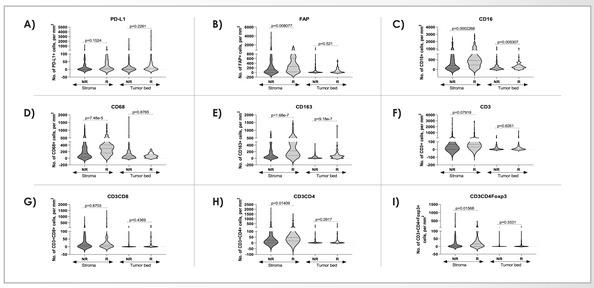


B)

		DPP9	
		Pos.	Neg.
Clinical Responder	Y	4	1
	N	4	8
Sensitivity		80.0	
Specificity		66.7	
False neg rate		20.0	
False pos rate		33.3	

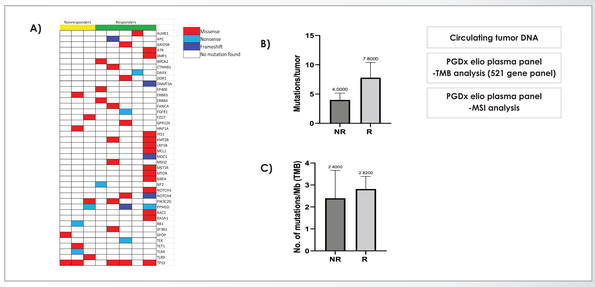
Quantification of DPP9-positive cells in the stroma and tumor bed **(A)**. In both responder and non-responder patient cohort samples, stromal DPP9 expression was found to be significantly higher compared to the tumor bed. The responder population had a significantly higher level of DPP9 expression in the stroma compared to the non-responder population. Response classification error showing sensitivity and specificity scores for a total median number of DPP9-positive cells per mm^2 cutoff of 21.6 for this sample set **(B)**. Test of significance was performed using Wilcoxon rank-sum test. A p-value < 0.05 is considered significant.
NR = non-responders, R = responders

BASELINE INNATE IMMUNE CELL INFILTRATION BUT NOT PD-L1 EXPRESSION CORRELATES WITH THE RESPONDER PATIENT POPULATION



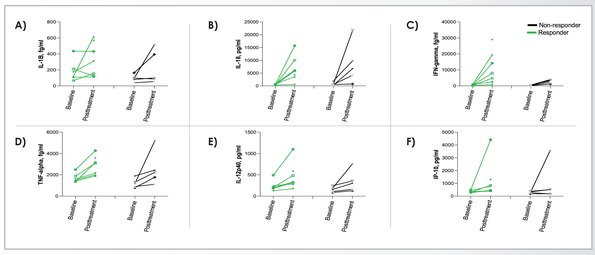
Quantification of biomarker expression in the stroma and tumor bed. The total number of PD-L1+ cells **(A)**, FAP+ cancer associated fibroblasts **(B)**, CD16+ innate immune cells **(C)**, CD68+ macrophages **(D)**, CD163+ macrophages/monocytes (E), CD3+ pan T cells (F), CD3+CD8+ T cells (G), CD3+CD4+ T cells **(H)**, and CD3+CD4+Foxp3+ T cells **(I)** in the stroma and tumor bed are shown. There was a separation according to clinical response status for tumor infiltrating innate immune cells particularly in the stroma including CD68+, CD163+, and CD16+ as well as the BXCL701 target FAP. No statistically significant difference was observed between responder and non-responder patient populations for PD-L1+ or CD3+ T cells. Test of significance was determined using Wilcoxon rank-sum test. A p-value < 0.05 is considered significant. NR = non-responders, R = responders

NEPC TUMORS SHOW GENOMIC HETEROGENEITY AND HAVE LOW TUMOR MUTATIONAL LOAD



The distribution of genomic alterations and mutational load in tumors as revealed by the PGDx elio plasma complete mutational profiling of circulating tumor DNA. Gene alterations **(A)**, total number of mutations found per tumor **(B)** and TMB **(C)** are shown. The average number of mutations and TMB are indicated by a number on each bar graph. The NEPC tumors showed genomic heterogeneity and low tumor mutation load. There was no association between the number of mutations or TMB and response status in this sample set although the responders trended higher in total number of mutations per tumor. TP53 was the most frequently mutated gene, occurring in 5/8 (62.5%) of tumors. All tumors were also MSI stable **(not shown)**. NR = non-responders, R = responders

BXCL701 IN COMBINATION WITH PEMBROLIZUMAB INDUCED INFLAMMATORY CYTOKINE PRODUCTION IN THE CIRCULATION POST DOSE



BXL701 in combination with Pembrolizumab evoked potent cytokine production in the circulation. Baseline and post-treatment levels of BXCL701 mechanism-based cytokines and other relevant immune effector cytokines including IL-1 beta **(A)**, IL-18 **(B)**, IFN-gamma **(C)**, TNF-alpha **(D)**, IL-12p40 **(E)**, and IP-10 **(F)** are shown. General increases in serum cytokine production post-dose were noted. Notably, interferon (IFN)-gamma showed the most difference in average increase from baseline in the responder vs. non-responder populations (15.1-fold vs. 7.4-fold), although this did not reach statistical significance in this small sample set. Other effector cytokines including IP-10 (range 0.6-14.0-fold), CXCL9 (range 1.3-55.6-fold) and TNF-alpha (range 1.3-4.3-fold) were also remarkably induced post-dose. NR = non-responders, R = responders

CONCLUSIONS

- Our data show that high baseline DPP9 protein expression is associated with response of BXCL701 and pembrolizumab combination treatment in mCRPC patients with SCNC phenotype
- Despite the small sample size, baseline DPP9 overexpression is predictive of response with good sensitivity and moderate specificity
- Immune cell infiltration, particularly CD16+ innate immune cells, CD68+ macrophages, CD163+ macrophages/monocytes and stromal FAP expression, but not CD3+ T cell marker or PD-L1 expression, were also significantly higher in the responder patient population compared to the non-responder population
- In addition, there were general increases in BXCL701 mechanism-based cytokines and other relevant immune effector cytokines in the circulation post-dose
- The low TMB and MSS status coupled with the low baseline T cell and PD-L1 expression signatures suggest a pharmacological mechanism where BXCL701 modulates an otherwise cold ICI-refractory TME into a permissive phenotype that promotes responsiveness to PD-1 blockade
- Additional analyses are ongoing to build on this finding and improve on the DPP9 protein expression by IHC as a response classifier

REFERENCES

- Fitzgerald et al, 2021, DPP inhibition alters the CXCR3 axis and enhances NK and CD8+ T cell infiltration to improve anti-PD1 efficacy in murine models of pancreatic ductal adenocarcinoma. J Immunother. Cancer, 9(11): e002837
- Antonarakis et al, 2020. Pembrolizumab for Treatment - Refractory Metastatic Castration - Resistant Prostate Cancer: Multicohort, Open-Label Phase II KEYNOTE-199 Study. J Clin Oncol. 38(5):395-405

ACKNOWLEDGEMENTS

We would like to acknowledge the team members at NeoGenomics Laboratories, 31 Columbia, Aliso Viejo, CA 92656, for running the multiplex immunofluorescence study; the team members of PGDx at 3600 Boston St, Suite 10, Baltimore, MD 21224, for running the ctDNA mutational profiling and the team members of NorthEast BioLab, 35 Worth Avenue, Hamden, CT 06518, for the serum cytokine analysis