Selection of a Bispecific Trivalent HER2 x CD137 TRIDENT™ Format Providing Optimal Tumor-anchored Immune Co-stimulation

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Abstract

Introduction: CD137 (4-1BB) signaling provides co-stimulation of CD8 or NK cells following antigen or FcγR engagement, respectively. Efforts to leverage CD137 co-stimulation via agonistic monoclonal antibodies (mAbs) have been thwarted by limited clinical efficacy or unacceptable toxicity. Bispecific targeting strategies linking CD137 activation to a tumor-targeting moiety provides an approach to localize CD137 activation to the tumor microenvironment. Here we evaluate a panel of Fc-bearing HER2 x CD137 bispecific molecules incorporating different valency and geometry to define the format providing optimal CD137 co-stimulation in a tumor-cell anchor-dependent manner.

Methods: An anti-HER2 mAb specificity that does not cross compete with margetuximab, trastuzumab or pertuzumab and a proprietary anti-CD137 mAb were utilized to assemble a set of HER2 x CD137 bispecific molecules in bivalent and tetravalent DART® or trivalent TRIDENT™ configurations. The resulting molecules were compared in binding, signaling and co-stimulation assays in the presence or absence of tumor cells expressing HER2. Combination studies were performed in vitro and in immune deficient mice reconstituted with human PBMCs.

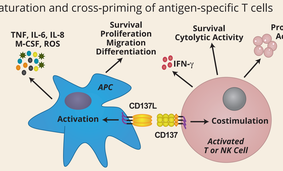
Results: TRIDENT molecules bearing bivalent CD137 and monovalent HER2 binding achieve optimal HER2-dependent tumor-cell anchored CD137 immune cell co-stimulation. CD137 co-stimulation increases proportionally with the level of HER2 expression as observed with HER2 1+ (MCF7 breast), HER2 2+ (JIMT1 breast) and 3+ (N87 gastric) tumor cells and was paralleled with increased HER2$^+$/CD137$^+$ cell association. No CD137 activation is observed in the absence of HER2-expressing tumor cells. HER2 x CD137 bispecifics enhance NK-cell proliferation and IFN-γ release induced by margetuximab, an Fc-optimized anti-HER2 mAb that up-regulates CD137 expression on NK-cells concomitant with enhanced ADCC against HER2-positive cells. Similarly, HER2 x CD137 bispecific molecules enhanced the in vitro activity of MGD009, a B7-H3 x CD3 bispecific DART molecule that up-regulates CD137 during T-cell redirected killing. Finally, in vivo mouse studies demonstrate the ability of HER2 x CD137 molecules to expand tumor-associated CD8 cells when co-administered with a tumor targeted CD3 bispecific molecule and support enhance anti-tumor activity.

Conclusions: An optimal HER2 x CD137 bispecific format providing maximal CD137 activation in a HER2- dependent manner was identified as a trivalent TRIDENT molecule bearing bivalent CD137 and monovalent HER2 binding. Combinatorial activity with HER2 x CD137 bispecifics was observed with both a HER2-directed therapeutic mAb and a CD3-engaging tumor-targeted bispecific molecule. HER2 x CD137 TRIDENT molecules have therapeutic potential and provide a structural template for incorporating alternate tumor- and/or co-stimulatory-targeting arms.

Background

CD137 (4-1BB)-Conditional Agonist: Rationale

- CD137 (4-1BB), a TNFR superfamily member expressed on immune cells, interacts with CD137L leading to CD137 pathway activation
  - ↑ T, NK-cell effector function, proliferation and survival
  - ↑ Endothelial adhesion molecules and chemokines resulting in CD8 homing to tumor site
  - ↑ DC maturation and cross-priming of antigen-specific T cells



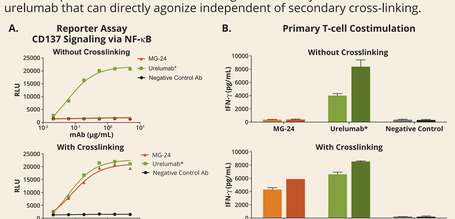
- CD137 expression is up-regulated on T cells following TCR engagement with cognate MHC:peptide complex
  - Expressed on tumor-reactive T-cell lineages[1]
  - Synergy of CD137 agonism with PD-1 checkpoint blockade[2]
  - CD3 based bispecifics induce CD137 expression on T cells
- Monoclonal antibodies can further induce CD137 expression on NK cells upon ligation of FcγRIIIA
  - Synergy of CD137 agonism with rituximab[3], trastuzumab[4], cetuximab[5]
- Agonistic CD137 mAbs exhibit potent antitumor activity in preclinical models but display unwanted toxicity or insufficient activity in the clinic[1]
- **Goal: Develop optimal bi-specific conditional CD137 pathway activation of tumor immune infiltrate**

HER2 x CD137 Bi-specific: Desired Molecule Profile

αHER2 x αCD137 Properties	Purpose
Bispecific format providing optimal CD137 co-stimulation contingent on tumor HER2 expression level	Co-stimulation of TILs to enhance anti-tumor immunity in HER2$^+$ tumors (breast, gastric, ovarian, CRC)
CD137 mediated agonistic activity dependent on cross-linking to HER2	Co-stimulatory activity restricted to tumor sites
αHER2 binding not competing with trastuzumab/margetuximab/pertuzumab	Enable combination with existing HER2 mAb-based therapies
Effector function "null" Fc	Avoid any FcγR interactions and cross-linking of CD137; retain FcRn binding for prolonged PK
Cyomolgus monkey αHER2 and αCD137 cross-reactivity	Enable NHP PK/Tox study
Combinatorial activity with mAbs (including Fc-optimized), checkpoint inhibitors and CD3-directed bispecifics	Combination therapy with other IO agents

Properties of anti-CD137 Arm

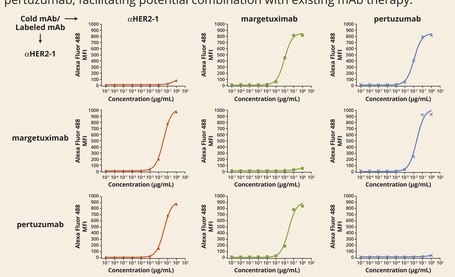
The CD137 antibody (MG-24) mAb was selected for incorporation into HER2 x CD137 bispecifics based on conditional agonistic activity. This is in contrast to urelumab that can directly agonize independent of secondary cross-linking.



Evaluation of CD137 mAb MG-24 to activate **(A)** Jurkat CD137 NF-κB-luc reporter line or **(B)** support primary T cell co-stimulation in the absence (upper panels) or presence (lower panels) of 4 x αhFC cross-linking reagent. For panel **(B)** 0.1 μg/mL and 1 μg/mL of each test article were evaluated. *A replica of Urelumab was employed as control for non-cluster dependent activation.

Properties of anti-HER2 Arm

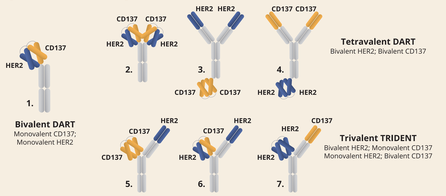
The HER2-1 mAb selected for incorporation into HER2 x CD137 bispecifics does not compete with margetuximab (same epitope as trastuzumab) or pertuzumab, facilitating potential combination with existing mAb therapy.



AlexaFluor 488-labeled mAbs binding to N87 cell line in the presence of 10 μg/mL unlabeled mAbs as competitor.

Results

Geometry and Relative Valency Critical for Optimal Activity

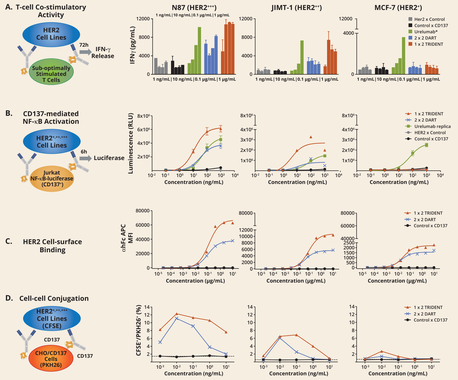


DART/TRIDENT proteins **(1–7)** with varying valency of CD137 and/or HER2 binding were expressed in CHO cells and purified to homogeneity. Binding of each molecule to soluble HER2 and CD137 was confirmed by ELISA and to natively expressed cell surface antigen by FACS analyses of JIMT-1 (HER2$^+$) tumor cells or activated CD8 T cells (CD137$^+$). Co-stimulatory activity on primary T cells was evaluated by co-incubating each test article with primary T cells sub-optimally primed with anti-CD3 in presence of HER2$^-$ N87 Gastric cancer cell line.

Format/ Valency	HER2 x CD137 Orientation	HER2 Binding (JIMT-1)	CD137 Binding (activated CD8)	Tumor Anchored Costimulatory Activity
DART/ Bivalent	**1.** HER2 x CD137	✓	✓	+
DART/ Tetravalent	**2.** (HER2/CD137) 2x	✓	✓	++
	3. (HER2)2 x (CD137)2	✓	✓	+++
	4. (CD137)2 x (HER2)2	✓	✓	++
TRIDENT/ Trivalent	**5.** HER2 x CD137 x CD137	✓	✓	++++
	6. HER2 x CD137 x HER2	✓	✓	+
	7. HER2 x HER2 x CD137	✓	✓	-

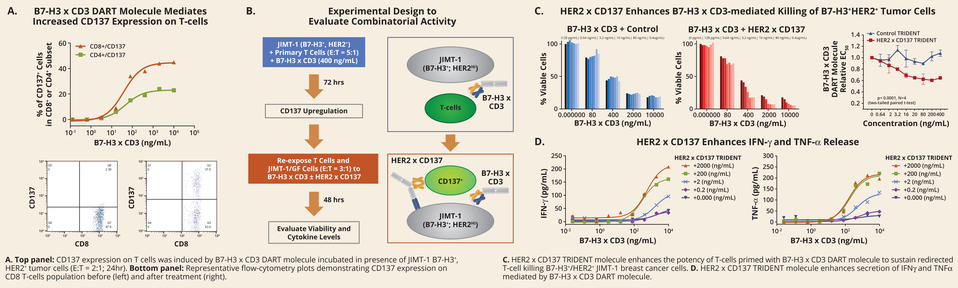
The (HER2)2 x (CD137)2 DART molecule **(3)**, a 2 x 2 DART and the HER2 x CD137 x CD137 TRIDENT molecule **(5)**, a 1 x 3 TRIDENT supported greatest conditional CD137 co-stimulation and were selected for further analyses.

HER2 x CD137 (1x2) TRIDENT Molecule Supports Optimal Conditional CD137 Agonism



- T-cell costimulation **(A)** and NF-κB pathway activation **(B)** strictly dependent on HER2 tumor cell surface expression; no activity observed in presence of low expressing HER2 (MCF-7) or absent HER2 expression (data not shown)
- Enhanced conditional CD137 activation correlates with increase HER2 cell surface binding **(C)** and consequential ability to support cell:cell association **(D)**

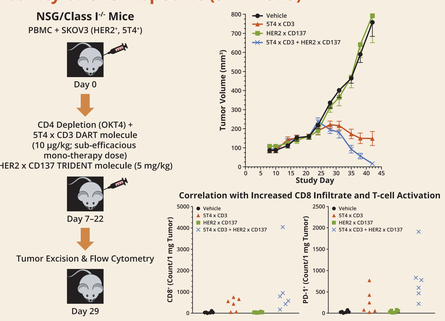
HER2 x CD137 TRIDENT Molecule: Combinatorial Activity with Redirected T-cell Killing



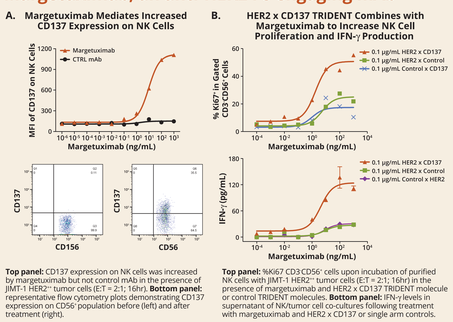
A. Top panel: CD137 expression on T cells was induced by B7-H3 x CD3 DART molecule incubated in presence of JIMT-1 B7-H3$^+$, HER2$^+$ tumor cells (E:T ~ 2:1; 24hr). **Bottom panel:** Representative flow-cytometry plots demonstrating CD137 expression on CD8 T-cells population before (left) and after treatment (right).

C. HER2 x CD137 TRIDENT molecule enhances the potency of T-cells primed with B7-H3 x CD3 DART molecule to sustain redirected T-cell killing B7-H3$^+$/HER2$^+$ JIMT-1 breast cancer cells. **D.** HER2 x CD137 TRIDENT molecule enhances secretion of IFNγ and TNFα mediated by B7-H3 x CD3 DART molecule.

HER2 x CD137 TRIDENT Molecule Enhances Anti-Tumor Activity of CD3 Bispecific (5T4 x CD3)



HER2 x CD137 Combinatorial Activity With Margetuximab, an anti-HER2 Fc-engaging mAb



Top panel: CD137 expression on NK cells was increased by margetuximab but not control mAb in the presence of JIMT-1 HER2^{++} tumor cells (E:T = 2:1; 16hr). **Bottom panel:** representative flow cytometry plots demonstrating CD137 expression on CD56$^+$ population before (left) and after treatment (right).

Top panel: %Ki67$^+$ CD3$^-$CD56$^+$ cells upon incubation of purified NK cells with JIMT-1 HER2^{++} tumor cells (E:T = 2:1; 16hr) in the presence of margetuximab and HER2 x CD137 TRIDENT molecule or control TRIDENT molecules. **Bottom panel:** IFN-γ levels in supernatant of NK/tumor cell co-cultures following treatment with margetuximab and HER2 x CD137 or single arm controls.

Conclusions

- Proof-of-concept studies confirm HER2+ tumor-cell conditional activation of the CD137 pathway by various HER2 x CD137 DART and TRIDENT proteins
- HER2 x CD137 (1x2) TRIDENT molecule bearing monovalent HER2 binding and bivalent CD137 engagement provides optimal activity
  - CD137 pathway activation and T-cell co-stimulation dependent on HER2$^+$ expression level on co-engaged tumor cells
  - Increased activity consistent with increased loading of HER2$^+$ cells and level of HER2$^+$ cell:CD137$^+$ cell association
- HER2 x CD137 TRIDENT molecule enhances anti-tumor activity of CD3-based bispecific DART molecules
  - Sustains CTL capacity of T cells primed by CD3-based DART molecule and increases secreted levels of IFN-γ and TNF-α
  - Enhances anti-tumor activity in xenograft model of HER2$^+$ ovarian cancer
- HER2 x CD137 TRIDENT molecule enhances activity mediated by an anti-HER2 antibody (margetuximab)
  - Binds an epitope distinct from trastuzumab (margetuximab) or pertuzumab
  - Increases NK cell proliferation and IFN-γ secretion
- Data support further evaluation of HER2 x CD137 to enhance anti-tumor immunity in HER2$^+$ cancers alone or in combination with existing anti-HER2 therapy, checkpoint inhibitors or with CD3-directed bispecific molecules
- The TRIDENT format, bearing monovalent tumor targeting and bivalent CD137 binding, provides a framework for other tumor antigen-anchored CD137 bispecifics[6]

References

1. Chester et al., (2018) Immunotherapy targeting 4-1BB: mechanistic rationale, clinical results, and future strategies. *BLOOD* 131 (1): 49-57. **2.** Chen et al (2015) Combination of 4-1BB agonist and PD-1 antagonist promotes antitumor effector/memory CD8 T cells in a poorly immunogenic tumor model. *Cancer Immunol. Res* 3 (2): 149-160. **3.** Kohrt et al., (2011) CD137 stimulates anti-lymphoma activity of anti-CD20 antibodies. *BLOOD* 117(8): 2423-2432. **4.** Masu et al., (2018) Anti-CD137 monoclonal antibody enhances trastuzumab-induced, natural killer cell-mediated cytotoxicity against pancreatic cancer cell lines with low human epidermal growth factor-like receptor 2 expression. *PLoS One*, Dec 31;13(12):e0200664. **5.** Kohrt et al., (2014) Targeting CD137 enhances the efficacy of cetuximab. *J.Clin. Invest.* 124: 2668-2682. **6.** Liu et al., (2019) Tumor-antigen 5T4-dependent Activation of the CD137 Costimulatory Pathway by Bispecific 5T4 x CD137 x CD137 TRIDENT™ Molecules. AACR 2019 Abstract 3476.