Enhanced HER2-dependent Immune Activation by Margetuximab, an Investigational Fc-engineered anti-HER2 mAb, Supports Combination with Checkpoint Blockade

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Abstract

Background: Margetuximab (M) has demonstrated anti-tumor activity in patients with advanced HER2+ gastric cancer and PFS superiority to trastuzumab (T) in pre-treated metastatic HER2+ breast cancer patients[1]. Similar to T, M inhibits HER2 signaling; additionally, M enhances engagement with the activating Fcγ receptor (FcR)IIIA, while diminishing interaction with the inhibitory FcRIIB. Given the role of FcRs in coupling innate and adaptive immune responses, clinical studies of M in combination with PD-1 or PD-1 plus LAG-3 blockade have been initiated[2,3]. The combination of M with tebotelimab, an investigational bispecific DART® molecule blocking PD-1 and LAG-3, has demonstrated encouraging early evidence of anti-tumor activity in patients with advanced HER2+ malignancies, including those with PD-L1-negative tumors[3]. Here we present data supporting the potential mechanisms underlying the anti-tumor activity elicited by M and tebotelimab combination therapy.

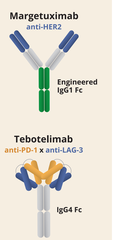
Methods: PBMCs were challenged with HER2-expressing tumor cell lines in the presence of M or T. Immune modulation was assessed by analysis of immune cell phenotype, gene expression profiles, and cytokine secretome. Functional activity of immune cells following exposure to the combination of M with tebotelimab was evaluated.

Results: M induced greater upregulation relative to T of both co-stimulatory (eg 4-1BB) and/or checkpoint molecules (eg PD-L1 and LAG-3) on NK cells and/or monocytes in PBMCs cocultured with HER2-expressing tumor cells. Compared with T, M also induced greater proinflammatory cytokine release, including IFNγ that in turn upregulates PD-L1 and MHC class I expression on co-cultured tumor cells. These effects were not observed with an Fc-null version of M or were inhibited by the addition of FcR-blocking Abs, demonstrating Fc:FcR dependency of M-induced immune activation. Interestingly, CD8 T cells, which lack FcR, also exhibited LAG-3 upregulation in the above cultures[3], suggesting the effects of M can also be mediated via a cascade mechanism. An IFNγ neutralizing mAb reduced tumor cell-associated PD-L1 and MHC class I upregulation, but not LAG-3 induction on T-cells. PBMCs, pre-treated with a combination of M and tebotelimab in the presence of HER2-expressing tumor cells, demonstrated enhanced cytolytic activity against both the canonical NK cell target, K562, or M-opsonized HER2-positive tumor cells.

Conclusions: M, through an Fc-dependent mechanism, can upregulate checkpoint molecules on NK-cells, CD8 T cells and tumor cells in vitro, thereby sensitizing them to immune checkpoint blockade. In turn, PD-1 and LAG-3 blockade by tebotelimab enhances M-mediated NK cell cytolytic activity in vitro.

Introduction

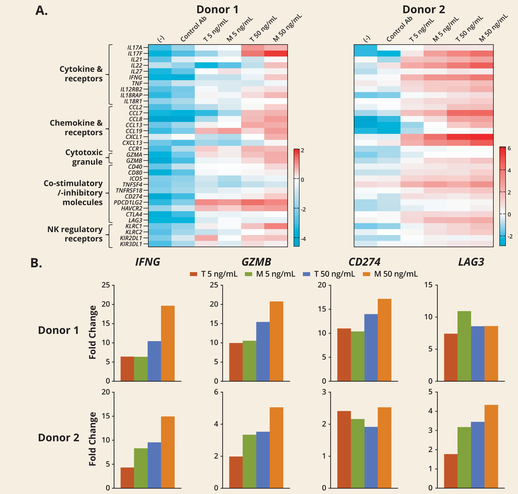
- **Margetuximab:** Fc-engineered anti-HER2 therapeutic mAb
  - Engineering
    - Fab: Same specificity and comparable affinity as trastuzumab
    - Fc: Increased affinity for both allelic variants of the activating FcγRIIIA (CD16A) compared to Trastuzumab; Binding to the low-affinity CD16A-158F variant by margetuximab exceeds that of trastuzumab for the high-affinity CD16A-158V variant; decreased affinity for the inhibitory FcγRIIB (CD32B)
  - Bioactivity
    - Enhanced in vitro ADCC compared to trastuzumab
  - Clinical studies
    - SOPHIA: Phase 3 study of margetuximab plus chemotherapy compared to trastuzumab plus chemotherapy in patients with metastatic HER2-positive breast cancer who had received prior chemotherapy and at least one form of anti-HER2 treatment. Improved PFS compared to control arm[1]
    - MAHOGANY: Phase 2/3 study in patients with HER2-positive gastric cancer in first-line therapy in combination with PD-1 blockade[2]



- **Tebotelimab:** PD1 x LAG-3 bispecific IgG4 DART protein
  - Engineering
    - Tetravalent (bivalent for each target) IgG4 DART molecule
  - Bioactivity
    - Binds to and blocks PD-1 and LAG-3 concomitantly or independently
    - Greater synergistic in vitro T-cell activation (IFNγ release) compared to the combination of its individual constituents
  - Clinical study
    - Anti-tumor activity in various advanced solid tumors[3]
- **Margetuximab** and **Tebotelimab** combination therapy
  - Evidence of anti-tumor activity in patients with advanced HER2+ malignancies, including those with PD-L1-negative tumors[4]

Results

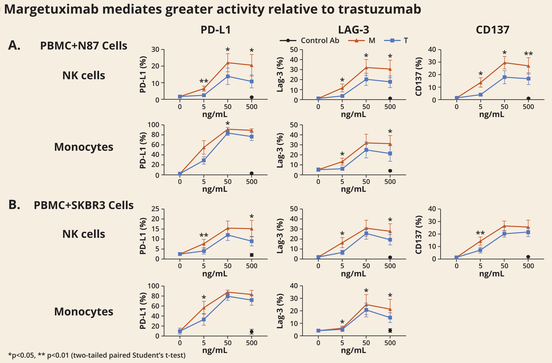
Margetuximab vs Trastuzumab-treated PBMC Gene Expression Profile — Upregulated Inflammatory Responses



Transcription profiles (RNA-seq) of margetuximab (M) or trastuzumab (T)-treated PBMCs from two healthy donors in the presence of HER2-expressing N87 cells (E:T ratio = 15:1, 48hr incubation; Donor 1: CD16A 158 V/F; Donor 2: CD16A 158 F/F). **A.** Immune-related genes with ≥ 2-fold increase following M or T treatment were grouped as indicated (Log_2 normalized expression values [DEseq]). **B.** Fold increase of *IFNG, GZMB, CD274* and *LAG3* gene expression.

Margetuximab-mediated Engagement With HER2-positive Tumor Cells in Vitro Induces Co-signaling Molecules on Effector Cells

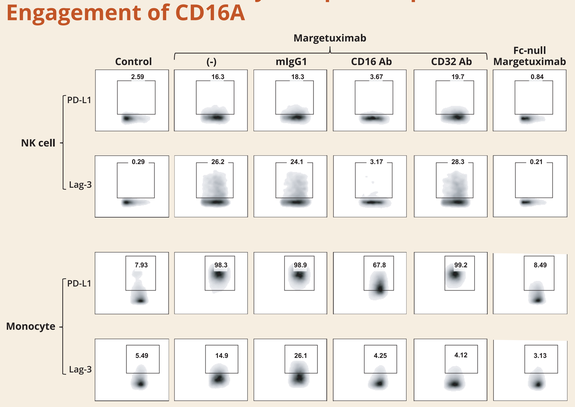
- **Induction of both checkpoint (PD-L1 and LAG-3) and co-stimulatory (CD137) pathways**
- **Margetuximab mediates greater activity relative to trastuzumab**



*p<0.05, ** p<0.01 (two-tailed paired Student's *t*-test)

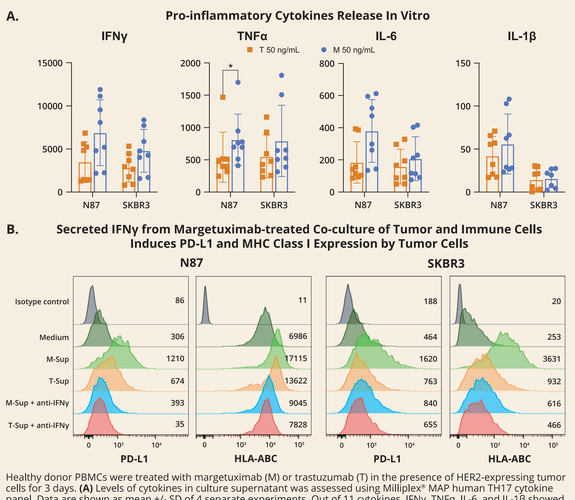
Cell surface expression of PD-L1, LAG-3 and CD137 (4-1BB) were assessed by FACS analysis of PBMCs treated by margetuximab (M) or trastuzumab (T) in the presence of HER2 expressing N87 gastric cancer cells **(A)** or SKBR3 breast cancer cells **(B)**. Data are shown as mean +/- SEM of 7 separate experiments.

Margetuximab-induced PD-L1 or LAG-3 Upregulation on NK Cells and Monocyte Depends upon the Engagement of CD16A



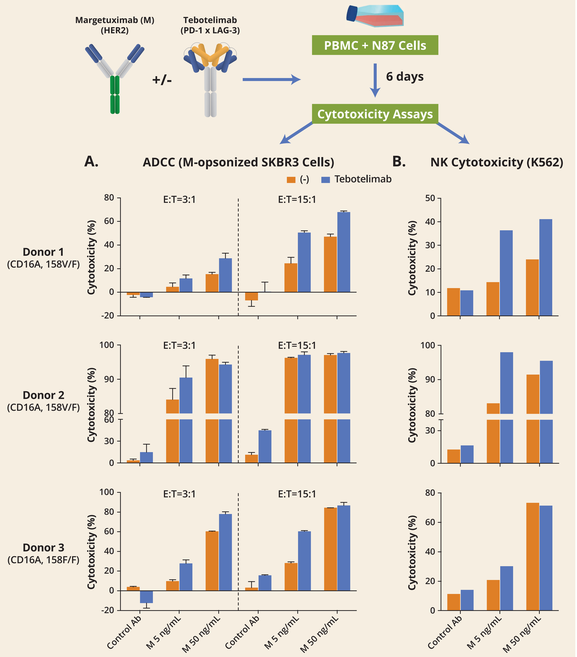
NK cells and monocytes from PBMCs treated with margetuximab upregulate PDL-1 and LAG-3, which was prevented by CD16A-blockade. An aglycosylated Fc-null version of margetuximab failed to upregulate PD-L1 or LAG-3. Notably, CD32 blockade prevented LAG-3 upregulation by monocytes.

Margetuximab Induces Enhanced PD-L1 and MHC Class I Expression on Tumor Cells via an IFNγ-dependent Mechanism



Healthy donor PBMCs were treated with margetuximab (M) or trastuzumab (T) in the presence of HER2-expressing tumor cells for 3 days. **(A)** Levels of cytokines in culture supernatant was assessed using Milliplex® MAP human TH17 cytokine panel. Data are shown as mean +/- SD of 4 separate experiments. Out of 11 cytokines, IFNγ, TNFα, IL-6, and IL-1β showed significant increase post M or T treatment. Differences in cytokine release were not significant, except for TNFα (*p < 0.05, two-tailed paired Student's *t*-test). **(B)** Supernatant (sup) was collected to treat N87 or SKBR3 cells for 24 hours in the presence or absence of anti-IFNγ-blocking Ab. The expression of PD-L1 and HLA-ABC on the surface of tumor cells was assessed by flow cytometry. Data are from a representative experiment of 3 performed.

Treatment of Margetuximab-targeted PBMC with Tebotelimab May Prime NK Cell Cytolytic Activity



Healthy donor PBMCs were pre-treated with margetuximab ± tebotelimab in the presence of HER2-expressing N87 gastric cancer cells for 6 days, as indicated. **A.** Natural killer cell activity (NK Activity) was assessed by FACS analysis of annexin V/ 7-AAD staining of K562 target cells. Percent of cytotoxicity represents sum of % apoptotic cells (Annexin V+7-AAD-) and % dead cells (Annexin V+7-AAD+). **B.** Antibody-dependent cell cytotoxicity (ADCC) was assessed by measuring the luminescence of luciferase-expressing, margetuximab-opsonized SKBR3 cells. Cytotoxicity was normalized by setting no treatment control as 0.

Conclusions

- Engagement of HER2+ tumor cells with margetuximab induces:
  - Enhanced inflammatory genes expression, upregulation of the co-inhibitory molecules, PD-L1 and LAG-3, and expression of the co-stimulatory molecule, CD137 (4-1BB) by immune effector cells relative to trastuzumab
  - Induction of LAG-3 and PD-L1 on NK cells and monocytes is mediated by Fc:CD16A interaction
  - Enhanced PD-L1 and MHC class I expression on tumor cells, mediated by IFNγ
- Exposure of margetuximab-targeted PBMC to tebotelimab, a bispecific DART molecule that blocks PD-1 and LAG-3, may prime NK cells to enhance their intrinsic cytolytic capacity

Acknowledgements

1. Rugo et al., 2021 *JAMA Oncology* doi:10.1001/jamaoncol.2020.7932. **2.** Catenacci et al., 2020 *Lancet Oncology* 21(8):1066-76. **3.** Luke et al., 2020 ASCO oral presentation. **4.** Patel et al., 2020 SITC poster presentation.

Presented at the American Association for Cancer Research 2021 Virtual Annual Meeting I, April 10–15, 2021