# Preclinical characterization of BGB-B3227, a MUC1 x CD16A bispecific engager, for the treatment of MUC1-expressing solid tumors

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## Background

- MUC1 (CD227 or CA15-3) is an attractive TAA frequently overexpressed in various human epithelial cancers, including NSCLC, gastrointestinal cancers, breast cancer, pancreatic, ovarian, and colon carcinomas. Despite the development of multiple MUC1 targeted therapies, none have shown significant clinical efficacy to date.
- Here, we describe the preclinical characterization of BGB-B3227, a novel bispecific antibody targeting MUC1 and CD16A, designed to induce NK cell activation and subsequent cytotoxicity against MUC1-expressing tumor cells.

## Methods

- The binding activity of BGB-B3227 to CD16A and MUC1 was characterized through SPR (Surface Plasmon Resonance) and cell-based assays.
- A competitive FACS assay using MUC1-expressing cells was employed to assess the ability of BGB-B3227 to avoid the interference from soluble MUC1.
- The antibody-dependent cellular cytotoxicity (ADCC) and phagocytosis (ADCP) activities of BGB-B3227 were evaluated in co-culture systems consisting of effector cells and tumor cell lines expressing MUC1, both in the presence and absence of human IgG proteins at physiologically relevant concentrations.
- Furthermore, the in vivo anti-tumor efficacy of BGB-B3227, either as a monotherapy or in combination with anti-PD-1 antibody, was evaluated in the MC-38/hMUC1X model in human CD16A knock-in mice.

## Results

- BGB-B3227 demonstrated high binding affinity to recombinant human CD16A protein and CD16A expressing cells, with comparable binding affinities to both CD16A-158V and 158F variants.
- BGB-B3227 demonstrated high binding affinity to the recombinant SEA domain of human MUC1 protein and MUC1 expressing tumor cells. Compared to HMFG1, which targets MUC1-N, soluble MUC1 interfered significantly less with the binding of BGB-B3227 to MUC1 expressing cells.
- In cellular assays, BGB-B3227 induced potent ADCC and ADCP activity against MUC1-expressing cells in a dose-dependent manner. Notably, the cytotoxic activity of BGB-B3227 was greater than that of Fc-enhanced monoclonal antibodies and was less affected by human IgG. No activity was observed in MUC1-negative tumor cells, indicating that the effect of BGB-B3227 is highly dependent on MUC1 expression.
- In mouse models, BGB-B3227 monotherapy demonstrated a dose-dependent anti-tumor efficacy. Furthermore, BGB-B3227 combined with an anti-PD-1 antibody further enhanced anti-tumor activity in the same model. Importantly, no significant changes in animal body weight were observed across all treatments, suggesting good tolerability of BGB-B3227 in mouse models.

## Conclusions

- BGB-B3227 is a bispecific MUC1xCD16A NK engager demonstrating potent ADCC activity, along with notable anti-tumor efficacy when used as a monotherapy or in combination with anti-PD-1 therapy.
- BGB-B3227 shows promise as a therapeutic option for MUC1-expressing cancers, with the potential to overcome the limitations of MUC1-N antibodies by mitigating the sink effect from soluble MUC1.
- Currently, BGB-B3227 alone and in combination with Tislelizumab is under clinical investigation in participants with advanced or metastatic solid tumors (NCT06540066).

## BGB-B3227 binds to human CD16A and MUC1 with high affinity

| $K_D$ (M) \ Antigen | Human CD16A V158 | Human CD16A F158 | Cynomolgus CD16A | Human MUC1 | Cynomolgus MUC1 |
|---|---|---|---|---|---|
| **BGB-B3227** | 3.54E-10 | 5.45E-10 | 9.43E-11 | 4.45E-10 | 4.37E-09 |
| **Isotype control** | 5.57E-07 | 1.29E-06 | 4.99E-07 | ND | ND |

**Table 1.** Binding affinity of BGB-B3227 to recombinant CD16A protein and MUC1 by SPR assay.

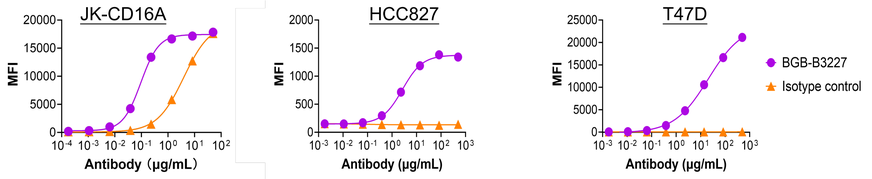


**Figure 1.** The binding activity of BGB-B3227 to native MUC1 and CD16A was determined by FACS in Jurkat cells that ectopically overexpress CD16A (JK-CD16A) or tumor cells that endogenously express MUC1 (HCC827 and T47D).

## The binding of BGB-B3227 to MUC1 is less interfered with by soluble-MUC1

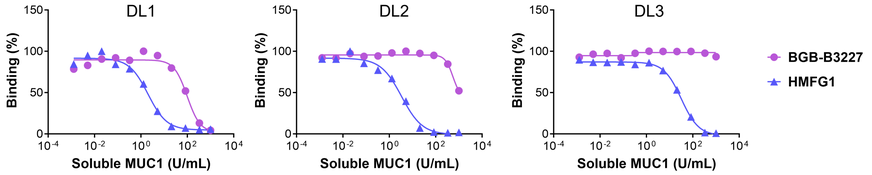


**Figure 2.** Human MUC1-expressing cells were incubated with serial dilutions (DL1, DL2, DL3) of BGB-B3227 and HMFG1, an anti-MUC1 antibody that binds to MUC1-N subunit, in the presence of serially diluted soluble MUC1. The interference of soluble MUC1 on the specific binding of BGB-B3227 to MUC1-expressing cells was determined by competitive FACS assay.

## BGB-B3227 exhibits superior ADCC activity and is less affected by IgG

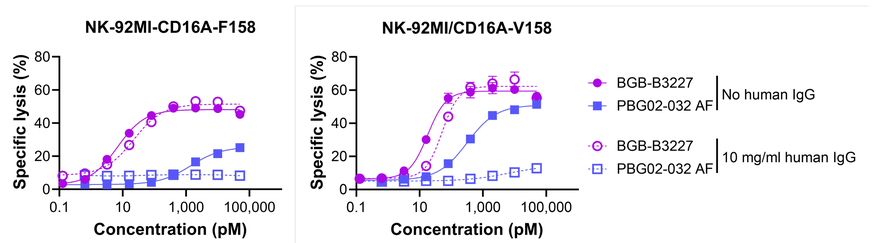


**Figure 3.** The ADCC Activity of BGB-B3227 was determined by co-culture of MUC1 expressing T47D cells and NK-92MI cells expressing human CD16A (F158 and V158 allotypes) in the presence or absence of human IgG, the cytotoxicity was measured by a NanoLuc®-release assay. PBG02-032 AF is an anti MUC1 afucosylated IgG1 antibody with the same anti MUC1 sequence as that of BGB-B3227.

## BGB-B3227 exhibits potent ADCP activity and is less affected by IgG

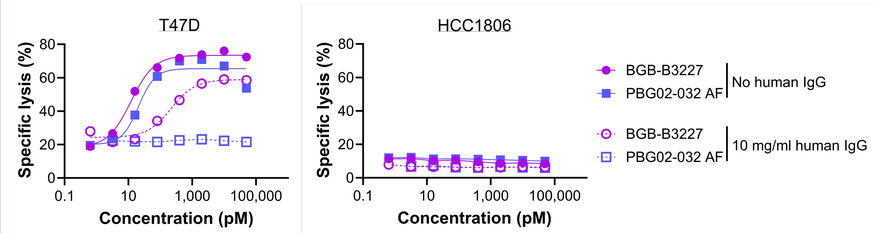


**Figure 4.** The phagocytotic activity of BGB-B3227 against T47D (MUC1+) and HCC1806 (MUC1-) cells was determined in a flow cytometry-based assay using human PBMC-derived macrophages as the effector cells in the presence or absence of human IgG. PBG02-032 AF is an anti MUC1 afucosylated IgG1 antibody with the same anti MUC1 sequence as that of BGB-B3227.

## Dose-dependent anti-tumor efficacy by BGB-B3227 monotherapy

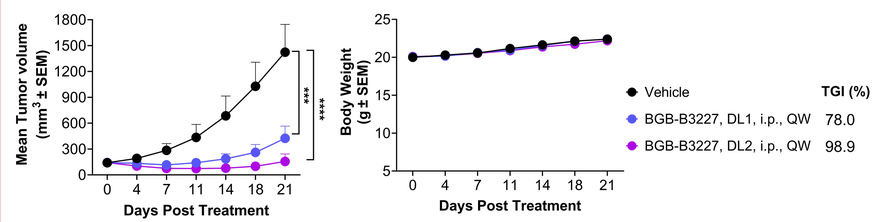


**Figure 5.** The in-vivo antitumor efficacy of BGB-B3227 was investigated in the MC-38/hMUC1X syngeneic model in human CD16A knock-in mice. Animals were treated with vehicle (DPBS) or two different dose levels (DL1 and DL2) of BGB-B3227 once per week for 3 weeks. On Day 21, the tumor volume was compared with vehicle group using Dunnett multiple comparison test *(*** $p < 0.01$, **** $p < 0.0001$)*. Abbreviations: i.p., intraperitoneal; QW, once weekly; SEM, standard error of the mean.

## Enhanced anti-tumor efficacy by BGB-B3227 in combination with anti-PD-1

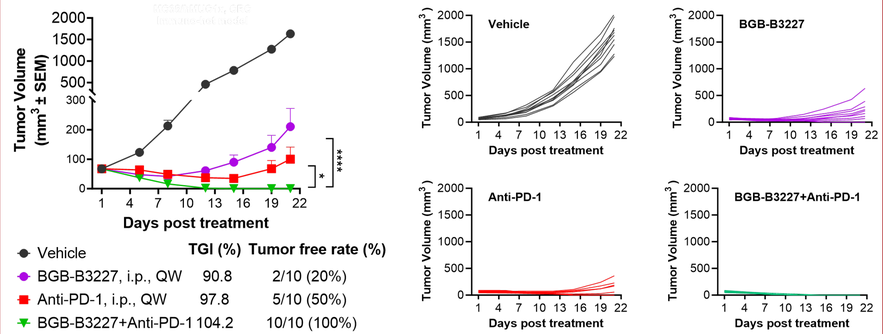


**Figure 6.** The antitumor efficacy of the combination of BGB-B3227 and an anti-PD 1 antibody was investigated in the MC-38/hMUC1X model in human CD16A knock-in mice. Animals were treated with vehicle, BGB-B3227, anti-mouse PD-1 or the combination once per week for 3 weeks. On Day 21, the tumor volume was compared with vehicle group using Dunnett multiple comparison test *(*** $p = 0.0109$, **** $p < 0.0001$)*. Abbreviations: i.p., intraperitoneal; QW, once weekly; SEM, standard error of the mean.

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