**INTRODUCTION**

Givastomig (TJ-426444.NH), or Giva, is a first-in-class bispecific antibody designed to engage tumor cells with a wide range of Claudin 18.2 expression. Giva potently and selectively killed tumor cells and T cells with a wide range of Claudin 18.2 expression at 100 nM. Claudin 18.2 or Giva-activated T cell activity was observed in xenograft assays using CD11c-positive tumor-draining lymphoid (CD11cPositive LN) tumor cells derived from mice with a wide range of Claudin 18.2 expression. Here we further investigate the in vivo pharmacological and pharmacodynamic effects of Givastomig in combination with first-line and second-line (2L) therapies for gastric cancer.

**METHODS**

- **The CLDN1.2 expression in formalin-fixed, paraffin-embedded (FFPE) tumors of three human gastric cancer cell lines (MKN-45 parental, MKN-45 #18, and MKN-45 #14) was determined by immunohistochemistry (IHC) staining.**
- **The T cell activation and tumor-killing mediated by Giva were investigated, either alone or in combination with other drugs, using a co-culture assay of T cell and PBMCs. T cell activation was evaluated by the production of IFN-γ, and soluble CLDN18.2 expression from PBMCs.**
- **Anti-tumor activity and pharmacodynamic effects of the combination treatment were also examined in an in vivo gastric cancer patient-derived xenograft (PDX) model.**

**RESULTS**

Givastomig-induced T cell activation in a dose- and CLDN1.2 expression-dependent manner

Figure 1. The CLDN1.2 expression in three human gastric cancer cell lines, MKN-45, MKN-45 #18, and MKN-45 #14, was determined by immunohistochemistry (IHC) staining in formalin-fixed, paraffin-embedded (FFPE) tumor tissues. T cell numbers in combination with different CLDN1.2 expression were analyzed by an IFN-γ ELISA. The T cell杀伤 was evaluated by the production of IFN-γ per cell in each condition.

Givastomig exerts bystander tumor killing: T cells activated by Giva and CLDN1.2-positive tumor cells leads to the killing of nearby CLDN1.2-negative tumor cells

Figure 2. Givastomig tumor killing was evaluated in co-culture of PBMCs with homogenous tumor cells (A) or CLDN1.2-negative tumor cells (B) as the effect of the whole tumor cell (T) or IFN-γ. In co-culture of PBMCs with heterogenous tumor (C), T cell-mediated tumor killing was observed in the PBMCs only (E:T = 1:1), in co-culture of PBMCs with a mixture of CLDN12-negative (parental) and CLDN12-expressing (45-18) at ratios 10:0, 10:1, 1:10, 1:100. Givastomig tumor killing by PBMCs in parental MKN45 cells was further enhanced by the addition of nivolumab (Nivo) + 5-fluorouracil (5-FU) or ramucirumab (Ram) to the mixture of tumor cells and normal PBMCs. The effect of the effect of the whole tumor cell (T) or IFN-γ on CLDN12-negative tumor cells (CLDN12-low tumor cells) is shown as CLDN12-expression dependent bystander killing (B).

Givastomig-induced tumor killing is enhanced in combination with chemotherapy used in 1L or 2L treatment for gastric cancer

Figure 3. The expression levels of CLDN12 or MKN45/45-18/45-18 were analyzed using Flow cytometry. Tumor-killing by Giva, alone or in combination with other therapies, was evaluated in co-culture of PBMCs with Givastomig or nivolumab. Giva, nivolumab, or 5-FU, PTX, Ram, or Oxa in combination with CLDN12-negative tumor cells at the E:T = 1:10 or 1:1 or 1:100. In co-culture of tumor cells with a mixture of CLDN12-positive and CLDN12-negative tumor cells, Giva (10nM) increased tumor killing in the presence of chemotherapy. The combination of nivolumab and Giva exhibited enhanced tumor killing activity compared to one-drug treatment alone. In summary, the minimal dose to achieve maximal tumor-killing activity in tumor cells is 2.5 nM (10% CLDN12-positive tumor cells) or 5 nM (20% CLDN12-positive tumor cells). CDDP control treatment (without Giva).

**CONCLUSION**

- **An in vitro co-culture system that mimics tumor microenvironment, givastomig (TJ-426444.NH) induced T cell activation in a dose- and CLDN1.2 expression-dependent manner and bystander tumor-killing in which co-cultured tumor cells were treated with givastomig T cell activation by CLDN12-positive tumor cells leads to the killing of nearby CLDN1.2-negative tumor cells, implying the therapeutic potential of givastomig in the treatment of solid tumors with broad and heterogenous CLDN1.2 expression.**
- **In the same co-culture system, givastomig-induced tumor killing is further enhanced in combination with chemotherapies used in 1L or 2L treatment for gastric cancer.**
- **Givastomig combined with nivolumab + POLYFOX exhibited synergistic anti-tumor activity, accompanied by increase in tumor-infiltrating lymphocytes, in a gastric cancer PDX model.**

**Givastomig, a novel Claudin18.2/4-1BB bispecific antibody, exerts bystander tumor-killing and synergistic anti-tumor activity with therapeutics in 1L2L treatment for gastric cancer**

Boojun Liu, Xiaoyi Fu, Zhen Meng, Xia Xia, Chenyu Pan, Ai Li, Zhenniu Lu, Zheng Wang, Jinho Jang, Andrew X. Zhu

1-1 Mab BioPharma, Shanghai, China; 2. ABL Bio IBC, Gyeongsang, Republic of Korea

Corresponding authors: Andrew.Zhui@mabbiopharma.com