XMT-2056, a HER2-directed STING agonist antibody-drug conjugate, exhibits ADCC function that synergizes with STING pathway activation and contributes to anti-tumor responses

Abstract ID: 4423

Jahna T. A. Soomer-James, Kelly Lancaster, Marc Damelin, Naniye Malli (ncetinbas@mersana.com)

Mersana Therapeutics, Inc., Cambridge, MA

ABSTRACT

XMT-2056 is a systemically administered Immunosynthen STING agonist antibody-drug conjugate (ADC; drug-to-antibody ratio = 8) that targets a novel HER2 epitope and induces complete tumor regressions with a single dose in multiple tumor models. We have previously shown that XMT-2056 delivers its STING agonist payload into tumor cells and Fcγ-RI (CD64)-expressing myeloid cells, activating STING signaling in both cell types, leading to type I interferon (IFN) and anti-tumor innate immune responses.

Here, we demonstrate that XMT-2056 exhibits ADCC (antibody-dependent cell-mediated cytotoxicity) function, which synergizes with STING pathway activation and induces potent cancer cell-killing activity in co-cultures of HER2-expressing cancer cells and Fcγ-RIII$^+$ (CD16$^+$) immune cells. We show that both XMT-2056 and HT-19 (the unconjugated parental anti-HER2 antibody) retain significant cancer cell-killing activity in an Fc-effector function dependent manner in PBMC co-cultures depleted of Fcγ-RI-expressing myeloid cells. This activity is abrogated by co-depletion of Fcγ-RIII$^+$ immune cells, illustrating the ADCC function of XMT-2056. In this setting, XMT-2056 cancer cell-killing activity was significantly increased compared to HT-19, suggesting that the STING agonist payload contributes to the differential activity observed with XMT-2056 treatment. Indeed, co-treatment of cancer cell and immune cell co-cultures with HT-19 and free STING agonist payload enhanced the anti-tumor responses, although to a lesser extent than XMT-2056, suggesting a synergy between the ADCC function and STING pathway activation.

Consistently, XMT-2056 treatment of HER2-expressing cancer cells co-cultured with unstimulated CD56$^+$/Fcγ-RIII$^+$ NK cells induced granzyme b and IFN-γ cytokine production, expression of NK cell activation markers, and cancer cell-killing activity. The ADCC activity of HT-19 was comparable to that of trastuzumab in NK cell co-cultures. Finally, we found that depletion of Fcγ-RI$^+$ cells inhibited the cancer cell-killing activity of XMT-2056 in cancer cell and PBMC co-cultures more substantially compared to depletion of Fcγ-RIII$^+$ cells or CD56$^+$ NK cells, indicating a greater contribution of myeloid cells to the XMT-2056 mechanism of action in this setting. Notably, XMT-2056 was capable of engaging both Fcγ-RI$^+$ myeloid cells and Fcγ-RIII$^+$ NK cells, activating both STING-mediated innate immune responses and ADCC function in triple cultures with HER2-expressing cancer cells.

Collectively, our data reveal a synergy between ADCC function and STING pathway induction both mediated by XMT-2056, which enhances the cancer cell-killing activity of Fcγ-RIII$^+$ cells. This additional mechanism of action of XMT-2056 can potentially impact the overall anti-tumor immune responses in tumors infiltrated by Fcγ-RIII$^+$ cells.

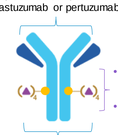
BACKGROUND

XMT-2056

- HER2-directed Immunosynthen STING-agonist ADC[1]
- Systemically administered
- Tumor targeted delivery of STING agonist
- Potent target-dependent anti-tumor activity in tumor models
- Improved efficacy and reduced systemic cytokines compared to a systemically administered diABZI STING agonist[2] in mice
- Well-tolerated in repeat dose toxicology studies in NHP

A Phase I clinical trial was initiated in January 2023

Binds to HER2 on tumor cells (does not compete with trastuzumab or pertuzumab) → **Opportunity for combinations**



Binds to Fcγ receptors and internalizes into tumor resident myeloid cells; dependent on HER2 binding

Hypothesized mechanism of the synergy between STING pathway activation & ADCC function of XMT-2056

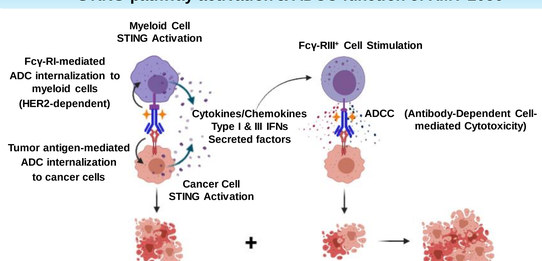


Figure 1. XMT-2056-mediated STING pathway activation in cancer cells and Fcγ-RI$^+$ myeloid cells[3] synergizes with its ADCC function in CD16 (Fcγ-RIII)$^+$ immune cells and contributes to the anti-tumor activity

RESULTS

XMT-2056 exhibits Fc-effector function & CD16$^+$ cell-dependent cancer cell-killing activity in PBMC co-cultures

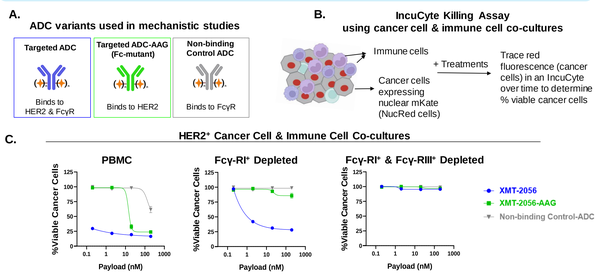


Figure 2. XMT-2056 retains significant cancer cell-killing activity in HER2$^+$ cancer cells co-cultured with PBMCs depleted of Fcγ-RI expressing cells in an Fc-effector function dependent manner, which is completely abrogated by co-depletion of Fcγ-RIII$^+$ immune cells, suggesting an ADCC function. (A) Schematics of the ADC variants used to investigate FcγR-mediated anti-tumor activity. **(B)** Schematic of the IncuCyte cancer cell-killing assay in HER2$^+$ cancer cell (NucRed) and immune cell co-cultures. **(C)** XMT-2056 retained significant cancer cell-killing activity in Fcγ-RI-depleted immune cell co-cultures, which was abrogated with co-depletion of Fcγ-RI$^+$ and Fcγ-RIII$^+$ immune cells. **(D)** Flow cytometry assay confirmed the efficient removal of Fcγ-RI$^+$ or Fcγ-RIII$^+$ cells from human PBMCs using positive selection magnetic beads.

HT-19 antibody exhibits potent ADCC reporter activity

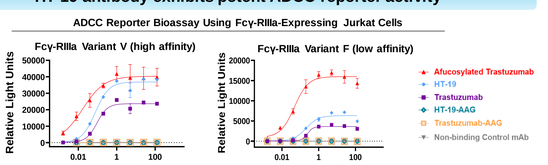


Figure 3. HT-19, the unconjugated anti-HER2 antibody of XMT-2056, exhibits ADCC reporter activity in Jurkat cells expressing high or low affinity variants of Fcγ-RIIIa. SKBR3 (HER2$^+$) cancer cells were treated with the indicated antibodies and co-cultured with Jurkat effector cells expressing the high affinity human Fcγ-RIIIa variant (V158), or low affinity variant (F158). Binding of the antibodies via their Fc region to the Fcγ-RIIIa on Jurkat reporter cells induces NFAT-mediated luciferase activity. In this assay, HT-19 and trastuzumab had comparable ADCC activity with both Fcγ-RIIIa variants.

XMT-2056 ADCC function synergizes with STING pathway activation

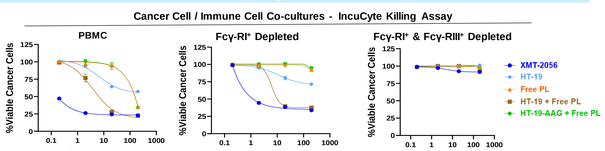


Figure 4. XMT-2056 ADCC function synergizes with STING pathway activation. Cancer cell-killing activity of HT-19 vs XMT-2056 via ADCC function in Fcγ-RI-depleted immune cell co-cultures was evaluated using the IncuCyte killing assay. Interestingly, XMT-2056 activity was greater compared to HT-19, suggesting an additional contribution of STING pathway activation mediated by the STING agonist payload (free payload (PL)) of XMT-2056. Indeed, combining HT-19 and free payload enhanced the cancer cell-killing activity, demonstrating a synergy between the ADCC function of HT-19 and STING pathway activation. The XMT-2056 response remained more potent highlighting the contribution of the ADC to more effectively deliver the STING agonist payload into target expressing cells. XMT-2056 and HT-19 (*wt*) activity was abrogated with co-depletion of Fcγ-RI$^+$ & Fcγ-RIII$^+$ cells. (HT-19 dose matches XMT-2056 antibody dose.)

XMT-2056 & HT-19 both exhibit ADCC activity with primary human NK cells

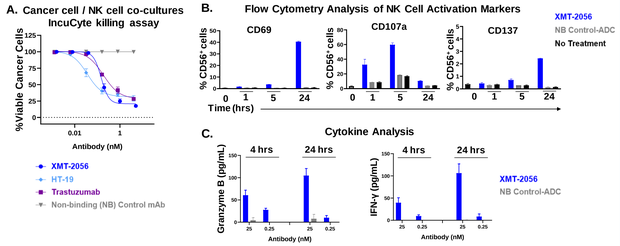


Figure 5. XMT-2056 and HT-19 exhibit ADCC activity with primary human NK cell co-cultures. (A) XMT-2056 and HT-19 both induce similar cancer cell-killing activity in co-cultures of SKBR3 (HER2$^+$)-NucRed cancer cells (15K) and unstimulated NK cells (30K). **(B)** Flow cytometry and **(C)** cytokine analysis of NK cell-activation markers and supernatants in the same cancer cell and NK cell cultures as in (A) demonstrates that XMT-2056 treatment stimulates NK cells.

XMT-2056-mediated STING pathway activation in myeloid cells and cancer cells synergizes with HT-19-mediated ADCC function in NK cell co-cultures

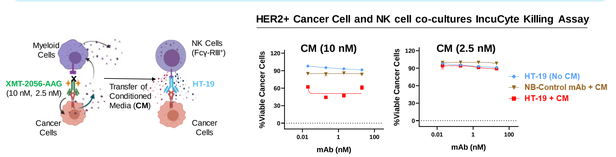
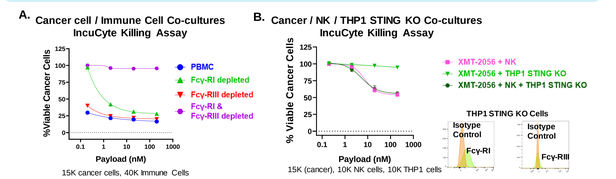


Figure 6. XMT-2056-mediated STING pathway activation in myeloid cells and cancer cells synergizes with HT-19-mediated ADCC function in NK cell co-cultures. Since we have seen a synergy between ADCC function and STING pathway activation in the Fcγ-RI-depleted PBMC (including CD56$^+$Fcγ-RIII$^+$ NK cells and a subpopulation of Fcγ-RIII$^+$ immune cells) co-cultures, we expected to see a differential cancer cell-killing activity by XMT-2056 compared to HT-19 alone in the NK cell co-cultures in Fig. 5. Lack of synergy in NK cell-only co-cultures suggested also lack of STING pathway activation in this setting. However, treatment of cancer cell (15K) and NK cell (15K) co-cultures with HT-19 in the presence of conditioned media (CM) collected 24 hours post treatment of cancer cell and myeloid cell co-cultures with Fc-mutant XMT-2056 markedly enhanced the HT-19-induced cancer cell killing activity of NK cells, demonstrating that STING pathway activation by XMT-2056 in cancer cells and myeloid cells synergizes with the ADCC function of HT-19 with NK cells.

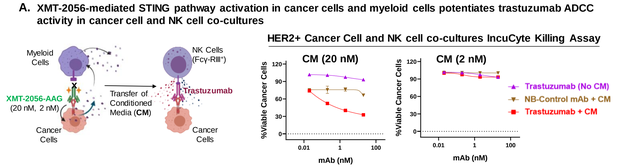
XMT-2056 engages both Fcγ-RI$^+$ and Fcγ-RIII$^+$ cells, with greater activity contributed by Fcγ-RI$^+$ cells

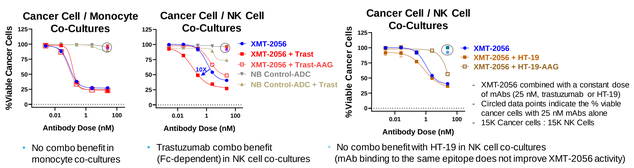


- Depletion of Fcγ-RI$^+$ cells from immune cell co-cultures resulted in a greater loss of XMT-2056 cancer cell-killing activity than depletion of Fcγ-RIII$^+$ cells
- THP1 cells express Fcγ-RI, NK cells express Fcγ-RIII
- THP1 STING KO cells were used to see the effect of Fcγ-RI presence on Fcγ-RIII engagement, without STING contribution
- Fcγ-RIII-mediated ADCC activity with NK cells is not impacted by the presence of Fcγ-RI expressing cells

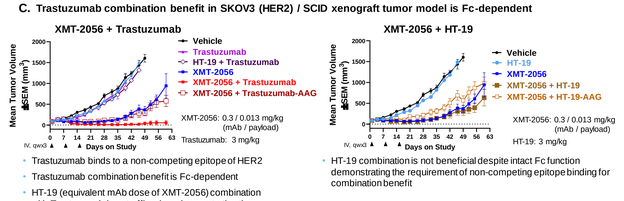
Figure 7. XMT-2056 engages both Fcγ-RI$^+$ myeloid cells and Fcγ-RIII$^+$ NK cells, and the presence of Fcγ-RI$^+$ cells does not interfere with Fcγ-RIII-mediated ADCC activity (A) IncuCyte analysis of SKBR3 (HER2$^+$)-NucRed cancer cells co-cultured with the indicated immune cell populations after 84 hours treatment with XMT-2056. Depletion of Fcγ-RI$^+$ cells from PBMC co-cultures by magnetic beads positive selection, resulted in a greater loss of cancer cell-killing activity compared to depletion of Fcγ-RIII$^+$ cells indicating a greater contribution of myeloid cells to the MOA in this setting. **(B)**. Addition of Fcγ-RI$^+$ THP1 STING KO myeloid cells to the cancer cell and NK cell co-cultures did not inhibit the ADCC-mediated cancer cell-killing activity of XMT-2056. Together these data suggest that XMT-2056 engages both Fcγ-RI$^+$ myeloid cells and Fcγ-RIII$^+$ NK cells.

XMT-2056-mediated STING activation synergizes with ADCC function of trastuzumab





- No combo benefit in monocyte co-cultures
- Trastuzumab combo benefit (Fc-dependent) in NK cell co-cultures
- No combo benefit with HT-19 in NK cell co-cultures (mAb binding to the same epitope does not improve XMT-2056 activity)
- XMT-2056 combined with a constant dose of mAbs (25 nM, trastuzumab or HT-19)
- Circled data points indicate the % viable cancer cells with 25 nM mAbs alone
- 15K Cancer cells : 15K NK Cells



- Trastuzumab binds to a non-competing epitope of HER2
- Trastuzumab combination benefit is Fc-dependent
- HT-19 (equivalent mAb dose of XMT-2056) combination with Trastuzumab is not efficacious demonstrating the contribution of STING pathway activation
- HT-19 combination is not beneficial despite intact Fc function demonstrating the requirement of non-competing epitope binding for combination benefit

Figure 8. XMT-2056-mediated STING pathway activation synergizes with ADCC activity of trastuzumab when combined *in vitro* and *in vivo*. (A) Treatment of cancer cell (15K) and NK cell (15K) co-cultures with trastuzumab in the presence of conditioned media (CM) collected 24 hours post treatment of cancer cell and myeloid cell co-cultures with Fc-mutant XMT-2056 markedly enhanced the trastuzumab-induced cancer cell killing activity of NK cells. **(B)** XMT-2056 and trastuzumab combination shows benefit specifically in the NK cell but not in myeloid cell co-cultures. **(C)** XMT-2056 and trastuzumab combination exhibits benefit in the SKOV3 (HER2)/CB.17 SCID tumor model in vivo, which requires intact Fc function of trastuzumab and binding to a non-competing epitope on HER2. Together these data demonstrate that STING pathway activation by XMT-2056 synergizes with the ADCC function of trastuzumab.

CONCLUSIONS

- We have previously shown that XMT-2056 anti-tumor activity involves STING activation in both cancer cells and Fcγ-R-expressing myeloid cells.[1,2]
- In this study we demonstrated;
  - XMT-2056 exhibits ADCC function, which synergizes with STING pathway activation and induces potent cancer cell-killing activity in co-cultures of HER2-expressing cancer cells and Fcγ-RIII$^+$ immune cells.
  - XMT-2056 confers greater synergy compared to mAb and free STING agonist combination.
  - XMT-2056 can engage both Fcγ-RI$^+$ myeloid cells and Fcγ-RIII$^+$ NK cells and the presence of Fcγ-RI$^+$ cells does not interfere with Fcγ-RIII-mediated ADCC activity
  - Contribution of the STING activation in cancer cells and Fcγ-RI$^+$ myeloid cells to the anti-tumor activity of XMT-2056 is greater than the ADCC activity via Fcγ-RIII$^+$ NK cells *in vitro.*
  - The synergy between the STING activation and ADCC function contributes to the MOA of the XMT-2056 and trastuzumab combination benefit.
- This unique MOA of the synergy between STING activation and ADCC function of XMT-2056 may potentially improve anti-tumor responses in tumors infiltrated with NK cells.

References:

1. Duvall et al. AACR 2022 https://www.mersana.com/publications/
2. Ramanjulu et al. Nature 2018
3. Malli Cetinbas et al. AACR 2022 https://www.mersana.com/publications/
4. Cartoons were generated using Biorender.