POTENTIAL PREDICTIVE BIOMARKERS FOR BXCL701 IN ACUTE MYELOID LEUKEMIA (AML)

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Abstract No #25

ABSTRACT

BACKGROUND

BXCL701 (talabostat), an oral innate immune activator is currently in a Phase 2 trial in combination with PD-1 checkpoint inhibitor in metastatic castration-resistant prostate cancer patients. In solid tumors, preclinical data suggest that BXCL701 inhibition of dipeptidyl peptidases (DPPs) enhances antitumor immune responses via two mechanisms: (1) DPP4 inhibition increases tumor content of CXCL9/10, which recruits CXCR3+ NK and T cells, and (2) DPP8/9 inhibition activates the inflammasome resulting in immune cell pyroptosis followed by Th1 proinflammatory cytokine release, and dendritic cells (DCs) activation which further enhances the CXCL9/10-CXCR3 axis[1]. Recent studies show that BXCL701 exhibits single agent cytotoxicity against human AML cells through a similar pyroptotic mechanism[2]. Here, we report the identification of potential predictive biomarkers by using BXCL701-responsive and -nonresponsive human leukemic cell lines.

RESULTS

The analysis identified 20 genes as potential predictive biomarkers. These, include 5 genes (DPP9, DPP8, caspase 1, CARD8 and PYCARD) involved in the inflammasome - pyroptosis pathway that is activated by BXCL701 and correlate with the BXCL701 cytotoxic activity. Most of the genes have 2-to-1,000-fold higher expression in at least 3 responding cell lines in comparison to non-responding cell lines. On the other hand, EPCAM gene has 7,000-fold higher expression in non-responding cell lines vs responding cell lines. Further, copy number was evaluated for BXCL701 target genes (DPP8, DPP9, DPP4 and FAP) by RT-PCR in 11 responding leukemic cell lines and 6 non-responding cell lines. The DPP9 copy number variation (CNV) was found to be directly correlated with BXCL701 cytotoxicity in BXCL701-responding human leukemic cell lines with correlation coefficient (R^2) of 0.813.

METHODS

The cytotoxic activity of BXCL701 was evaluated in a panel of 17 hematological and non- hematological cell lines and confirmed that mostly but not all the leukemic cells were responsive to BXCL701. Three responding cell lines (KG1, MV4-11, and EOL-1, IC50 = 100 – 700 nM) and 2 non-responding cell lines (K562 and Kasumi1, IC50 >60 μM) were selected based on the cytotoxicity data and their gene expression profiles were compared to identify predictive biomarkers for BXCL701. Nanostring analysis was performed followed by qRT-PCR using cDNA from the cell lines.

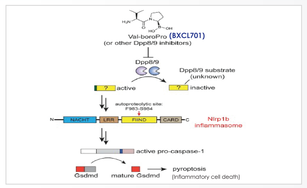
CONCLUSIONS

A gene panel consisting of genes involved in BXCL701 mechanism of action has been identified as a potential predictive biomarker for BXCL701 in leukemia, which can help in selecting patients susceptible to respond to BXCL701 treatment.

REFERENCES

1. Fitzgerald AA, 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 ductal adenocarcinoma. J Immunother. Can 2021, 9(11): e002837
2. Johnson DC, Taabazuin CY, Okondo MAC, et al. DPP8/DPP9 inhibitor-induced pyroptosis for treatment of acute myeloid leukemia. Nat Med. 2018; 24:1151-1156.

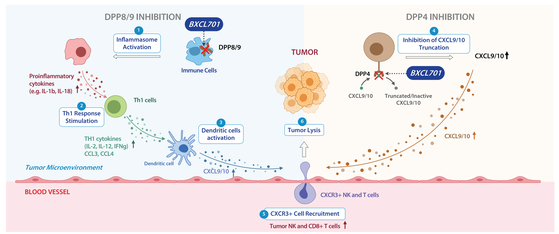
DPP8/9 INHIBITION STIMULATES THE INNATE IMMUNE SYSTEM



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 MC et al. Cell Chem Biol. 2018

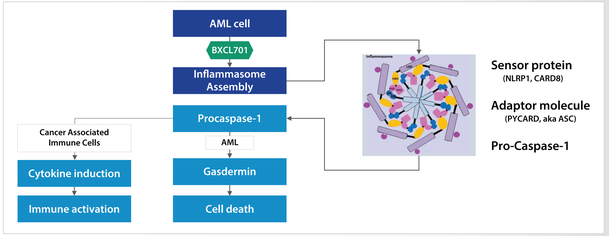
MOA: BXCL701 MODULATES THE TUMOR MICROENVIRONMENT BY ACTIVATING THE INNATE IMMUNITY FOLLOWED BY ADAPTIVE IMMUNITY LEADING TO CANCER CELL DEATH



Adopted from Fitzgerald AA, Wang S, Agarwal V, et al. J Immunother. Can 2021, 9(11): e002837).

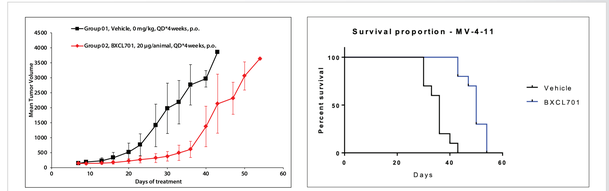
Broad DPP inhibition enhances antitumor immune response via two mechanisms: (1) DPP4 inhibition increases tumor content of CXCL9/10, which recruits CXCR3 + NK and T cells, and (2) DPP8/9 inhibition activates the inflammasome followed by induction of immune cell pyroptosis, resulting in proinflammatory cytokine (such as IL-18, IL-1β, TNFa, GM-CSF, and Eotaxin) release and stimulation of Th1 response (IFNγ, CCL2, IL-12 and IL-2). It is followed by dendritic cell (DC) activation further enhancing the CXCL9/10-CXCR3 axis.

BXCL701 ACTIVATES INFLAMMASOME FOLLOWED BY PYROPTOSIS IN ACUTE MYELOID LEUKEMIA



By inhibition of DPP8/9 BXCL701 induces caspase-1 that cleaves GSDMD to induce pyroptosis in human myeloid cells (Nat Med. 2018; 24:1151-1156).

BXCL701 AS A SINGLE AGENT REDUCED TUMOR GROWTH IN MV-411 AML XENOGRAFT MODEL AND PROLONGED SURVIVAL AS COMPARED TO MICE TREATED WITH VEHICLE ALONE



BXCL701 was administered at 20 μg per animal (Female NOD/SCID) in micet, QD for 4 weeks while the control group was administered vehicle only. The ability of BXCL701 to reduce tumor growth in xenograft model for AML shows its ability to act as a cytotoxic agent for AML.

RESULTS

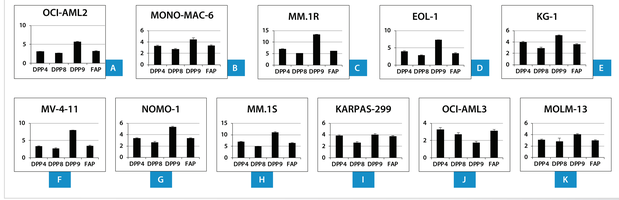
Seventeen cell lines of hematological cancers including AML were first evaluated for the effect of BXCL701 on its cytotoxicity. 39.5-100% was observed with the treatment of BXCL701 in eleven cell lines at the concentrations evaluated. Out of the 11 responding cell lines, 6 were AML, two were myeloma, one was lymphoma while two were of other leukemias: Eosinophilic leukemia and monocytic. BXCL701 demonstrated between 0-30% cytotoxicity in the remaining 6 cell lines. Next, genomic DNA was prepared from the 13 leukemic cell lines and gene copy number were evaluated for four BXCL701 targets (DPP9, DPP8, DPP4 and FAP) by qPCR. As shown in the figure below, target gene copy numbers were in general highest among responding compared to non-responding cell lines all genes. Interestingly, DPP9 copy numbers were the highest in responding cell lines and lowest in non-responding cell lines. In all responding cell lines DPP9 gene copy number were more than four except in OCI-AML3, although another variant of this cell line OCI-AML2 had higher copy number. The 6 non-responsive cell lines had less than two DPP9 copy numbers.

BXCL701 DEMONSTRATED CYTOTOXIC EFFECTS (> 30%) IN 11 HEMATOLOGICAL CANCER CELL LINES INCLUDING 6 AML CELL LINES

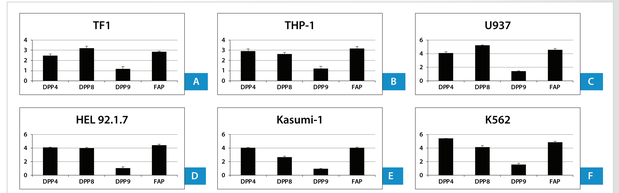
CELL LINE	CANCER TYPE	% Kill (10 μM)
OCI-AML2	AML	99.91%
MM.1R	Blood/Myeloma	98.26%
EOL-1	Eosinophilic leukemia	89.45%
KG-1	AML	84.75%
MV-4-11	AML	79.77%
NOMO-1	AML	78.16%
MM.1S	Blood/Myeloma	75.68%
KARPAS-299	Blood/Lymphoma	66.17%
MONO-MAC-6	Monocytic	55.37%
MOLM-13	AML	39.93%
OCI-AML3	AML	39.53%
THP-1	AML	29.75%
HEL	AML	NA
K562	CML	NA
Kasumi-1	AML	NA
U937	Blood/Lymphoma	14.52%
TF1	AML	NA

NA: Not Active

DNA COPY NUMBERS OF DPPS AND FAP GENES ARE HIGHER IN CELL LINES (A-K) THAT RESPONDED TO BXCL701 TREATMENT IN VITRO

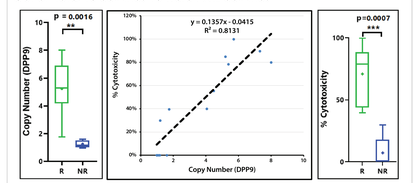


DNA COPY NUMBERS OF DPPS AND FAP GENES ARE LOWER IN CELL LINES (A-F) THAT DID NOT RESPOND TO BXCL701 TREATMENT IN VITRO



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

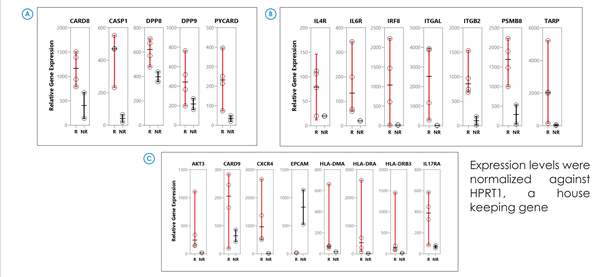
Out of total 13 cell lines 10 were AML cell lines



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

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).

DIFFERENTIAL EXPRESSION OF 20 GENES IN BXCL701-RESPONDER (R) AND NON-RESPONDER (NR) HUMAN LEUKEMIC CELL LINES AS DETERMINED BY NANOSTRING ANALYSIS (A-C)

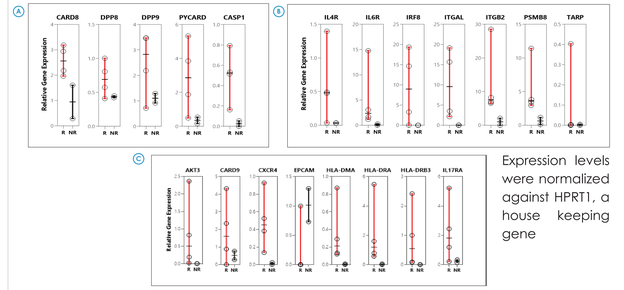


Expression levels were normalized against HPRT1, a house keeping gene

As a next step gene expression analysis was done in different human leukemic cell lines. RNA was prepared, and transcripts level were evaluated by Nanostring as per the protocol by Canopy Biosciences. The absolute gene expression values were normalized against HPRT1, a house keeping gene, to provide relative gene expression for all the genes.

Genes showing differential expression between responder and non-responder cell lines were selected. Genes including CARD8, CASP1, DPP8, DPP9 and PYCARD are directly associated with BXCL701 mechanism of action and inflammasome assembly and activation. All these genes were found to be upregulated in responder cell lines as compared to non-responders. Other found to have higher expression in responder cell lines included the genes involved are serine/threonine kinase AKT3, along with other immune signaling molecules IL4R, IL6R, IL17RA, CARD9, IRF8, CXCR4, TARP, ITGAL and ITGB2. Cell adhesion gene EPCAM was found to be elevated in non-responders while MHC class II associated genes HLA-DMA, HLA-DRA and HLA-DRB3 and an associated protease PSMB8 were found to be upregulated in responder cell lines.

RELATIVE GENE EXPRESSION OF 20 DIFFERENTIALLY EXPRESSED GENE IN RESPONDER (R) AND NON-RESPONDER (NR) HUMAN LEUKEMIC CELL LINES AS DETERMINED BY RT-PCR (A-C)



Expression levels were normalized against HPRT1, a house keeping gene

To validate the results obtained by nanostring technique gene expression of 20 genes was evaluated by RT-PCR. RNA was prepared, and transcripts level were evaluated by RT-PCR technique following the protocol by Canopy Biosciences.

The RT-PCR analysis confirmed the (results from) Nanostring data for the 20 genes which showed differential expression between responder and non-responder human leukemic cell lines.

CONCLUSIONS

- Our data show that DPP9 copy number is highly correlated with BXCL701 cytotoxicity in human leukemic cell lines and is a potential predictive biomarker in Leukemias.
- In addition, twenty genes known to be involved in the inflammasome pathway (including DPP9) and other immune signaling were found to be differentially expressed (transcripts levels) in responding (R) vs non-responding (NR) human leukemic cell lines.
- These data suggest that the expression levels of gene(s) in this set could be predictive biomarkers for BXCL701 responsiveness in patients.
- Validation of DPP9 copy number and expression levels of these 20 genes will be performed in our upcoming AML clinical trial and will be evaluated in our ongoing solid tumor trials.

REFERENCES

1. Fitzgerald AA, 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 ductal adenocarcinoma. J Immunother. Can 2021, 9(11): e002837
2. Johnson DC, Taabazuin CY, Okondo MAC, et al. DPP8/DPP9 inhibitor-induced pyroptosis for treatment of acute myeloid leukemia. Nat Med. 2018; 24:1151-1156.
3. Marian C.Okondo MC, Rao SD, Taabazuing CY, et al. Inhibition of Dpp8/9 Activates the Nlrp1b Inflammasome 2018, 25 (3), 262-267.e5.

ACKNOWLEDGEMENTS

We would like to Acknowledge the team members at Crown Bioscience (Beijing) Inc. Ground Floor, Light Muller Building, Changping Sector of Zhongguancun Scientific Park, No.21 Huoju Street, Changping District, Beijing, China, 102200 for running the in vivo study. Team members of Crown Bioscience (Suzhou) Inc., Room 501, Building A6, No. 218, Xinghu Street, Industrial Park, Suzhou, Jiangsu Province, P.R. China 215000 are acknowledged for performing cytotoxicity and DNA copy number. We thank Ajay Bhargava at NorthEast BioLab, 925 Sherman Avenue, Hamden, CT 06514 for preparing RNA from cell lines for nanostring and RT-PCR analyses, and team members at Canopy Biosciences/Bruker, 3535 Breakwater Avenue, Hayward, CA 94545 for gene expression analyses by nanostring and RT-PCR.