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Transcriptional Modulations of Tumor Microenvironment by BH3120, a PD-L1x4-1BB Bispecific Antibody, in Patient-derived Organotypic Tumor Spheroids (PDOTS) Model

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Abstract

BH3120 is a heterodimeric IgG like bispecific antibody in clinical stage targeting PD-L1 (CD274) and 4-1BB (CD137) simultaneously. It binds 4-1BB with moderate affinity while binding PD-L1 with relatively higher affinity, with the aim to focus PD-L1 binding dependent 4-1BB agonism in tumor microenvironment. De-coupling of immune modulation between tumor and normal tissues was confirmed in multiple non-clinical studies, and the results were consistent with favorable safety profiles. In the meantime, BH3120 shows synergistic anti-tumor efficacy with PD-1 inhibitors without systemic safety concerns *in vivo*.

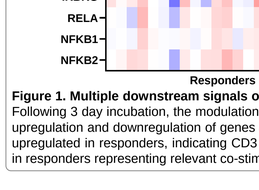
To understand how BH3120 and the combination with a PD-1 inhibitor modulate the tumor microenvironment, *ex vivo* studies were performed with patient-derived organotypic tumor spheroids (PDOTS)[1], which were generated from freshly obtained tumor tissues of non-small cell lung cancer (NSCLC) patients, and cultured in microfluidic devices containing extracellular matrix. After incubation of BH3120, a PD-1 inhibitor, and the combination in the *ex vivo* model, anti-tumor activities and immune modulations were observed by RNA analysis.

In responding tumor spheroids, multiple genes were significantly upregulated or downregulated. In particular, RNAs encoding CD4$^+$ T cell, CD8$^+$ T cell, NK cell, and B cell signals, and certain co-stimulators, pro-inflammatory cytokines, and chemokines were notably increased. The combination of BH3120 and PD-1 inhibitor resulted in the relevant genetic modulations with high magnitudes implicating potential synergism in RNA level.

Study design and background

BH3120 has demonstrated therapeutic potential in multiple non-clinical evaluations[2-4]. To understand the early stage transcriptional modulations by BH3120 and the combination with a PD-1 inhibitor in patient's tumor tissues, PDOTS model was selected as a system partially mimicking the nature of tumor microenvironment. This model enables testing of untreated control, which is often not available in clinical trials, and retains originally tumor-infiltrated immune cells with a variety of gene signatures. Total 15 PDOTS samples were prepared with the tumor tissues dissected from treatment naïve NSCLC patients.

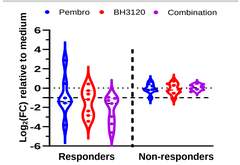
Study scheme



Definition of "Response"

- ❖ Tumor burden (EPCAM, EGFR, etc.): RNA downregulated≥2 folds, $Log_2(FC) \leq -1$ (Fold Change)
- ❖ Additionally,
- ✓ 4-1BB co-stimulation signals (TRAF1, BIRC3, etc.): upregulated≥1 folds, $Log_2(FC) \geq 0.5$
- ✓ PD-1/PD-L1 inhibition signals (CD3, ZAP70, etc.): upregulated≥1 folds, $Log_2(FC) \geq 0.5$

Tumor burden modulation



- ❖ 7 PDOTSs respond to the combination, in which
- ❖ 5 PDOTSs respond to Pembrolizumab, and
- ❖ 4 PDOTSs respond to BH3120

NSCLC sample demographics

Early stage gene modulations reflect relevant MOAs

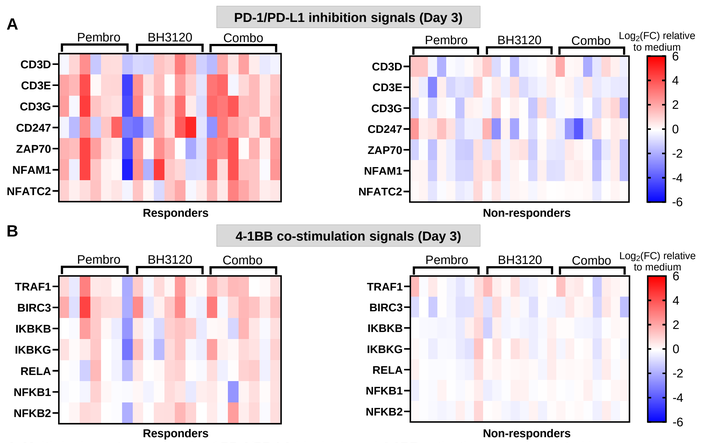


Figure 1. Multiple downstream signals of PD-1/PD-L1 blockade and 4-1BB activation are upregulated in responders
Following 3 day incubation, the modulation of downstream genes of PD-1/PD-L1 blockade and 4-1BB activation were analyzed, and the upregulation and downregulation of genes were compared with the control group. A. Downstream signals of PD-1/PD-L1 inhibition were upregulated in responders, indicating CD3 signal transduction. B. 4-1BB activation signals including TRAF and NFκB were upregulated in responders representing relevant co-stimulatory signals.

Synergistic upregulation of NK and T cell signature by the combination

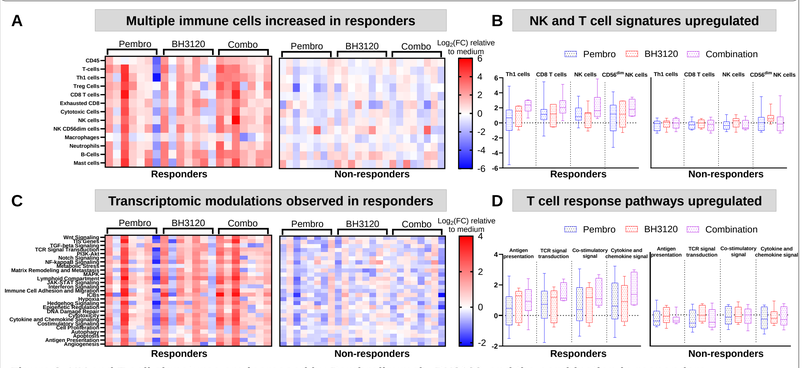


Figure 2. NK and T cell signatures are increased by Pembrolizumab, BH3120, and the combination in responders
A. Certain lineages of T cell, NK cell, and B cell were upregulated by Pembrolizumab, BH3120 and the combination in responders. B. Compared with Pembrolizumab or BH3120, the combination further increased NK and T cell signals in responders. C. Immune response related pathways were activated by Pembrolizumab, BH3120, and the combination in responders (antigen presentation, TCR signal transduction, co-stimulatory signals, and cytokine/chemokine related signals). D. The combination showed enhanced upregulation of these signals in comparison with Pembrolizumab or BH3120.

Significant RNA modulation by the combination at early stage of treatment

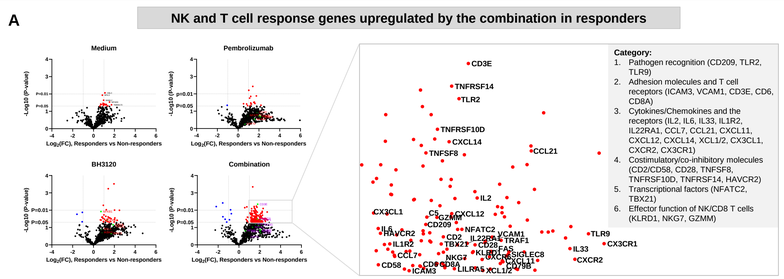


Figure 3. NK and T cell response related genes are significantly upregulated by the combination
A. Variety of genes were upregulated by each treatment in responders. In particular, genes related to NK and T cell response including adhesion molecules, T cell receptors, co-stimulators, and cytokines/chemokines were significantly upregulated by the combination. B. The correlation of upregulated signals with response to the combination was analyzed. Among cytokines and chemokines, IL-1β, IL-24, IL-34, CCL5, CCL14, and CCL21 showed certain level of correlation with response. Co-stimulators and co-inhibitors closely related to NK and T cell activation were upregulated: CD27/CD70, CD40L, ICOSLG, TNFRSF8 (CD30), PDCD1 (PD-1). Upregulation of cytotoxic granules, Granzyme B and M, was observed indicating potential effector function of NK cells and CTLs.

[Case study] RNA modulations in a responding PDOTS

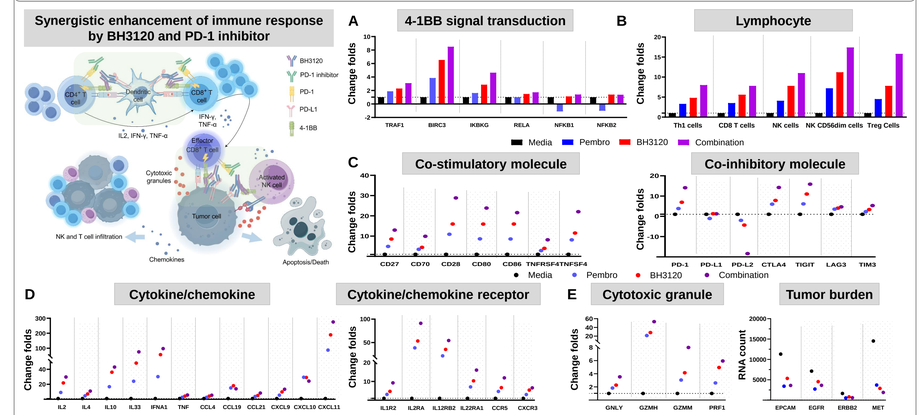
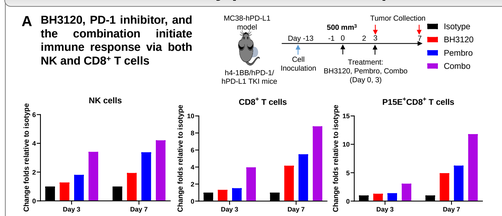


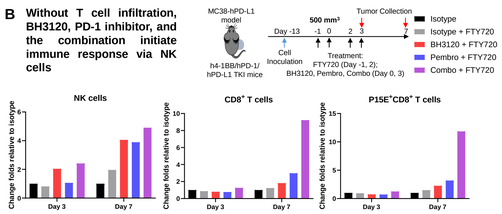
Figure 4. BH3120 in combination with PD-1 inhibitor shows synergism in gene modulation in a representative sample
The combination synergistically enhanced immune response compared to mono-treatments. A. 4-1BB down-stream signals were upregulated by the combination in synergistic manner. B. T cells and NK cells were upregulated in PDOTS system. C. Co-stimulatory receptors and checkpoint receptors were significantly regulated. D. Certain cytokines/chemokines and the corresponding receptors were upregulated. E. Cytotoxic granules were upregulated while tumor burden was downregulated.

Early period T cell response with or without T cell infiltration

A BH3120, PD-1 inhibitor, and the combination initiate immune response via both NK and CD8$^+$ T cells



B Without T cell infiltration, BH3120, PD-1 inhibitor, and the combination initiate immune response via NK cells



Cytokine/chemokine and lymphocyte in TME

Fold change relative to Isotype	Day 3		Day 7	
	Combination	Combination + FTY720	Combination	Combination + FTY720
IFN-γ / TNF-α	**3 / 2.2**	**1.9 / 1.8**	**2.2 / 2.2**	**0.8 / 1.7**
CCL3 / CCL4	**3.4 / 5.1**	**4.9 / 7.1**	**4.7 / 8.9**	**3.3 / 6.5**
CD8$^+$ T cells	**4.0**	**1.3**	**8.8**	**9.2**
P15E$^+$ CD8$^+$ T cells	**3.1**	**1.3**	**11.8**	**11.8**
NK cells	**3.4**	**2.4**	**4.2**	**4.9**

Figure 5. Immune response initiated by BH3120 and the combination treatment
A. Both NK cell and CD8$^+$ T cell response were significantly induced by BH3120 and Pembrolizumab, while the combination showed further enhanced signals. B. FTY720 treatment was employed to exclude T cell infiltration from lymph nodes[5-6]. NK cells played dominant roles in early response. FTY720 delayed T cell response which was triggered by BH3120 and the combination, meanwhile NK cell response was maintained.

Discussion

- ❖ Clinical evaluation of BH3120 as a monotherapy and in combination with a PD-1 inhibitor is ongoing (NCT06234397). To understand the immune modulations in tumor microenvironment by the treatments, tumor spheroids derived from treatment naïve NSCLC patient's tumor tissues were prepared, and NK and T cell response modulations were detected following 3 day incubation with BH3120 or the combination by NanoString RNA analysis.
- ❖ BH3120 and Pembrolizumab induce significant modulation of immune response related genes following short term incubation period, and synergism of the combination was observed in gene regulation, which was in line with the results of non-clinical MOA studies previously reported[4].
- ❖ These data suggest that combination of 4-1BB co-stimulation and PD-1 inhibition may enhance transcriptional modulations from the early stage of treatment and initiate re-structuring of tumor microenvironment.
- ❖ The widespread and significant upregulation of cytotoxicity related RNAs in the combination setting may provide clues for potential PD markers.
- ❖ Even without tdLN and T cell supplementation from external organs, which will attenuate the activity of 4-1BB agonism, the study provides valuable insights on the early stage mechanism of action in TME. The data can be discussed together with findings in clinical trials of BH3120 and the combination.

Reference:
1. Cancer Discov. 2018 Feb;8(2):196-215.
2. Cancer Res (2022) 82 (12_Supplement): 5605.
3. Cancer Res (2023) 83 (7_Supplement): 1799.
4. Cancer Res (2024) 84 (6_Supplement): 5300.
5. Pharmacol Ther. 2005 Dec;108(3):308-19.
6. Oncotarget. 2016 Apr 26;7(17):23106-27.

The schematic diagrams were drawn by FigDraw

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