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

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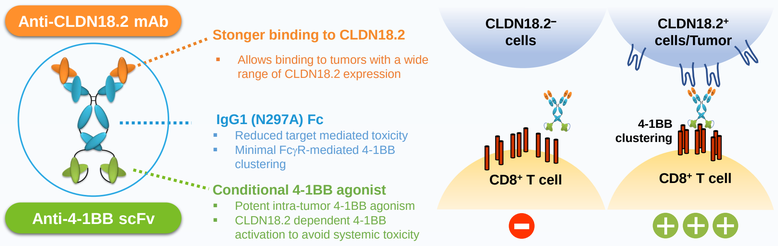
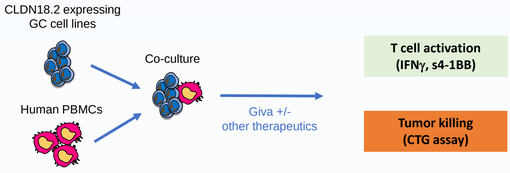
INTRODUCTION

- Givastomig (TJ-CD4B/ABL111, or Giva) is a first-in-class bispecific antibody designed to target tumors with a wide range of Claudin 18.2 (CLDN18.2) expression and elicit 4-1BB-mediated T cell activation upon engagement with CLDN18.2-expressing tumor cells.
- Here we further investigate the mechanism of Giva and explore its potential in combination with first-line (1L) and second-line (2L) therapeutics for gastric cancer.



METHODS

- The CLDN18.2 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; obtained from Genomeditech) 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 therapies, using a co-culture system of tumor cells and PBMCs. T cell activation was evaluated by the production of IFNγ and soluble 4-1BB (s4-1BB), while tumor-killing was evaluated by the CellTiter-Glo® (CTG) Assay.
- Anti-tumor activity and pharmacodynamics effects of the combination treatment were also examined by an *in vivo* gastric cancer patient-derived xenograft (PDX) model.



RESULTS

Givastomig-induced T cell activation in a dose and CLDN18.2 expression dependent manner

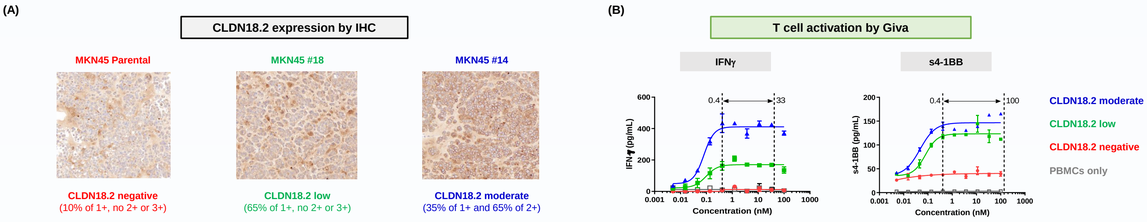


Figure 1. The CLDN18.2 expression in three human gastric cancer cell lines, MKN-45, MKN-45#14, and MKN-45#18, was determined by immunohistochemistry (IHC) staining in formalin-fixed, paraffin-embedded (FFPE) tumor tissues collected from cell line-derived xenograft models. MKN-45, MKN-45#18, and MKN-45#14 exhibited negative (<10% of 1+), low (65% of 1+, no 2+/3+), and moderate (35% of 1+ and 65% of 2+) CLDN18.2 expression, respectively (A). In the co-culture of tumor cells and human PBMCs at the effector and target ratio (E:T) of 1:10, Giva-induced T cell activation, as illustrated by the increased production of IFNγ and soluble 4-1BB (96h), in a dose and CLDN18.2 expression dependent manner (B).

Givastomig exerts bystander tumor killing: *T cells activated by Giva and CLDN18.2-positive tumor cells leads to the killing of nearby CLDN18.2-negative tumor cells*

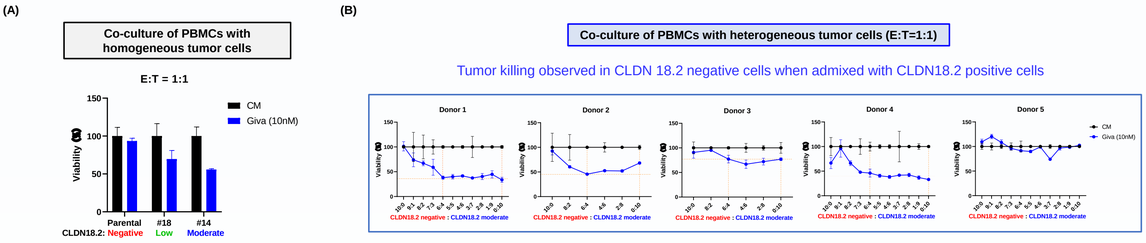


Figure 2. Giva-induced tumor-killing was evaluated in co-culture of PBMCs with homogeneous tumor cells (A) or heterogeneous tumor cells (B), at the effector and target ratio (E:T) of 1:1 for 6 days. In co-culture of PBMCs with homogeneous tumor cells, Giva elicited tumor-killing activity against CLDN18.2-moderate (MKN45 #14) and CLDN18.2-low (MKN45 #18) cells, but not CLDN18.2-negative (MKN45 parental) cells (A). In the co-culture of human PBMCs with a mixture of CLDN18.2-negative (MKN45 parental) cells and CLDN18.2-moderate (MKN45 #14) at various ratios (10:0 to 0:10), Giva-induced T cell activation by CLDN18.2-positive MKN-45#14 cells led to the killing of nearby CLDN18.2-negative MKN-45 cells, a bystander tumor-killing. The bystander tumor-killing activity was observed in co-culture experiment from multiple donors (B). In summary, the minimal ratio to achieve maximal tumor killing in 4 out of 5 donors was 6:4 (40% CLDN18.2 moderate tumor cells or 26% CLDN18.2 2+ tumor cells). CM: control medium (without Giva).

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

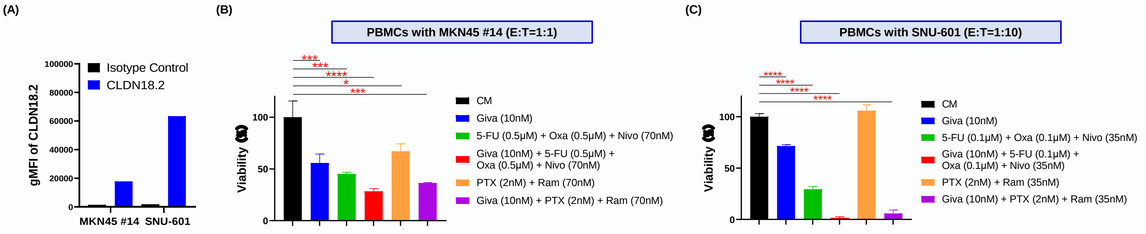


Figure 3. The expression levels of CLDN18.2 on MKN45#14 and SNU-601 were analyzed using flow cytometry (A). Tumor-killing by Giva, alone or in combination with other therapeutics, was evaluated in co-culture of PBMCs with CLDN18.2-positive MKN-45#14 tumor cell at the E:T of 1:1 for 6 days (B), or with CLDN18.2-positive SNU-601 tumor cell at the E:T of 1:10 for 5 days (C). Giva-induced tumor-killing was enhanced in combination with chemotherapies used in 1L or 2L treatment for gastric cancer, including nivolumab (Nivo) plus 5-fluorouracil and oxaliplatin (5-FU+Oxa or FOLFOX) for 1L or ramucirumab (Ram) plus paclitaxel (PTX) for 2L. CM: control medium. Data was shown as Mean ± SD, analyzed using one-way ANOVA followed Dunnett compared with control medium.

Givastomig in combination with nivolumab + FOLFOX exhibited synergistic anti-tumor activity, accompanied by increase in tumor-infiltrating lymphocytes, in a gastric cancer PDX model

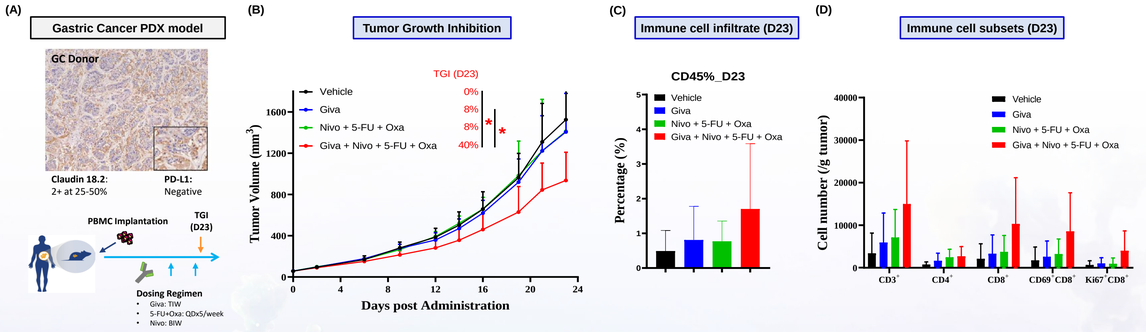


Figure 4. The *in vivo* anti-tumor activity and pharmacodynamics effect on tumor-infiltrating lymphocytes (TILs) by Giva (25 mpk), in monotherapy or in combination with nivolumab (Nivo, 10 mpk), and FOLFOX (5-fluorouracil (5-FU, 8mpk) plus oxaliplatin (Oxa, 3mpk)), was evaluated in a humanized gastric cancer patient-derived xenograft (PDX) model in NCG mice subcutaneously injected with human gastric cancer cells with moderate CLDN18.2 expression (25-50% of 2+) and human PBMCs (A). At Day 23 after treatment, the triple-combination of Giva, nivolumab and FOLFOX resulted in better tumor growth inhibition (TGI=40%), compared to Giva alone (TGI=8%) or nivolumab plus FOLFOX (TGI=8%) (B). The average percentage of tumor-infiltrating lymphocytes in the vehicle group (measured as % of hCD45 cells) was less than 1.0% at day 23 after treatment, indicating a characteristic of "cold tumor" for this PDX model (C). Compared to Giva alone or nivolumab plus FOLFOX , the triple-combination of Giva, nivolumab and FOLFOX showed an increase in densities of TILs ($CD3^+/CD8^+$) and activated $CD8^+$ T cells ($CD69^+/Ki67^+$) at day 23 after treatment (D). Data was shown as Mean ± SD, analyzed using unpaired t-test.

CONCLUSION

- In an *in vitro* co-culture system that mimics tumor microenvironment, givastomig (TJ-CD4B/ABL111) induced T cell activation in a dose- and CLDN18.2 expression- dependent manner and exerts bystander tumor-killing in which givastomig-mediated T cell activation by CLDN18.2-positive tumor cells leads to the killing of nearby CLDN18.2-negative tumor cells, implying the therapeutic potential of givastomig in the treatment of solid tumors with broad and heterogenous CLDN18.2 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.
- In a gastric cancer PDX model, givastomig in combination with nivolumab + FOLFOX exhibited synergistic anti-tumor activity, accompanied by increase in tumor-infiltrating lymphocytes.
- The synergistic anti-tumor activity by givastomig in combination with current therapeutics in 1L/2L treatment for gastric cancer warrants further investigation of these combinations in clinics.