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Novel Multifunctional Tetravalent CD38 NKp46 FLEX-NK™ Engagers Actively Target and Kill Multiple Myeloma Cells

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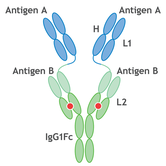
ABSTRACT

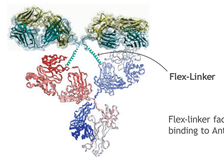
Given that CD38 is a clinically validated target for NK cell mediated cytotoxicity in multiple myeloma, we sought to leverage our FLEX-NK™ platform to create a NK engager antibody targeting CD38. FLEX-NK™ is a proprietary platform for production of tetravalent IgG1-like multifunctional NK engager antibodies with a novel FLEX-linker to allow for simultaneous binding of both the targeted cancer cells and NK cells. NK engagement and activation is mediated through a binder directed against the natural cytotoxicity receptor NKp46. With the FLEX-NK™ scaffold, we created two novel tetravalent NK cell engagers targeting CD38, one with a wild type Fc (CYT-338 WT) and one with a Fc null mutant (CYT-338 MUT). Both CD38 NKp46 engagers showed dose dependent binding to CD38 expressing multiple myeloma cell lines MM1S and KMS11 and no binding to a CD38 knock out MM1S cell line. Both engagers also bound multiple myeloma cell lines with ~ 3-fold higher mean fluorescence intensity than anti-CD38 monoclonal antibody (mAb) or daratumumab alone. Epitope mapping studies for our CD38 mAb using alanine scanning mutagenesis showed that amino acid S232 on CD38, critical for binding to daratumumab, is not important for our CD38 mAb binding to CD38, suggesting a distinct epitope detected by our antibody.

Interestingly, the NKp46 mAb alone without a functional Fc induced peripheral blood NK cell cytolysis of the multiple myeloma cells, consistent with a prior report that NKp46 plays a key role in NK-cell mediated killing of myeloma cells. Both CYT-338 WT and MUT showed further enhanced dose dependent NK cell redirected cytolysis and degranulation against multiple myeloma cells compared to anti-NKp46 mAb or daratumumab alone. CYT-338 WT is more potent in induction of TNF-α and IFN-γ production compared to daratumumab and CYT-338 MUT. No IL-18 or IL-6 was induced by the engagers or daratumumab. Daratumumab treatment resulted in NK cell fratricide, while minimal to low fratricide was observed with CYT-338 WT and MUT respectively. In peripheral blood mononuclear cell hemato-toxicity studies depletion of monocytes and NK cells were observed with daratumumab but minimal depletion was observed with CYT-338 MUT. Daratumumab and CYT-338 WT induced modest to low levels of cytokine release in the in-vitro human PBMC cytokine release assay while T cell agonist anti-CD3 and CD28 mAbs (TGN1412) readily induced cytokine release. These results suggest that the CD38 NKp46 engagers have a favorable NK cell engager profile for targeting CD38 expressing multiple myeloma distinct from daratumumab.

FIGURE 1: Proprietary BsAb Technology Leading to Novel Multifunctional FLEX Format



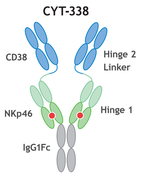
- Tetravalent: higher avidity for target, improved affinity and specificity
- Structure and novel FLEX-linker allow for plug & play design
- Low immunogenicity
- Excellent stability
- FLEX-NK™ construct enhances NK Cell function against target cells
- Manufacturability established



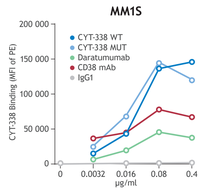
Flex-linker facilitates binding to Antigen AB

Reference: Golay et al. J. Immunol 2016

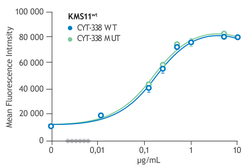
FIGURE 2: CYT-338 structure and binding to multiple myeloma cell lines expressing CD38

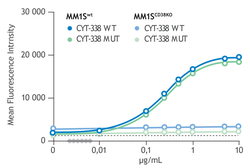


Structure of CYT-338 Flex NK™ tetravalent multifunctional hIgG1 antibody with a flexible linker for simultaneous binding to CD38 and NKp46.

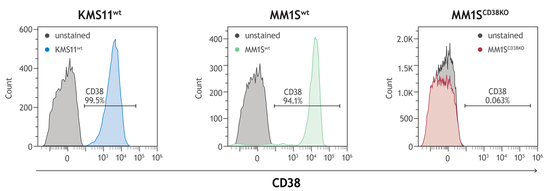


CYT-338 WT and CYT-338 MUT showed ~ 3 fold higher dose dependent binding to MM1S multiple myeloma cells compared to daratumumab



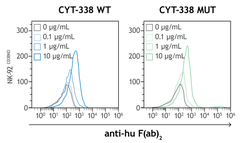


Similar dose dependent binding was observed with CYT-338 WT or CYT-338 MUT to MM1S and KMS11 multiple myeloma cells.

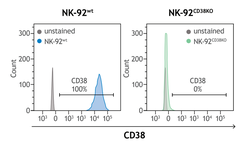


CD38 expression in KMS11, MM1S and MM1S^{CD38KO} determined by anti-CD38 mAb binding.

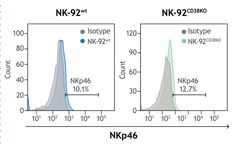
FIGURE 3: CYT-338 showed dose dependent binding to NK cells



Similar dose dependent binding was observed with CYT-338 WT and CYT-338 MUT in NK92^{CD38KO} cells expressing NKp46 detected with an anti-human IgG F(ab)2 antibody.



CD38 expression in NK92^{WT} and NK92^{CD38KO} cells detected with an anti-CD38 mAb.



NKp46 expression in NK92^{WT} and NK92^{CD38KO} cells detected with an anti-NKp46 mAb

FIGURE 4: CYT-338 epitope mapping studies indicate CYT-338 binds to CD38 differently than daratumumab

CD38 beta strand 1 residues

CD38 Residue	CD38 Mutant	CD38 mAb Binding
191	E191A	1.5
192	K192A	0.2
193	V193A	0.8
194	Q194A	0.2
195	T195A	0.2
196	L196A	0.6
197	E197A	0.5
198	A198S	0.7
199	W199A	0.3
200	V200A	0.6
201	I201A	0.9
202	H202A	0.8

CD38 beta strand 2 residues

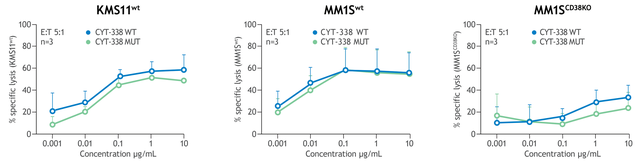
CD38 Residue	CD38 Mutant	CD38 mAb Binding
225	S225A	1.1
226	K226A	1.1
227	R227A	0.9
228	N228A	0.6
229	I229A	0.4
230	Q230A	0.2
231	F231A	0.3
232	S232A	1.1
233	C233A	0.6
234	K234A	1.1
235	N235A	1.1
236	I236A	1.7
237	Y237A	0.9
238	R238A	1.0

Only CD38 mAb binds
Only Dara binds
CD38 mAb and Dara bind

CYT-338 epitope mapping by CD38 alanine scanning mutagenesis indicates CYT-338 and daratumumab bind non-overlapping distinct sites on CD38. CD38 alanine mutants of beta strand 1 and 2 residues 191-202 and 225-238 respectively were derived and tested for binding to CD38 mAb used for the CYT-338 engager. CD38 mutant expression levels are evaluated by measuring FASEBA CD38 mutant fusion protein. CD38 mAb binding is expressed as ratio of binding to CD38 mutant vs binding to FASEBA CD38 mutant fusion protein. Binding < 0.3 is considered as significant perturbation of CD38 mAb binding.

References: M Weers et al. J. Immunol 2011; H Lee et al Biochem Biophys Res Comm 2021

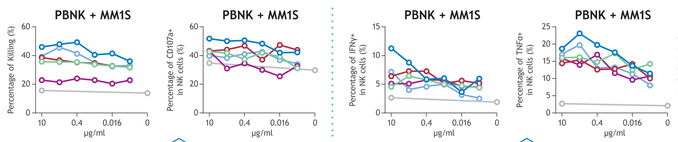
FIGURE 5: CYT-338 showed dose dependent PBNK redirected cytolysis of multiple myeloma cell lines



CYT-338 WT and CYT-338 MUT dose dependent PBNK redirected cytolysis of KMS11 and MM1S multiple myeloma cells was dependent on CD38 expression as minimal cytolysis was observed with MM1S^{CD38KO} cells.

CYT-338 WT showed slightly higher cytolysis compared to CYT-338 MUT. PBNK cytotoxicity assay was conducted at the indicated CYT-338 concentrations at a fixed E/T ratio of 5 for 20 hrs and target cytolysis assessed by flow cytometry.

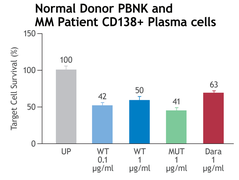
FIGURE 6: CYT-338 PB-NK cell redirected cytolysis, degranulation and cytokine production against multiple myeloma MM1S cells

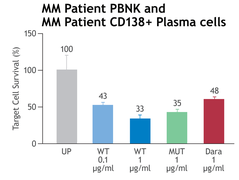


CYT-338 WT showed significantly higher dose dependent PBNK cell redirected cytolysis and degranulation against multiple myeloma MM1S cells compared to daratumumab, CYT338 MUT or single CD38 and NKp46 mAbs. These results indicate that co-engagement of NKp46 and CD38 on NK and MM.1S cells by CYT-338 contributes to the higher potency observed by this NK cell engager. PBNK cytolysis experiments were conducted at the indicated concentrations of CYT-338 at a fixed E/T ratio of 1 for 24 hrs and target lysis analyzed by flow cytometry. PBNK degranulation was assessed at an E/T ratio of 1 for 5 hrs and assessed by flow cytometry using an anti-CD107a antibody.

CYT-338 WT also induced higher % of IFN-γ and TNF-α producing PBNK cells compared to daratumumab, CYT-338 MUT and single CD38 and NKp46 mAbs following incubation with MM cells at E/T ratio of 1 for 5hrs. Cytokine production was assessed by intracellular staining for IFN-γ and TNF-α by flow cytometry.

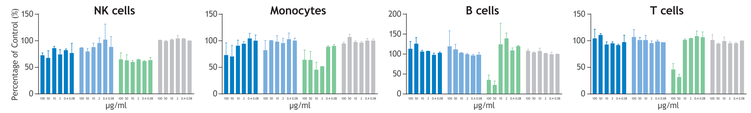
FIGURE 7: CYT-338 mediated donor and patient PBNK cell redirected cytolysis of multiple myeloma patient plasma cells

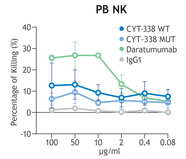




Normal donor and autologous multiple myeloma PBNK redirected cytolysis of multiple myeloma patient CD138+ plasma cells was higher with CYT-338 WT and CYT-338 MUT compared to daratumumab. A MicroC3TM device system (LynxBio®) was used to evaluate PBNK redirected cytolysis of multiple myeloma patient CD138+ plasma cells in the presence of CYT-338 or daratumumab. PBNK cocultures with patient multiple myeloma cells were incubated for 24 hrs in the presence of the indicated antibodies at an E/T ratio of 1 and cytolysis was assessed using fluorescence microscopy.

FIGURE 8: CYT-338 showed minimal immune subset depletion, NK cell fratricide, and cytokine release compared to daratumumab in human PBMCs in-vitro

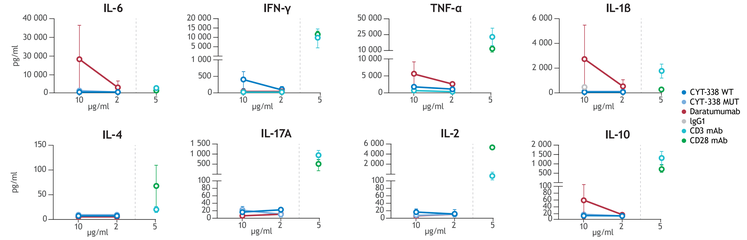




NK cell fratricide was evaluated using purified PBNK in the presence of CYT-338 or daratumumab or human IgG by flow cytometry using the live dead cell dye. Daratumumab showed higher fratricide of PBNK cells compared to CYT-338 WT and CYT-338 MUT.

Effect of CYT-338 on human PBMC immune cell subset depletion was evaluated following incubation with CYT-338 or daratumumab or hIgG1 for 24 hrs followed by immune subset analysis by flow cytometry. While daratumumab showed depletion of NK cells and monocytes, minimal immune cell depletion was observed with CYT-338 WT and CYT-338 MUT.

The potential for CYT-303 to induce cytokine release was evaluated in the human PBMC assay following incubation with CYT-338 or anti-CD3 or CD28 mAbs (TGN1412) or hIgG1 for 48 hrs and supernatants tested for the presence of cytokines by multiplex ELISA assay. While robust cytokine release was observed with anti-CD3 and CD28 (TGN1412) mAbs, minimal cytokine release was observed with CYT-338.



SUMMARY

- CYT-338 is a tetravalent human IgG1 multifunctional NK cell engager antibody with a flexible linker that allows for simultaneous binding to CD38 and NKp46 on multiple myeloma and NK cells respectively.
- CYT-338 WT and CYT-338 MUT showed ~ 3 fold increase in binding to MM1S multiple myeloma cells compared to daratumumab.
- CYT-338 epitope mapping studies based on CD38 alanine scanning mutagenesis indicate CYT-338 binds to CD38 differently than daratumumab.
- CYT-338 WT and CYT-338 MUT molecules showed significantly higher dose dependent PBNK redirected cytolysis, degranulation and cytokine production against multiple myeloma MM1S cells compared to daratumumab or single CD38 and NKp46 mAbs indicating co-engagement of NKp46 and CD38 on NK and MM cells by CYT-338 contributes to the higher potency observed with this NK cell engager.
- Although CYT-338 WT and CYT-338 MUT molecules showed similar binding to multiple myeloma and NK92 cells, CYT-338 WT showed slightly higher PBNK redirected cytolysis of MM1S cells compared to CYT-338 MUT.
- PBNK derived from healthy donor and multiple myeloma patients induced greater redirected cytolysis of multiple myeloma patient plasma cells with CYT-338 WT and CYT-338 MUT compared to daratumumab.
- In-vitro PBMC studies with CYT-338 WT and CYT-338 MUT showed minimal immune subset depletion and fratricide compared to higher immune subset depletion and fratricide observed with daratumumab.
- CYT-338 induced minimal cytokine release in the human PBMC assay while robust cytokine release was observed with anti-CD3 and CD28 mAbs (TGN1412).

CONCLUSIONS

- The FLEX-NK™ multifunctional engager antibody CYT-338 directed against NKp46 and CD38 demonstrated in vitro activity against multiple myeloma tumor targets.
- CYT-338 binds to CD38 differently than daratumumab.
- The binding, cytokine release, cytotoxicity, and fratricide profiles of CYT-338 were superior to daratumumab.
- These data support further development of CYT-338 as a therapeutic for targeting CD38 expressing multiple myeloma distinct from daratumumab.