BH3120, a PD-L1x4-1BB Bispecific Antibody, plus a PD-1 Antagonist Elicits Synergistic Anti-tumor Efficacy with Excellent Safety Profile

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ABSTRACT

BH3120 is a clinical stage PD-L1 binding dependent 4-1BB agonist with moderate affinity against 4-1BB. In multiple preclinical evaluations, BH3120 decouples T cell modulation in tumor microenvironment (TME) from that in normal tissues, indicating potential decoupling of anti-tumor efficacy from safety issues.

In humanized mouse models, the combination of BH3120 and a PD-1 antagonist shows synergistic and dose dependent efficacy potentially with multiple synergistic mechanisms. For example, BH3120 and a PD-1 antagonist mutually modulate the exposure and expression of targets in TME.

The synergism results in rapid eradication of tumor tissue shortening the time to regression, indicating fast control of tumor burden and associated symptoms. With the characteristics decoupling 4-1BB agonism in TME from that in normal tissues, BH3120 either as a monotherapy or in combination with a PD-1 antagonist is not associated with significant signs of toxicity in different organs including liver.

BH3120 is under evaluation as a monotherapy, and planned to be combined with a PD-1 antagonist in early-stage clinical trials.

BH3120 Provides 4-1BB Agonism in addition to PD-L1 Blockade, and Shows Synergism with PD-1 Antagonists

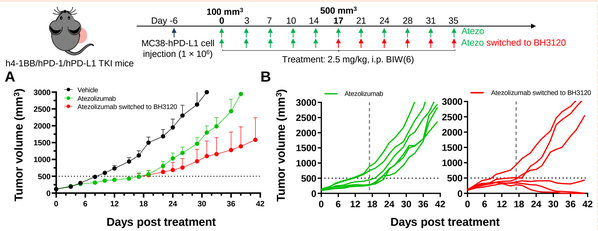


Figure 3. BH3120 provides 4-1BB agonism (additional mechanism) to PD-L1 blockade. MC38-hPD-L1 tumor bearing mice are treated with Atezolizumab[4] to screen less-sensitive mice. These less-sensitive mice are divided into 2 groups, one with continuous treatment with Atezolizumab and the other switched to the same dose of BH3120. **A)** Mean tumor volume; **B)** Individual tumor volume

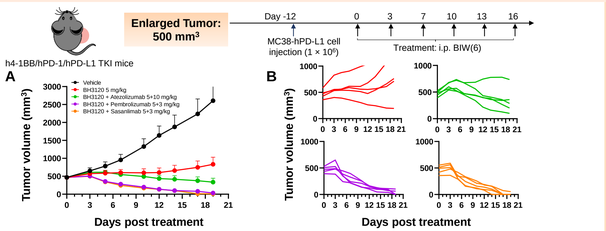


Figure 4. BH3120 in combination with PD-1 antagonists shows synergistic anti-tumor efficacy in enlarged tumor model (500mm³). MC38-hPD-L1 tumor bearing mice are treated with BH3120 alone or in combination with different PD-1/PD-L1 antagonists (Pembrolizumab, Sasanlimab[5], and Atezolizumab[4]) **A)** Mean tumor volume; **B)** Individual tumor volume

Modulation of BH3120 Dose Changes the Predominant Roles in Combination Synergism

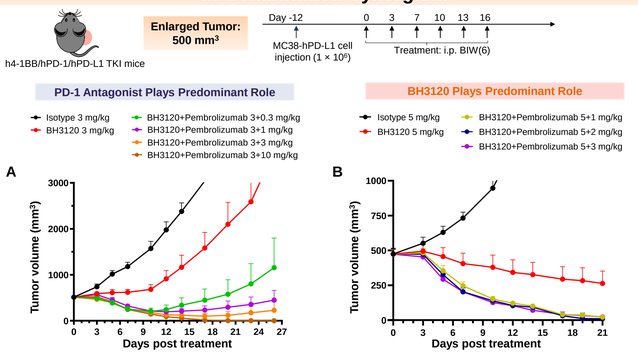


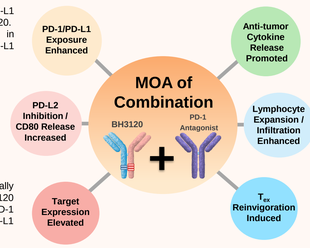
Figure 7. Sufficient level of BH3120 in the combination maintains strong synergism with different doses of PD-1 antagonist. When the dose of BH3120 is insufficient, the combination shows PD-1 antagonist dose-dependent anti-tumor efficacy. When the dose of BH3120 is sufficient, low dose of PD-1 antagonist in combination can fully eradicate tumor. **A)** Insufficient BH3120 dose level; **B)** Sufficient BH3120 dose level

BH3120 and PD-1 Antagonist: Potential Mechanisms of Synergism

PD-1 antagonist frees up PD-L1 enhancing 4-1BB clustering by BH3120. BH3120 frees up PD-1 resulting in enhanced blockade of PD-1/PD-L1 interaction by PD-1 antagonist

In addition to PD-1/PD-L1 blockade, the combination inhibits the interactions of PD-1/PD-L2 and PD-L1/CD80 simultaneously

BH3120 and PD-1 antagonist mutually induce the target expression (BH3120 induces PD-1 expression, while PD-1 antagonist induces 4-1BB and PD-L1 expression in TME)[1]



Combination of BH3120 and PD-1 antagonist enhances the release of multiple cytokines (e.g. IL-2, IFN-γ, TNF-α)

BH3120 and PD-1 antagonist together enhance expansion and/ or infiltration of lymphocyte, remodeling TME[2]

4-1BB agonism reinvigorates exhausted T cells and rescues anti-tumor function[3]

PD-1 Antagonist Frees Up PD-L1

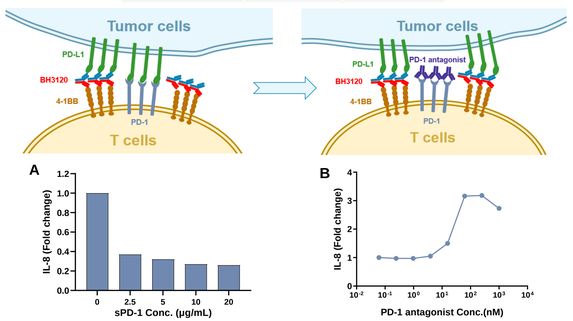


Figure 1. Soluble PD-1 suppresses but PD-1 antagonist rescues BH3120 mediated 4-1BB agonistic activity. A) Soluble PD-1 suppresses 4-1BB agonism by BH3120 in a reporter cell system **B)** PD-1 antagonist rescues 4-1BB agonistic activity

Target Induction in TME

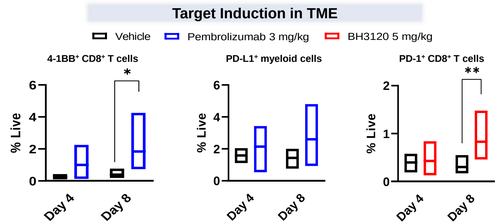
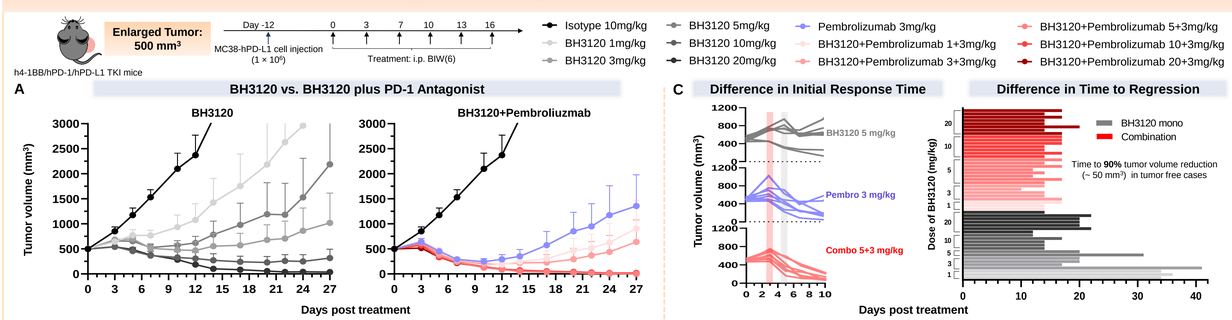


Figure 2. BH3120 and PD-1 antagonist mutually induce the target expression. In a humanized mouse model (MC38-hPD-L1 tumor bearing mice), PD-1 antagonist induces 4-1BB and PD-L1 expression promoting 4-1BB clustering by BH3120, meanwhile BH3120 induces PD-1 expression which increases the accumulation and activity of PD-1 antagonist in TME (n=5). Statistical analysis: *ps0.05; **ps0.01 vs. Vehicle, two-way ANOVA

Combination with a PD-1 Antagonist Rapidly Controls Tumor Burden in BH3120 Dose Dependent Manner



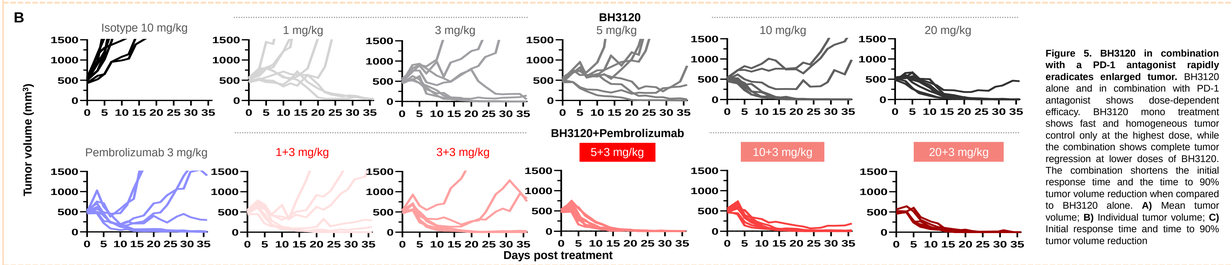
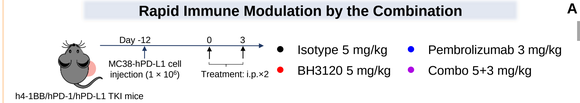


Figure 5. BH3120 in combination with a PD-1 antagonist rapidly eradicates enlarged tumor. BH3120 alone and in combination with PD-1 antagonist shows dose-dependent efficacy. BH3120 mono treatment shows fast and homogeneous tumor control only at the highest dose, while the combination shows complete tumor regression at lower doses of BH3120. The combination shortens the initial response time and the time to 90% tumor volume reduction when compared to BH3120 alone. **A)** Mean tumor volume; **B)** Individual tumor volume; **C)** Initial response time and time to 90% tumor volume reduction

Rapid Immune Modulation by the Combination



Early Stage Immune Modulation in TME

Test article	Intra-tumor Cytokines						TILs			
	Day 0 (2h post 1st dose)			Day 3 (3 days post 1st dose)			Day 0 (2h post 1st dose)		Day 7 (4 days post 2nd dose)	
	IL-2	IFN-γ	TNF-α	IL-2	IFN-γ	TNF-α	NK	CD8⁺ T	NK	CD8⁺ T
BH3120	2x	1.2x	1.2x	1.1x	2.8x	1.4x	No	No	2.6x	1.8x
Pembrolizumab	16.3x	4.1x	3.4x	2.0x	3.9x	1.7x	No	No	3.0x	3.5x
Combo	18.4x	6.5x	5.2x	1.8x	7.7x	2.2x	No	No	3.9x	14.5x

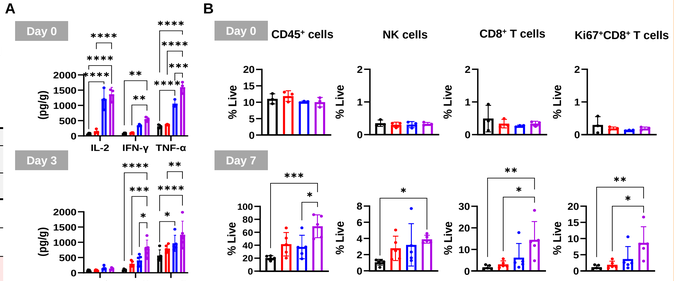


Figure 6. At early stage of treatment, PD-1 antagonist initiates immune modulation which is further enhanced by the combination with BH3120. PD-1 antagonist immediately induces IL-2, IFN-γ and TNF-α release in TME, while BH3120's response is delayed. The combination shows immediate and enhanced release of the cytokines. Both BH3120 and PD-1 antagonist monotherapies induce proliferation and/or infiltration of NK and CD8⁺ T cells, while the combination further increases the lymphocytes in TME. **A)** Cytokines and **B)** Lymphocytes in TME. Statistical analysis: *ps0.05; **ps0.01; ***ps0.001; ****ps0.0001 vs. Vehicle, one-way ANOVA

BH3120 plus PD-1 Antagonist Induces Minimal Changes in Normal Tissues

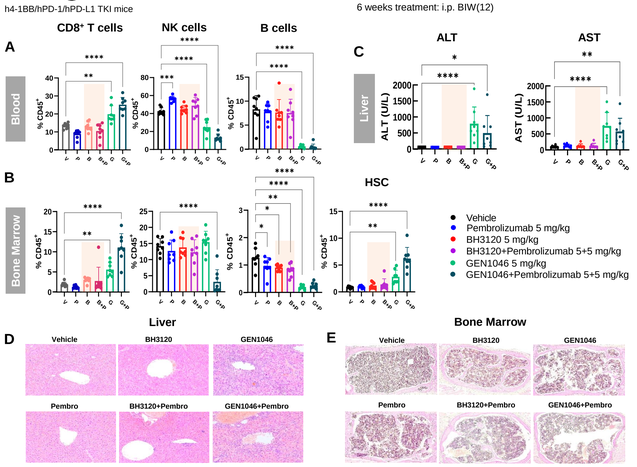


Figure 8. BH3120 plus PD-1 antagonist induces minimal changes in normal tissues with long term treatment in tumor free mice. BH3120 alone or combination with Pembrolizumab induces minimal changes in peripheral blood, bone marrow and liver. **A)** Lymphocytes in peripheral blood; **B)** Lymphocytes and hematopoietic stem cells (HSC) in bone marrow; **C)** Serum ALT and AST levels; Pathology examination of **D)** liver and **E)** bone marrow. Reference molecule: GEN1046[6]. Statistical analysis: *ps0.05; **ps0.01; ***ps0.001; ****ps0.0001 vs. Vehicle, one-way ANOVA

CONCLUSION

- **BH3120, with PD-L1 targeted immune modulation, shows synergism with PD-1 antagonists by different mode of actions.**
- **In different preclinical studies designed with a humanized mice model, BH3120 and PD-1 antagonist synergistically enhance anti-tumor efficacy shortening the time to regression in homogeneous pattern, and the efficacy of the combination shows correlation with the doses of BH3120 and PD-1 antagonist. BH3120 can minimize the risk of adverse events even when combined with a PD-1 antagonist via tumor localized immune stimulation.**
- **Clinical evaluation of BH3120 as a monotherapy is underway (NCT06234397), and the study in combination with a PD-1 antagonist is planned.**

Note: Test articles were generated based on relevant patents.

Reference:

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