BH3120, a Bispecific Antibody Targeting 4-1BB and PD-L1 Simultaneously, Stimulates T Cells in Tumor Tissue Preferred Manner

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ABSTRACT

BH3120 is an IgG like bivalent bispecific antibody based on Pentambody® platform with biased binding affinities against PD-L1 and 4-1BB. Monovalent anti-4-1BB arm (moderate affinity) and anti-PD-L1 arm (high affinity) together elicited strong antitumor activities in tumor microenvironment (TME), while no significant immune activation was observed in peripheral blood and normal tissues. Anti-tumor efficacy of BH3120 was in PD-L1 binding and dose dependent manner without clear hook effect in various models[1].

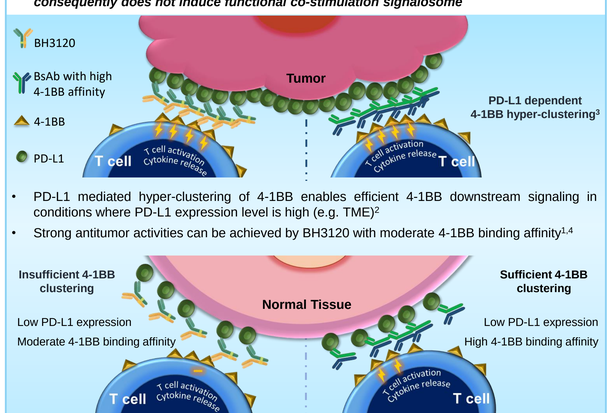
Biased binding against each target results in tumor focused distribution of BH3120, and makes TME an ideal environment (PD-L1 high and BH3120 high) for conditional activation of 4-1BB[2], while immune boosting is not observed with immune cells in circulation and liver (PD-L1 low and BH3120 low) of different animal models. The no observed adverse effect level (NOAEL) was determined to be 200 mg/kg without significant systemic immune modulation.

Antitumor activity of BH3120 is further enhanced by synergism with a PD-1 antagonist without additional risk of systemic toxicities indicating clear decoupling of T cell activation between TME and non-tumor tissues, and rapid eradication of tumor tissue de-risks tumor burden derived inflammation.

All these evidences verify the hypothesis that BH3120 stimulates T cells in tumor tissue preferred manner and reduces the risk of systemic immune related adverse events (irAEs).

Decoupling Tumor and Normal Tissue

- ***BH3120 with biased binding affinities against PD-L1 and 4-1BB[1] shows preferred distribution to PD-L1 positive tumor tissue (data not shown)***
- ***BH3120 with monovalent anti-4-1BB arm with moderate affinity efficiently co-stimulates lymphocytes in PD-L1 binding dependent manner in TME***
- ***In normal tissues, BH3120 does not induce sufficient 4-1BB clustering (hyper-clustering), consequently does not induce functional co-stimulation signalosome***



- PD-L1 mediated hyper-clustering of 4-1BB enables efficient 4-1BB downstream signaling in conditions where PD-L1 expression level is high (e.g. TME)[2]
- Strong antitumor activities can be achieved by BH3120 with moderate 4-1BB binding affinity[1,4]
- In PD-L1 low expressing normal tissues (e.g. liver), the PD-L1/4-1BB BsAb with high affinity against 4-1BB may result in certain level of unwanted 4-1BB clustering and subsequent risks
- In low PD-L1 conditions, BH3120 with moderate 4-1BB affinity and biased distribution may avoid excessive 4-1BB agonism and subsequent systemic toxicities

Favorable Safety in NHP Toxicity Study

BH3120 was weekly administrated at 30, 100 and 200 mg/kg (QW X 5) in cynomolgus monkeys*. No BH3120 related adverse effects were found in all treatment groups. The NOAEL was determined to be 200 mg/kg/dose without significant systemic immune modulation.

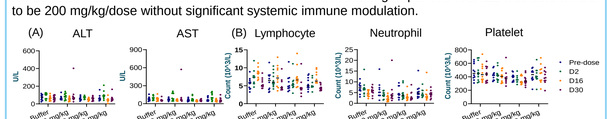
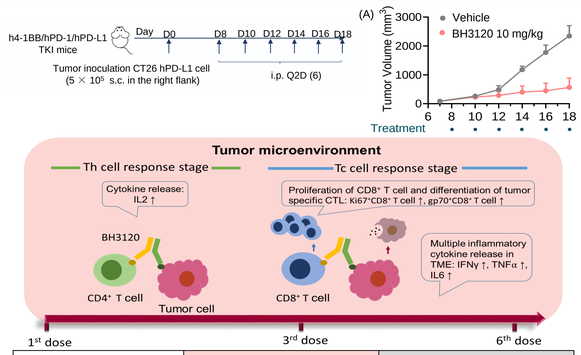
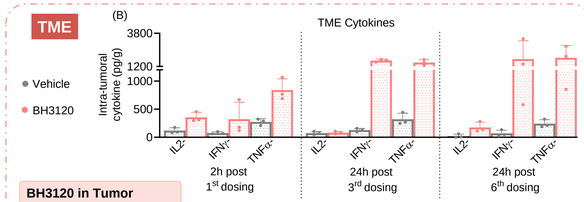


Figure 1. GLP repeat-dose non-human primate toxicity study of BH3120 (n=10 for each group, male and female cynomolgus monkeys) (A) Individual serum concentration of ALT and AST (B) Individual number of circulating lymphocytes, neutrophils and platelets. *Conducted by WuXi AppTec

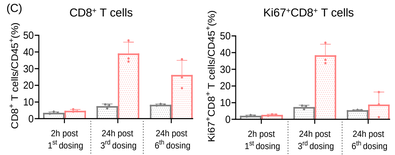
Different Immune Modulation: TME vs. Blood

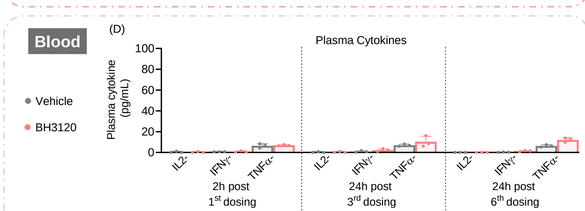


Immune Activation Stage	TME	Peripheral Blood
Th cell (CD4⁺) response	IL2↑	No change
Tc cell (CD8⁺) response	CD8⁺ T cell proliferating↑, IFNγ↑, TNFα↑	No change



BH3120 in Tumor Microenvironment:
- Initially enhances Th cell signals
- Followed by Tc cell response
- Cytokine release enhanced after T cell activation





BH3120 in Peripheral Blood:
- No activation or proliferation of Th and Tc
- No change of IFNγ and TNFα

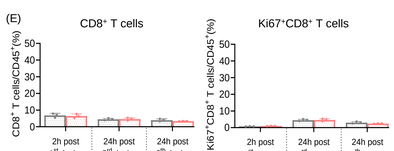
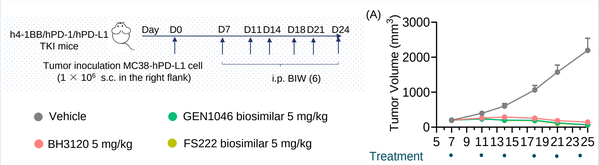
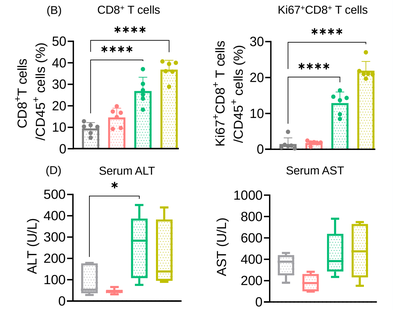


Figure 2. Decoupling of immune modulation of BH3120 in h4-1BB/hPD-1/hPD-L1 knock-in mice bearing CT26 hPD-L1 cells (A) Tumor volumes of CT26 hPD-L1 tumors, the mice in each group were euthanized on day 8, 13 and 19 post-tumor implantation (n=3 for each time point). Sample collection at 2 hours after 1st treatment and 24 hours after 3rd, and 6th treatment with indicated BH3120 or vehicle for (B) Cytokine level in tumor (C) Proportion of CD8⁺ T cells and proliferating CD8⁺ T cells in tumor (D) Cytokine level in plasma (E) Proportion of CD8⁺ T cells and proliferating CD8⁺ T cells in peripheral blood

Decoupling of Efficacy and Hepatotoxicity



BH3120 shows:
- Equivalent antitumor efficacy comparable to bispecific antibodies with high 4-1BB affinity without systemic activation of T cells or Kupffer cells.



Biosimilars of GEN1046 and FS222 were generated based on relevant patents[5,6]

Figure 3. Decoupling of efficacy and liver toxicity of BH3120 in h4-1BB/hPD1/hPD-L1 knock-in mice bearing MC38-hPD-L1 tumor cells. Blood was collected at 24 hours after 6th treatment for analysis. (A) Tumor volumes in mice treated with the indicated antibodies (n=6). (B) Proportion of CD8⁺ T cells and proliferating CD8⁺ T cells in peripheral blood (C) IFNγ level in plasma (D) Serum concentration of ALT and AST. (E) Livers from the mice treated with the indicated antibodies were stained for CD8 and F4/80 via IHC, quantification of positive cells analyzed by QuPath 0.4.2 in slides of each mice. (F) Representative captures for CD8 and F4/80 IHC Statistical analysis: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 vs. vehicle, one-way ANOVA

No Immune Modulation by BH3120 in Combination with an ICI in Non-tumor Bearing Triple KI Mice

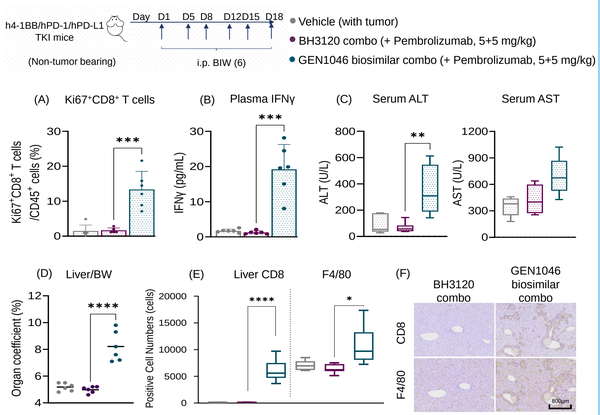
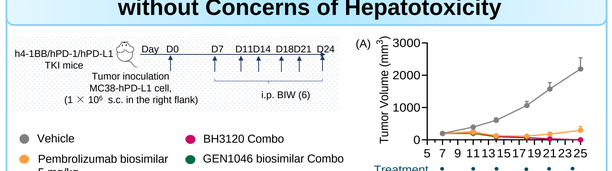


Figure 4. Immune modulation by BH3120 in combination with anti PD-1 antibody in h4-1BB/hPD-1/hPD-L1 knock-in mice without tumor. Mice were treated with the indicated antibodies on day 1, 5, 8, 12, 15, and 18 (n=6). Blood was collected at 24 hours after 6th treatment for analysis (A) Proliferating CD8⁺ T cells in peripheral blood (B) IFNγ level in plasma (C) Serum concentration of ALT and AST (D) Organ coefficient of liver to body weight, livers were collected after mice were euthanized on day 19. (E) Livers from the mice treated with the indicated antibodies were stained for CD8 and F4/80 via IHC, quantification of positive cells analyzed by QuPath 0.4.2 in slides of each mice. (F) Representative captures for CD8 and F4/80 IHC Statistical analysis: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, T-test

Synergism with an ICI in a Hot Tumor Model without Concerns of Hepatotoxicity



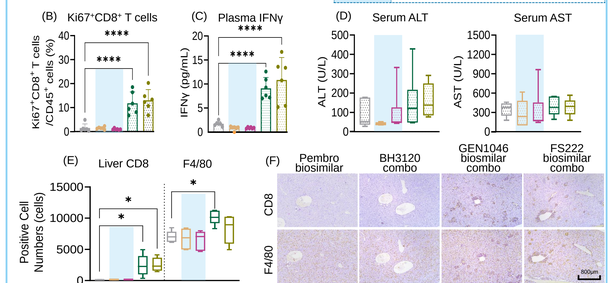
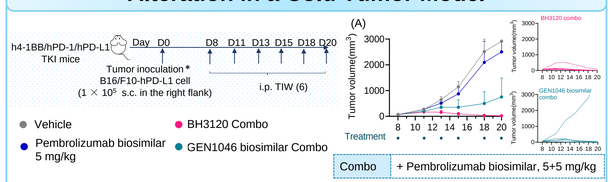


Figure 5. BH3120 synergism with anti PD-1 antibody in h4-1BB/hPD-1/hPD-L1 knock-in mice bearing MC38-hPD-L1 tumor cells. Blood was collected at 24 hours after 6th treatment for analysis. (A) Tumor volumes in mice treated with the indicated antibodies (n=6) (B) Proportion of proliferating CD8⁺ T cells in peripheral blood (C) IFNγ level in plasma (D) Serum concentration of ALT and AST (E) Livers from the mice treated with the indicated antibodies were stained for CD8 and F4/80 via IHC, quantification of positive cells analyzed by QuPath 0.4.2 in slides of each mice. (F) Representative captures for CD8 and F4/80 IHC Statistical analysis *p<0.05, ****p<0.0001 vs. vehicle, one-way ANOVA

Synergism with an ICI without Systemic Immune Alteration in a Cold Tumor Model



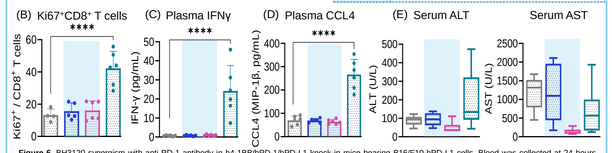


Figure 6. BH3120 synergism with anti PD-1 antibody in h4-1BB/hPD-1/hPD-L1 knock-in mice bearing B16/F10-hPD-L1 cells. Blood was collected at 24 hours after 6th treatment for analysis. (A) Left, tumor volumes in mice treated with the indicated antibodies (n=6) Right, individual tumor volumes in combination treatment groups (B) Proportion of proliferating CD8⁺ T cells in peripheral blood (C) IFNγ level in plasma (D) Representative chemokine (CCL4) level in plasma (E) Serum concentration of ALT and AST Statistical analysis: ****p<0.0001 vs. vehicle, one-way ANOVA

* Conducted by Biocytogen

Conclusion

- **In various models studied so far, BH3120 stimulates T cells in tumor tissue preferred manner by biased binding affinities against PD-L1 and 4-1BB.**
- **BH3120 decouples immune modulation in TME from that in normal tissues, consequently decoupling antitumor efficacy from systemic safety issues.**
- **Antitumor activity of BH3120 is strong as monotherapy[1], and further enhanced in combination with PD-1 antagonist without systemic toxicities.**
- **Clinical evaluation of BH3120 to test the safety and efficacy are planned to be initiated in 2023.**

Reference:

1. Xu J, et al. Cancer Res. 2022;82:5605.
2. Melero I, et al. Cancer Discov. 2023;13:552–69.
3. Claus C, et al. MAbs. 2023;15:2167189.
4. Yu X, et al. Nature. 2023;614:539–47.
5. Isil A, et al. WO2020094744.
6. Matthew L, et al. WO2020011964.