

# BGB-B2033, a novel 4-1BB/GPC3 bispecific antibody, exhibits potent in vitro and in vivo antitumor activity in preclinical models

## Abstract

The tumor necrosis factor receptor superfamily member 4-1BB (CD137) is a key costimulatory receptor of T cells and a promising therapeutic target in cancer. Glypican-3 (GPC3) is a well-established tumor-associated antigen (TAA) overexpressed in a variety of solid tumors, including hepatocellular carcinoma, germ cell tumors, squamous non-small cell lung cancer, alpha-fetoprotein producing gastric cancer, pancreatic carcinoma, and to a lesser extent in other tumors. GPC3 is not expressed in adult normal tissues (except endometrium and placenta), thus is recognized as an ideal TAA.

BGB-B2033 is a novel IgG-based bispecific antibody targeting 4-1BB and GPC3 and is under clinical development for the treatment of advanced or metastatic solid tumors in humans. BGB-B2033 binds to its target proteins with high specificity and affinity. Potent and GPC3-dependent functional activities were demonstrated using the human peripheral blood mononuclear cell (PBMC)-based immune cell activation and cytotoxicity assays. In humanized 4-1BB knock-in mice bearing human GPC3-expressing tumors, BGB-B2033 exhibited potent, dose-associated single-agent efficacy as well as synergistic antitumor activity in combination with anti-PD-1 antibody.

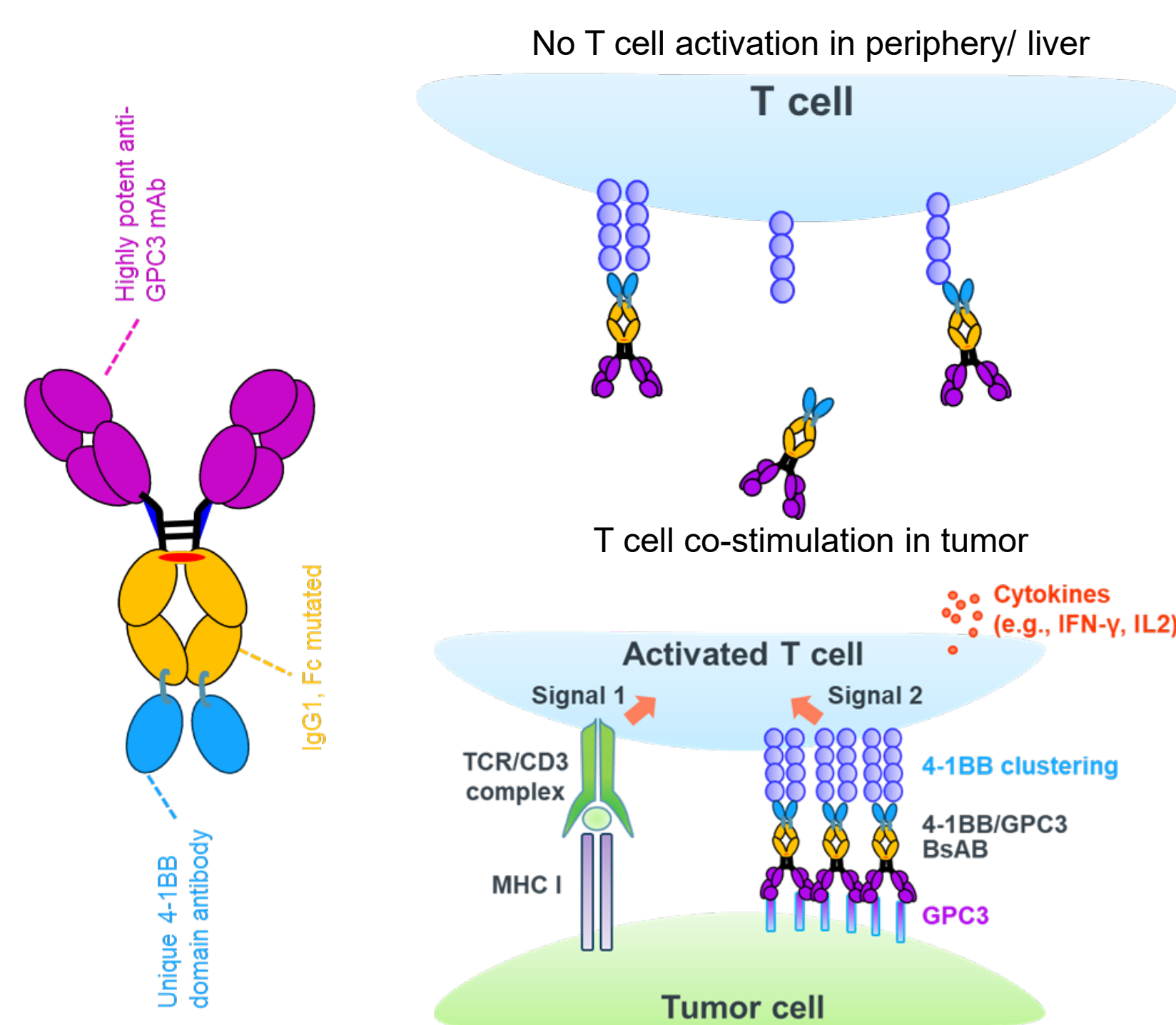
The phase 1 study of BGB-B2033, alone or in combination with Tislelizumab is ongoing (NCT06427941). Here, we describe the characterization of BGB-B2033 regarding its mechanism of action and preclinical activities.

## Molecule Design

BGB-B2033 is a novel IgG-based bispecific antibody (BsAb) targeting GPC3 and 4-1BB. It includes a bivalent F(ab')<sub>2</sub> fragment that binds to GPC3, a fusion of 4-1BB-binding heavy chain variable (VH) domain fragments, and a silenced Fc that prevents binding to FcγRs (Figure 1). BGB-B2033 can only activate 4-1BB receptors when GPC3 is present, thus resulting in the immune cell stimulation in the tumor microenvironment while greatly reducing risk of systemic toxicity (Figure 2).

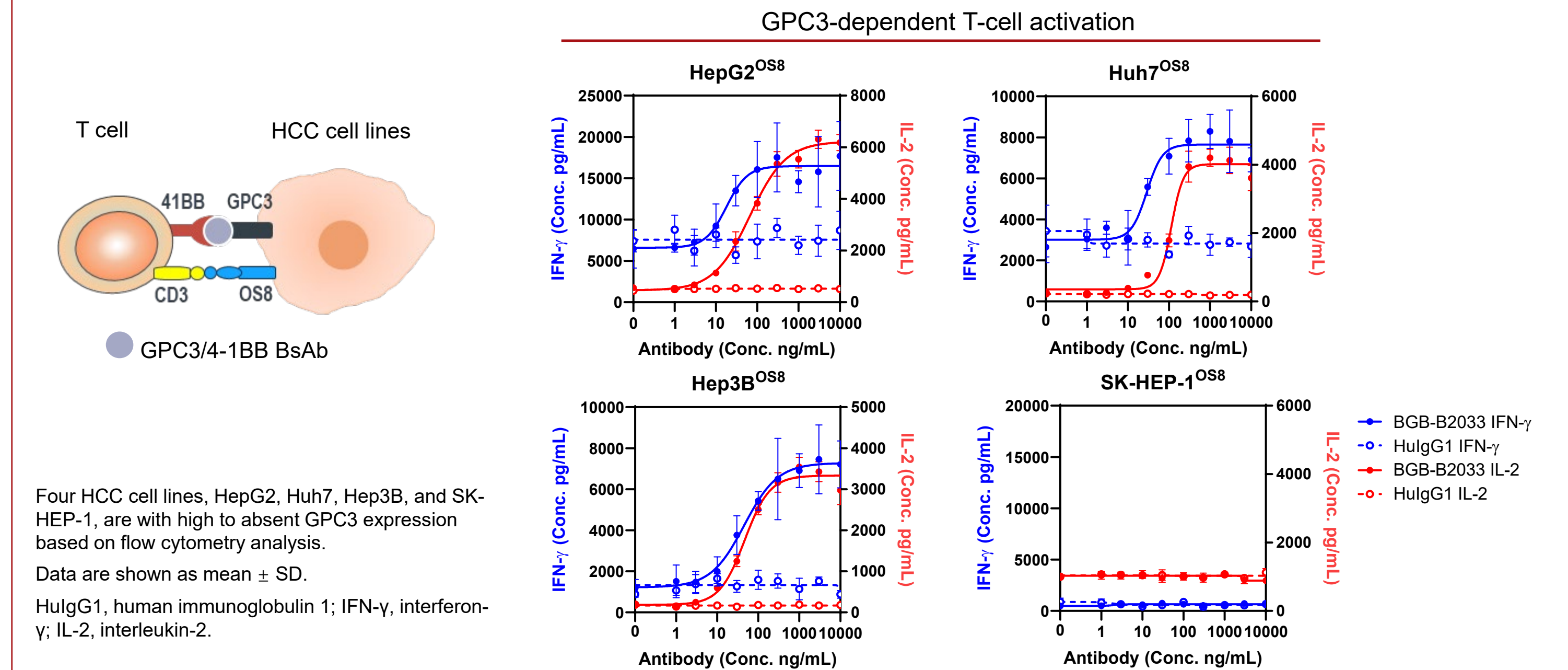
Figure 1. BsAb design

Figure 2. Mechanism of action



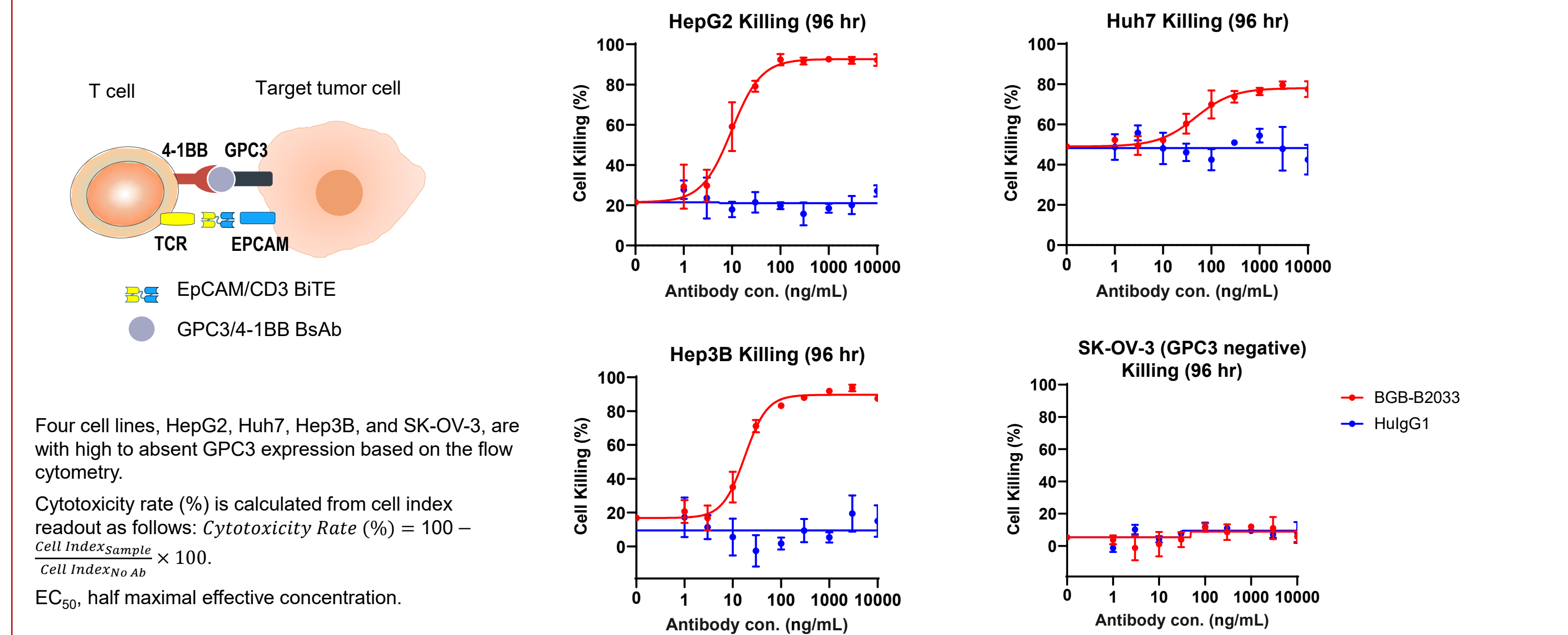
## Enhanced IFN-γ and IL-2 Secretion from PBMCs in a GPC3-Dependent Manner

Figure 3. PBMCs were co-cultured with GPC3-expressing HCC cells in the presence of serial diluted BGB-B2033. BGB-B2033 enhanced IFN-γ and IL-2 secretion in GPC3-dependent manner.



## Potent Cytotoxicity of PBMCs Against Cancer Cells

Figure 4. PBMCs were co-cultured with GPC3-expressing target tumor cell in presence of BGB-B2033. BGB-B2033 dose-dependently enhanced T-cell killing activity in GPC3-expressing cells, and no killing activity observed in GPC3-negative cells.



## Dose-Dependent Antitumor Monotherapy Activity in Human 4-1BB Knock-in Mice

Figure 6. Hepa1-6/hGPC3 cells were implanted into human 4-1BB knock-in mice. BGB-B2033 significantly inhibited tumor growth in the Hepa1-6/hGPC3 model; tumor-free rates at study end were 30% (3/10) and 90% (9/10) for dose level 1 and 2 treatment groups.

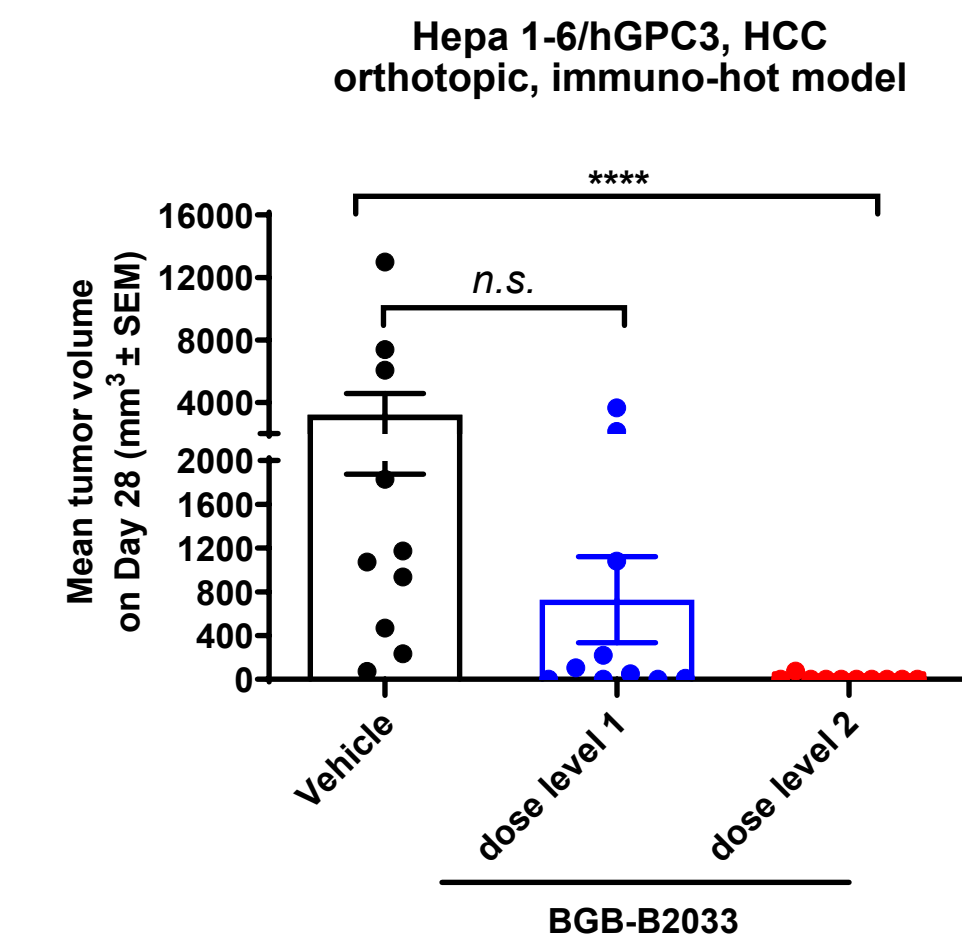
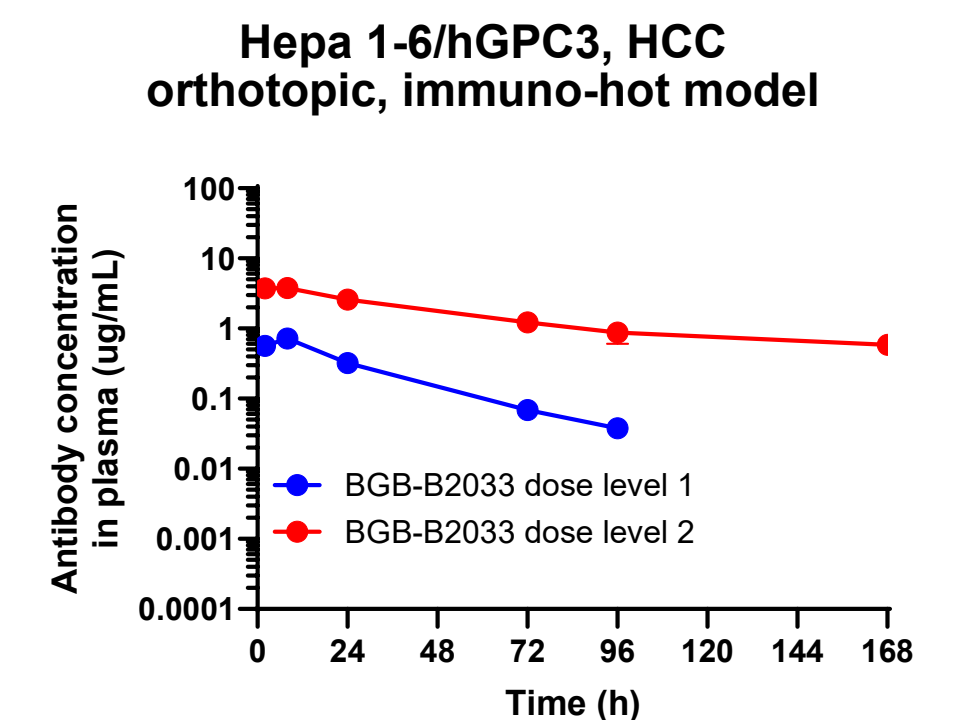


Figure 7. Hepa1-6/hGPC3 tumor bearing human 4-1BB knock-in mice were treated with BGB-B2033. Plasma concentration was quantified by ELISA.

Hepa1-6/hGPC3 model: human GPC3 expressed in mouse hepatoma cell line Hepa1-6.



## Enhanced Antitumor Activity when Combined with Anti-PD1 Ab

H22/hGPC3 or LL2/hGPC3 cells were implanted into human 4-1BB knock-in mice. The mice were treated with BGB-B2033, anti-mouse PD-1 antibody Ch15mt, or a combination of both.

Figure 8. Tumor growth inhibition rate in the combination group was significantly higher in H22/hGPC3 HCC model

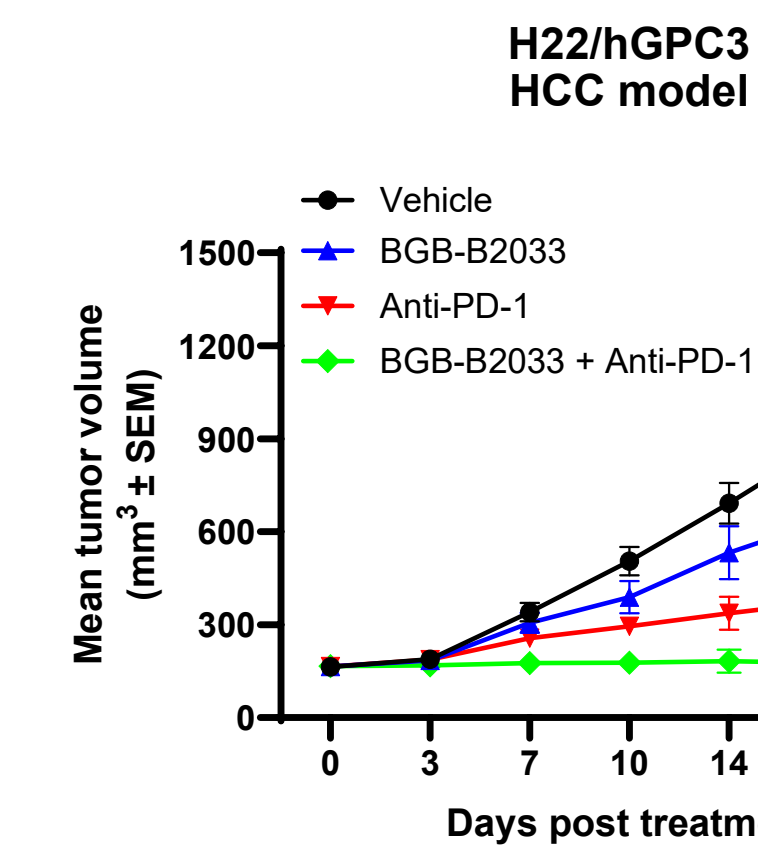
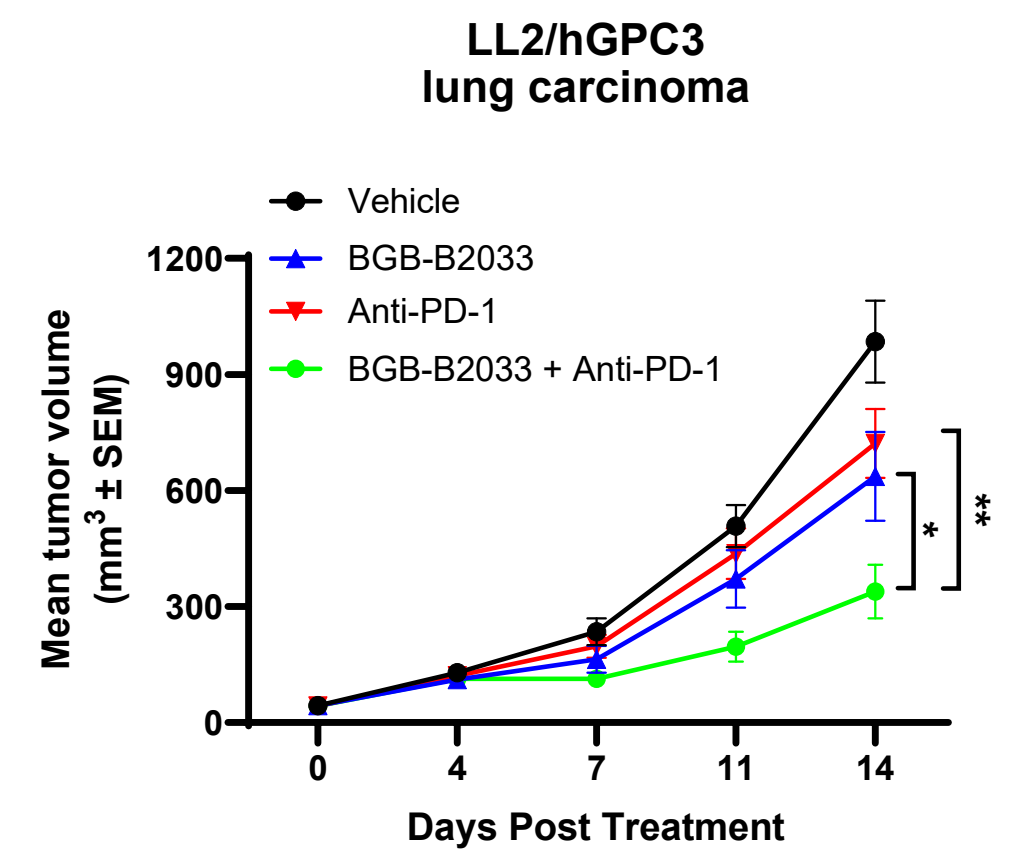
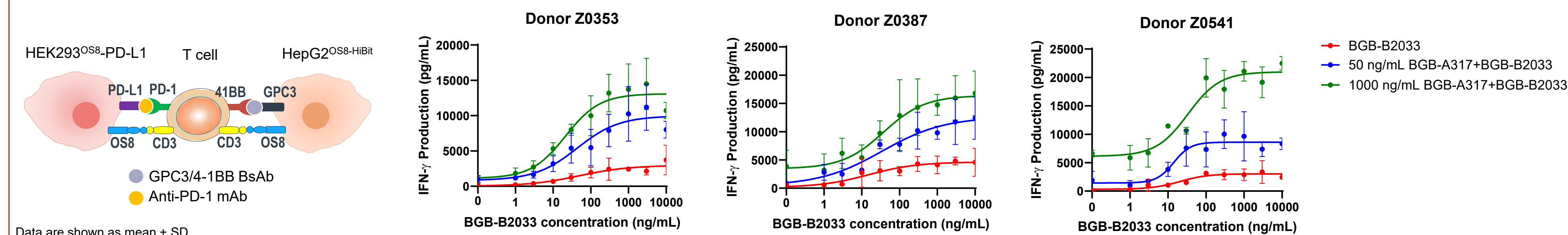


Figure 9. Tumor growth inhibition rate in the combination group was significantly higher in LL2/hGPC3 lung carcinoma model



## Combination with Anti-PD1 Ab Promotes IFN-γ Secretion from PBMCs

Figure 5. PBMCs were co-cultured with GPC3-expressing HepG2 cells and engineered HEK293 cells (with PD-L1) in the presence of serial diluted BGB-B2033 and BGB-A317 (anti-PD1 Ab). Combination of BGB-A317 and BGB-B2033 enhanced the maximum IFN-γ production.



## Conclusion

BGB-B2033 is a selective GPC3-dependent 4-1BB targeting bispecific antibody and demonstrated strong antitumor effects as monotherapy or in combination with anti-PD1 antibody in pre-clinical setting with the following evidences:

- GPC3-dependent T-cell activation in *in vitro*
- Strong antitumor effects in animal studies
- Enhanced antitumor activity in combination with anti-PD1 antibody.