Abstract #2628

BGB-A425: a humanized anti-human Tim-3 antibody that exhibits strong immune cell activation

Tong Zhang, Liu Xue, Jing Zhang, Qi Liu, Jie Ma, Yi Zhang, Yingdi Shi, Hongjia Hou, Hao Peng, Ning Liu, Yilu Zhang, Xiaomin Song, Yucheng Li, Jing Song, Lai Wang, Min Wei and Kang Li*

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

Abstract

Background: Tim-3 (T-cell immunoglobin and mucin-domain containing-3) is a "checkpoint" inhibitory receptor, which is primarily expressed in activated or "exhausted" T cells, NK cells, macrophages and DCs. Engagement of Tim-3 receptor to its ligand phosphatidylserine (PtdSer) or galectin-9 leads to negative regulatory signaling in T cells, promoting functional exhaustion of tumor-infiltrating T lymphocytes. BGB-A425 is a novel humanized IgG1 (variant) anti-Tim-3 antibody under pre-clinical development. The immunomodulatory activity of BGB-A425 was evaluated both *in vitro* and *in vivo*.

Materials and methods: BGB-A425 was generated through hybridoma fusion, humanized by CDR grafting and structural simulation. The Fc region (IgG1) of BGB-A425 was engineered to remove Fc gamma receptor (FcγR) binding. The binding affinity and specificity were studied by ELISA, FACS and SPR (Biacore). The immunomodulatory functions of BGB-A425 were evaluated using primary immune cells as well as cell lines.

Results: BGB-A425 binds to the extracellular domain of human Tim-3 with high affinity (K_D = 0.36 nM) and specificity. In a competition assay, BGB-A425 efficiently blocks the interactions between Tim-3 and PtdSer. *In vitro,* BGB-A425 significantly enhances IFN-γ production of primary T cells and NK-mediated cytotoxicity against tumor cells. In a MLR assay, BGB-A425 augments T-cell response to allogeneic antigens either alone or in combination with an anti-PD-1 antibody BGB-A317. Besides blocking Tim-3, BGB-A425 can also induce the internalization of Tim-3 receptor on cell surface. In vivo, BGB-A425 in combination with BGB-A317 inhibits tumor growth in a mouse xenograft cancer model.

Conclusions: BGB-A425 demonstrates strong immune cell activation both *in vitro* and in vivo, supporting its clinical development for the treatment of human cancers.

BGB-A425 binds to human Tim-3 with high affinity

Table 1 Summary of SPR determined kinetic parameters and affinities of BGB-A425 Fab to human Tim-3.

Antigen	K_{on} (1/Ms)	K_{off} (1/s)	K_D (M)
Human Tim-3	1.60 x 10^6	5.7 x 10^{-4}	3.56 x 10^{-10}

Note: BGB-A425 Fab was expressed from HEK293G cells using BeiGene's Fab expression constructs.

Figure 1 Tim-3 binding analysis by FACS. (A) Tim-3-expressing NK-cell line NK92MI/Tim-3 was stained with anti-Tim-3 mAbs (BGB-A425, Ref Ab-1 and Ref Ab-2). MFI: mean fluorescence intensity. Ref Ab-1: ABTIM-3-Hum11 from patent # US20150218274 A1; Ref Ab-2: lead Ab from patent # WO2016161270 A1. **(B)** BGB-A425 binds to monkey Tim-3 as shown in FACS.

BGB-A425 inhibits Tim-3 binding to PtdSer

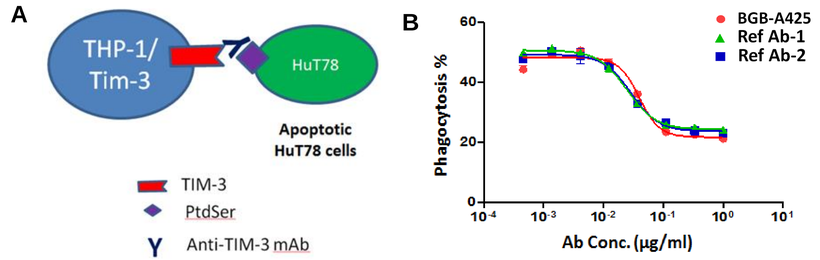

Figure 2 Tim-3 mAbs inhibit Tim-3 binding to PtdSer. (A) Assay set-up was shown in the diagram. Tim-3-expressing THP-1 cells were co-cultured with CFSE-labeled apoptotic Hut78 cells for 5h in the presence of anti-Tim-3 mAbs. The % (phagocytosis) of $CFSE^+$ THP-1/Tim-3 cells was determined by FACS. **(B)** Three Tim-3 mAbs were compared in their Tim-3-PtdSer binding blockade activity.

BGB-A425 activates primary PBMCs to release IFN-γ

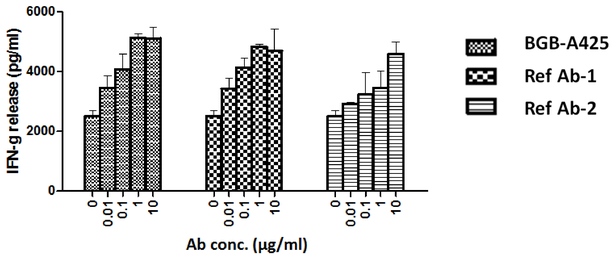

Figure 3 BGB-A425 activates human PBMCs to produce IFN-γ. Pre-activated PBMCs were co-incubated with T-cell engager-positive HepG2 cells for overnight. The results shown are a representative experiment using PBMCs isolated from healthy donors.

Combo of PD-1 and Tim-3 blocking Abs promotes IFN-γ production in MLR

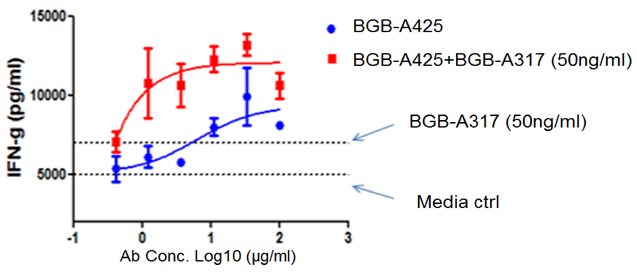

Figure 4 BGB-A425 synergizes with BGB-A317 in MLR. Mitomycin-C-pretreated "stimulator PBMCs" were co-cultured with "responder PBMCs" in the presence of BGB-A425 or BGB-A425 plus an anti-PD-1 Ab BGB-A317 (50 ng/ml) for 4 days.

BGB-A425 induces the internalization of Tim-3

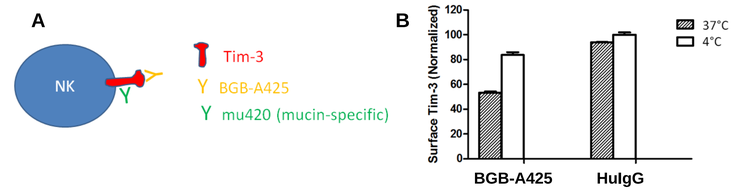

Figure 4 BGB-A425 induces Tim-3 internalization. (A) Primary human NK cells were incubated with BGB-A425 (10 μg/ml) at either 37° C or 4° C for 1 hr. Surface Tim-3 expression was determined by staining with a non-competing Tim-3 Ab mu420 (generated in house). **(B)** A representative data was plotted.

BGB-A425 does not induce ADCC or CDC

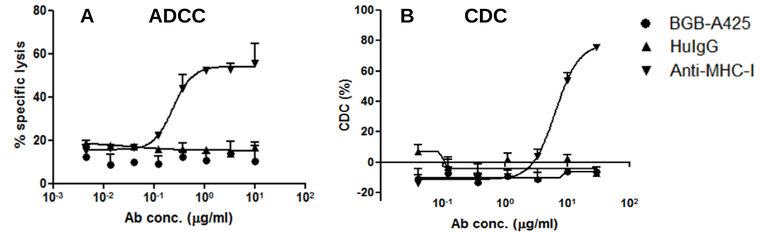

Figure 5: BGB-A425 does not induce ADCC or CDC. (A) NK92MI/CD16 cells were co-cultured with target HuT78/Tim-3 cells in the presence BGB-A425 for 5h. The % of cytotoxicity was calculated based on LDH release assay. **(B)** After overnight co-culture of pre-activated PBMCs with BGB-A425 containing 15% autologous sera, % of CDC was measured by Celltiter-Glo. HuIgG: negative control; Anti-MHC-I: positive control.

Combination of PD-1 and Tim-3 Abs inhibits tumor growth in a mouse xenograft cancer model

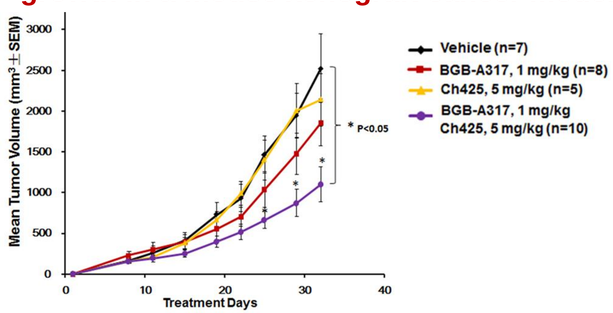

Figure 6 BGB-A425 synergizes with PD-1 Ab BGB-A317 to inhibit tumor growth in a xenograft mouse model. Cyclophosphamide-pre-conditioned NOD/SCID mice were co-implanted with PBMCs and A431 tumor cells in Matrigel *s.c.*. Four days later, tumor-bearing mice were treated with either vehicle (PBS) or mAbs (QW) *i.p.*. Tumor size was monitored twice a week.