A Fc-competent anti-human TIGIT blocking antibody BGB-A1217 elicits strong immune responses and potent anti-tumor efficacy in pre-clinical models

Abstract #1854

Xin Chen, Liu Xue, Xiao Ding, Qi Liu, Jing Zhang, Lei Jiang, Sha Liu, Hongjia Hou, Qing Zhu, Bin Jiang, Lijie Zhang, Xiaosui Zhou, Jie Ma, Yucheng Li, Wei Jin, Min Wei, Zhirong Shen, Mike Liu, Kang Li, Tong Zhang*

BeiGene (Beijing) Co., Ltd. No. 30 Science Park Rd, Zhong-Guan-Cun Life Science Park, Changping District, Beijing 102206, China. Correspondence: tong.zhang@beigene.com

Abstract

Background: TIGIT (T-cell immunoglobulin and ITIM domain) is a "checkpoint" inhibitory receptor, which is primarily expressed on activated and "exhausted" T and NK cells. Engagement of TIGIT to its ligands (i.e., PVR and PVR-L2) leads to inhibitory signaling in T cells, promoting functional exhaustion of tumor-infiltrating T lymphocytes. BGB-A1217 is a novel humanized IgG1 anti-TIGIT antibody under clinical development. The immunomodulatory and anti-tumor activity of BGB-A1217 was evaluated in pre-clinical models.

Materials and methods: BGB-A1217 was generated through hybridoma fusion, humanized by CDR grafting and structural simulation. The binding affinity and specificity were measured by FACS and SPR. The immunomodulatory functions of BGB-A1217 were evaluated using primary immune cells and pre-clinical animal models.

Results: BGB-A1217 binds to the extracellular domain of human TIGIT with high affinity (K_D = 0.135 nM) and specificity. In a competition assay, BGB-A1217 efficiently blocks the interaction between TIGIT and PVR. *In vitro*, BGB-A1217 significantly enhances T-cell functions, induces potential ADCC against Treg cells, activates NK and monocytes, and removes TIGIT from T cell surfaces in an Fc function dependent manner. *In vivo*, the Fc effector function is critical for the anti-tumor activity of BGB-A1217 in a CT26WT syngeneic mouse model. The observed anti-tumor efficacy is associated with a pharmacodynamic change of TIGIT down-regulation, CD226 up-regulation and Treg reduction. Moreover, TIGIT antibody shows combination activity with PD-1 antibody in both PD-1 sensitive and PD-1 resistant tumor models.

Conclusions: BGB-A1217, either alone or in combination with anti-PD-1 mAb elicits strong immune responses and potent anti-tumor efficacy in pre-clinical models, supporting its clinical development for the treatment of human cancers.

BGB-A1217 binds to human TIGIT with high affinity and blocks the binding of TIGIT to PVR

Table 1. Summary of SPR determined kinetic parameters and affinity of BGB-A1217 to human TIGIT.

Antigen	K_{on} (1/Ms)	K_{off} (1/s)	K_D (M)
Human TIGIT	1.76x 10⁶	2.38 x 10⁻⁴	1.35 x 10⁻¹⁰
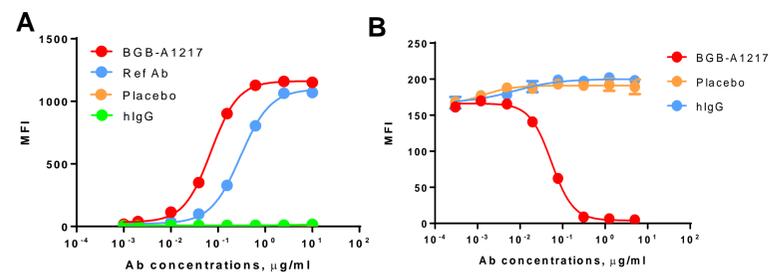
Figure 1. BGB-A1217 binds to TIGIT and inhibits TIGIT binding to PVR in the FACS binding assay. (A) TIGIT-expressing cell line BW5147.3/TIGIT was stained with anti-TIGIT mAbs (BGB-A1217 or Ref Ab-1) and AF488 labeled anti-human F(ab')₂ secondary antibody. (B) Biotinylated PVR-mIgG2a was incubated with BW5147.3/TIGIT cells in the presence of increasing amounts of BGB-A1217. Streptavidin-APC was used to detect PVR binding signal. MFI: mean fluorescence intensity.

BGB-A1217 enhances T cell responses
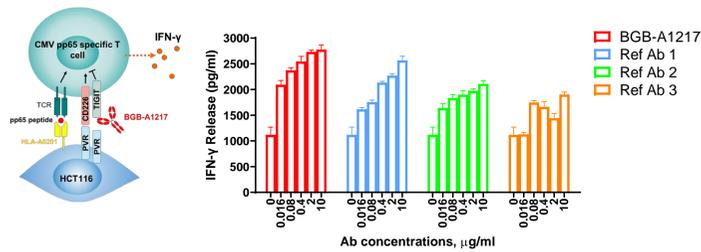
Figure 2. BGB-A1217 potentiates human T cell response to produce IFN-γ. PBMCs isolated from healthy donors were pre-stimulated by 2µg/ml CMV pp65 peptide for 7 days. HCT116 tumor cells were pulsed by 10µg/ml CMV pp65 peptide and washed. Pre-stimulated PBMC (5x10⁴/well) were co-incubated with peptide-pulsed HCT116 (2x10⁴/well) overnight. Secreted IFN-γ in the conditioned media was measured by ELISA. Data are shown as mean ± SEM. N=3.

BGB-A1217 augments T cell response in combination with anti-PD-1 mAb BGB-A317
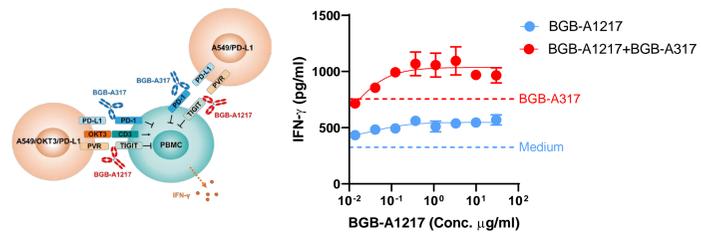
Figure 3. BGB-A1217 activates human T cells in combination with an anti-PD-1 mAb BGB-A317. PBMC isolated from healthy donors were stimulated with OKT3 (40 ng/ml) for 3 days. Pre-activated PBMCs (1x10⁴/well) were co-cultured with a mixture of A549/OKT3-PD-L1 (5x10³/well) and A549/PD-L1 (3.5x10⁴/well) in 96-well plates for 18 hours. The indicated concentrations of BGB-A1217 together with 10 ng/ml BGB-A317, or without BGB-A317 were added to the co-culture system. Secreted IFN-γ in the conditioned media was measured by ELISA. Data are shown as mean ± SEM. N=3.

BGB-A1217 activates human NK cells *in vitro*
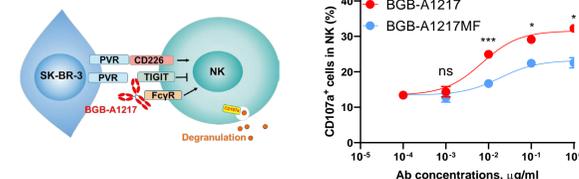
Figure 4. BGB-A1217 up-regulates CD107a on NK cells co-culturing with tumor cells. The indicated anti-TIGIT antibodies, BGB-A1217 or BGB-A1217MF (MF: mutant Fc with Fc effector function abolished), were incubated with SK-BR-3 human breast cancer cell line (5x10⁴/well), and NK cells isolated from PBMC of healthy donors (5x10⁴/well). NK cells were pre-stimulated with 25 U/ml IL-2 overnight before the co-culture assay. CD107a expression on NK cells was measured by FACS. Data are shown as mean ± SD. N=2. *p<0.05, ***p<0.001, ns: no significant difference.

BGB-A1217 treatment reduces Tregs in human PBMC from cancer donors *in vitro*
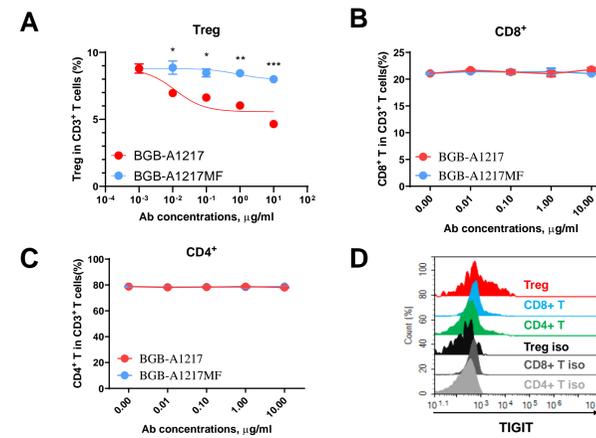
Figure 5. BGB-A1217 preferentially decreases Tregs. The indicated anti-TIGIT antibodies BGB-A1217 or BGB-A1217MF, were incubated with human PBMC from a lung cancer donor (5x10⁴/well), and NK cells isolated from PBMC of a healthy donor (5x10⁴/well) in 96-well plates overnight. Cells were collected for FACS analysis. Frequencies of (A) Treg, (B) CD8⁺, and (C) CD4⁺ T cells in CD3⁺ T cells. Data shown as mean ± SEM. N=3. (D) TIGIT expression on T cells. *p<0.05, **p<0.01,***p<0.001, ns: no significant difference.

BGB-A1217 removes TIGIT from T cell surface through Fc-dependent trogocytosis *in vitro*
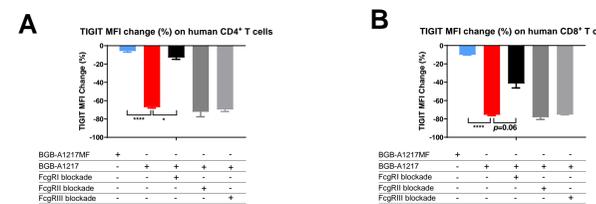
Figure 6. BGB-A1217 induces trogocytosis on T cells in a Fc function dependent manner. T cells (4x10⁴/well) and monocytes (8x10⁴/well) isolated from the same healthy donor were incubated with 10 µg/ml CF633 labeled BGB-A1217 or CF633-labeled BGB-A1217MF as indicated overnight. Dependence on FcγR was determined by treatment with FcγR blocking antibodies (10 µg/ml). Changes of TIGIT (CF633) MFI on T cells were measured by FACS. (A) CD4⁺ T cells. (B) CD8⁺ T cells. Data shown as mean ± SD. *p<0.05, ****p<0.0001.

BGB-A1217 activates monocytes *in vitro*
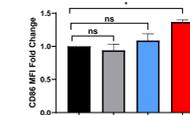
Figure 7. BGB-A1217 up-regulates CD86 on myeloid cells *in vitro*. Human PBMC from healthy donors were incubated with 10 µg/ml antibodies as indicated overnight and analyzed by FACS. CD86 MFI fold change on monocytes was calculated. Data shown as mean ± SEM, 4 donors. Suvi HIgG1: anti-V3 antibody Suvizumab, human IgG1 format. *p<0.05, ns: no significant difference.

Fc effector function is required for anti-tumor efficacy of TIGIT blockade Ab *in vivo*
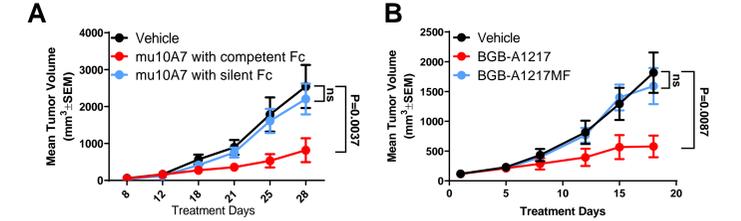
Figure 8. TIGIT blockade antibody shows potent efficacy in CT26WT syngeneic mouse model. (A) murine TIGIT blockade antibody mu10A7 with indicated Fc was administered to CT26WT tumor-bearing mice (5 mg/kg, QW). N=13. **(B)** CT26WT tumor-bearing humanized TIGIT knock-in mice were treated with indicated antibodies (10 mg/kg, Q5D), N=10. Data shown as mean ± SEM.

BGB-A1217 shows combination activity with PD-1 antibody *in vivo*
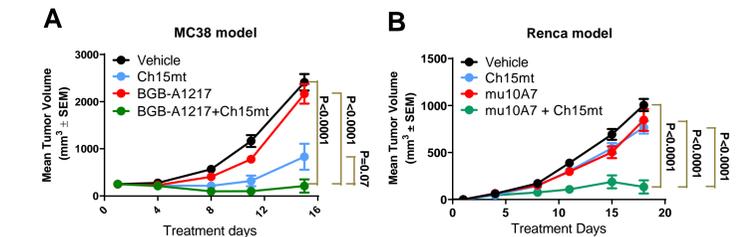
Figure 9. TIGIT antibody shows combination activity with PD-1 antibody in both PD-1 sensitive and PD-1 resistant models. (A) MC38 tumor-bearing humanized TIGIT knock-in mice were treated with vehicle (DPBS), Ch15mt (1 mg/kg, Q5D), BGB-A1217 (3 mg/kg, Q5D) or the combination; N=10. **(B)** Renca tumor-bearing mice were treated with murine TIGIT blockade antibody mu10A7 (5 mg/kg, QW), murine PD-1 blockade antibody Ch15mt (3 mg/kg, QW), or the combination as indicated. N=15; Data shown as mean ± SEM.

BGB-A1217 reduces Tregs, down-regulates TIGIT and up-regulates CD226 on T cells *in vivo*
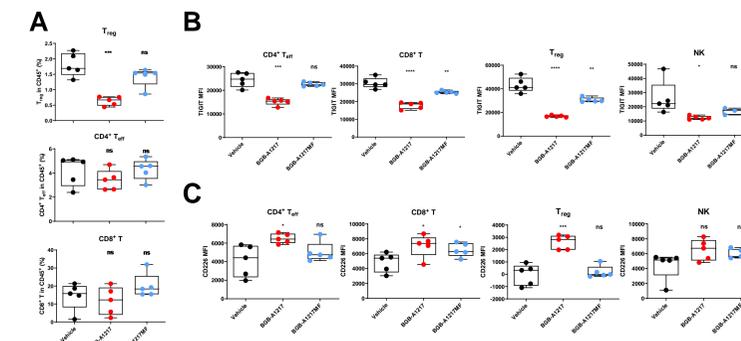
Figure 10. BGB-A1217 reduces Tregs, down-regulates TIGIT and up-regulates CD226 on T cells in a Fc effector function dependent manner *in vivo*. CT26WT tumor-bearing human TIGIT knock-in mice were treated with BGB-A1217 or BGB-A1217MF at 3 mg/kg. Tumor infiltrating lymphocytes were analyzed by FACS 48h after treatment. **(A)** Intra-tumor Treg, CD4⁺ T_eff, CD8⁺ T cell frequencies. **(B)** hTIGIT or **(C)** CD226 MFI on T cell subsets and NK cells. Data shown as mean ± SEM, N=5. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.