GDA-601: NAM-NK CELLS WITH CD38 KNOCKOUT EXPRESSES ENHANCED CD38 CHIMERIC ANTIGEN RECEPTOR AND TARGETS MULTIPLE MYELOMA CELLS WITH INCREASED CYTOTOXICITY

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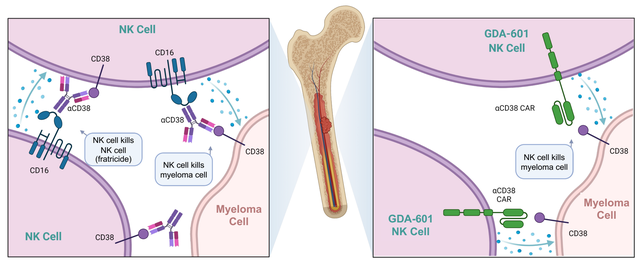
BACKGROUND

- Natural killer (NK) cells are a vital component of cancer immune surveillance, generating a rapid and potent immune response. The therapeutic potential of NK cells in the treatment of hematologic malignancies, including multiple myeloma (MM), has been the focus of extensive translational research. CD38 is an ectoenzyme ubiquitously expressed on the surface of MM cells. Monoclonal antibodies (mAbs) targeting CD38 induce lysis of MM cells through antibody-mediated mechanisms including antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity, and antibody-dependent cellular phagocytosis.[1] However, an important limitation of this therapeutic strategy is the expression of CD38 on normal hematologic cells including NK cells, potentially leading to depletion of CD38-expressing NK cells.[2] Ex vivo expansion of allogeneic NK cells using our proprietary nicotinamide (NAM) platform enhances NK cell functionality by (1) preventing cell exhaustion, (2) enhancing cytotoxic activity, (3) protecting against oxidative stress, and (4) improving homing to lymphoid tissues. These attributes provide opportunities to explore the therapeutic potential of NK cells in the clinic. This study evaluated the cytotoxicity of GDA-601, a genetically engineered NAM-NK cell product designed to target MM cells.

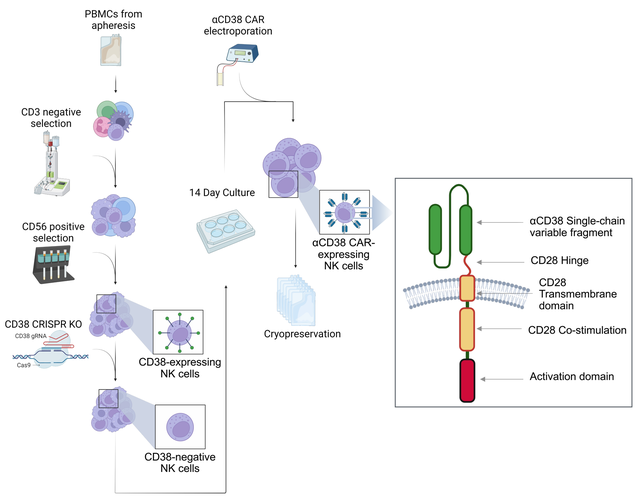


OBJECTIVE

- To create a potent, CD38-mediated, fratricide-resistant, NAM-NK cell therapeutic strategy targeting MM cells.

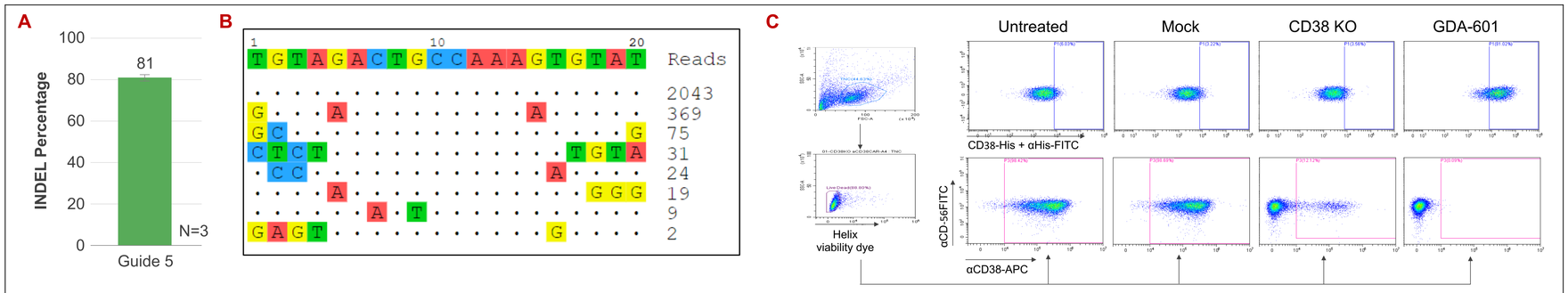
PROCESS DESIGN AND METHODS

Peripheral blood mononuclear cells (PBMCs) were obtained by apheresis from a healthy volunteer. Cells were purified by CD3-negative selection using the CliniMACS System followed by a CD56-positive selection using MACS Cell Separation columns. After a short recuperation in interleukin-2 (IL-2)–supplemented media, the purified cells were subjected to CRISPR-Cas9 editing aimed to knockout (KO) CD38. CD38 KO cells were then cultured for 14 days in NAM-supplemented media in the presence of irradiated feeder cells and IL-15. On day of harvest, an mRNA anti-CD38 chimeric antigen receptor (CAR) was introduced into cells by electroporation. Approximately 5 hours post-electroporation, the cells were either cryopreserved or used fresh for further experimentation.



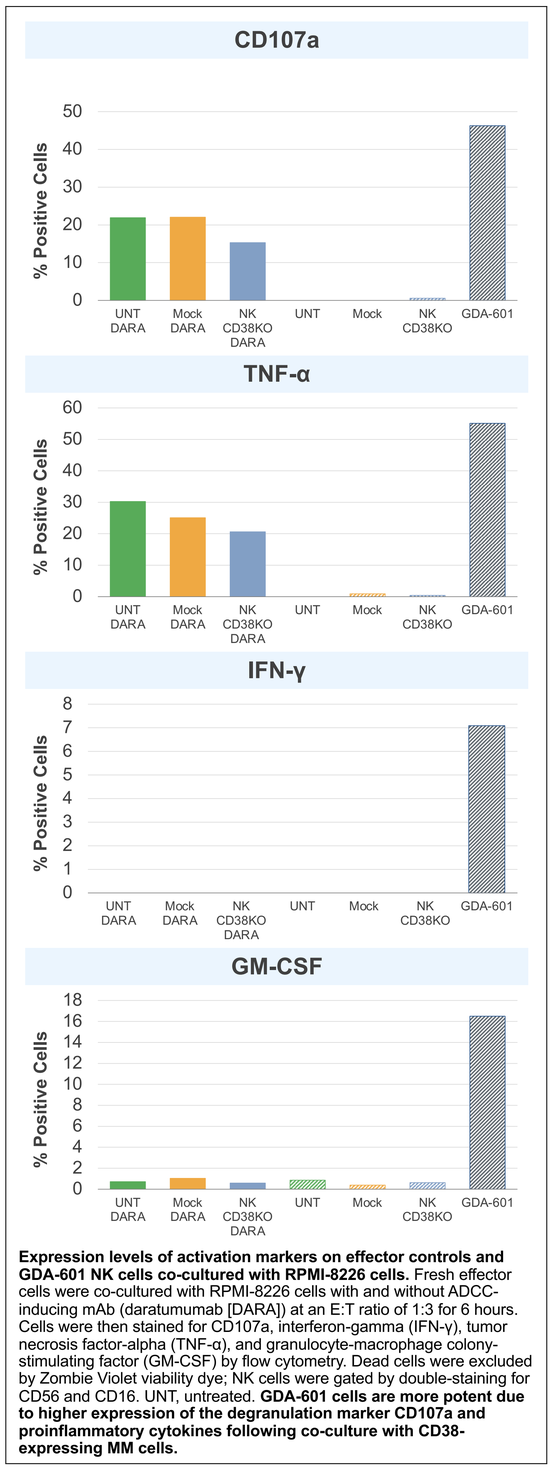
RESULTS

GDA-601: CD38 KO



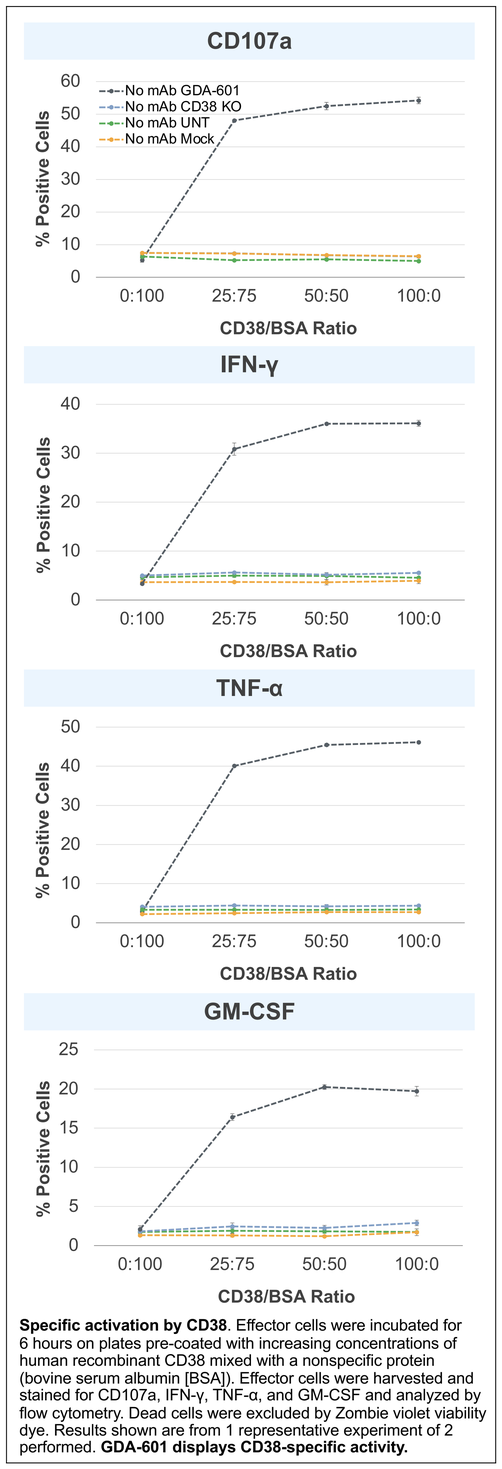
CD38 KO editing strategy. (**A**) To KO CD38, a CRISPR-Cas9–based system was designed. Human primary NK cells were electroporated with 4 µM ribonucleoprotein (RNP) complex targeting CD38. To evaluate editing efficiency, DNA from the cells was extracted after 14 days in culture. Sanger sequencing was performed and analyzed via insertions and deletions (INDELs) by decomposition (TIDE) to determine INDEL frequencies. CD38 single-guide RNA (sgRNA) yields a high editing rate (81% INDEL frequency); mean ± SD, n=3. (**B**) To study the specificity profile of the CD38 sgRNAs, the GUIDE-Seq method was used. Double-stranded oligodeoxynucleotides were co-delivered together with CD38 XT 2-part gRNA to a Cas9-expressing HEK293 stable cell line. The intended target sequence is shown in the top line with cleaved sites shown underneath and with mismatches to the on-target site shown and highlighted in color. GUIDE-Seq sequencing read counts are shown to the right of each site. GUIDE-Seq analysis of CD38 gRNA editing revealed only 7 off-target cleavage sites (OTS). (**C**) GDA-601 phenotyping: On day of harvest, 4 hours after electroporation, all effector cells were stained with FITC-conjugated anti-CD56 mAb and an allophycocyanin (APC)-conjugated anti-CD38 mAb. Alternatively, cells were incubated with His tag-conjugated CD38 to induce complex formation with αCD38 CAR, followed by staining with FITC-conjugated αHis tag mAb. Staining was followed by addition of the Helix viability dye immediately prior to analysis by flow cytometry. Results shown are from 1 representative experiment of 5 performed.

GDA-601: POTENCY



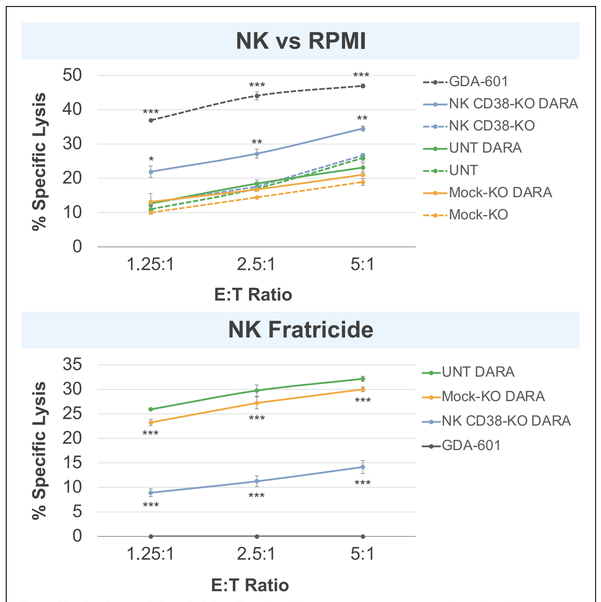
Expression levels of activation markers on effector controls and GDA-601 NK cells co-cultured with RPMI-8226 cells. Fresh effector cells were co-cultured with RPMI-8226 cells with and without ADCC-inducing mAb (daratumumab [DARA]) at an E:T ratio of 1:3 for 6 hours. Cells were then stained for CD107a, interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), and granulocyte-macrophage colony-stimulating factor (GM-CSF) by flow cytometry. Dead cells were excluded by Zombie Violet viability dye; NK cells were gated by double-staining for CD56 and CD16. UNT, untreated. **GDA-601 cells are more potent due to higher expression of the degranulation marker CD107a and proinflammatory cytokines following co-culture with CD38-expressing MM cells.**

GDA-601: CD38 CAR ACTIVITY



Specific activation by CD38. Effector cells were incubated for 6 hours on plates pre-coated with increasing concentrations of human recombinant CD38 mixed with a nonspecific protein (bovine serum albumin [BSA]). Effector cells were harvested and stained for CD107a, IFN-γ, TNF-α, and GM-CSF and analyzed by flow cytometry. Dead cells were excluded by Zombie violet viability dye. Results shown are from 1 representative experiment of 2 performed. **GDA-601 displays CD38-specific activity.**

GDA-601: SPECIFIC KILLING CAPABILITIES



Specific lysis and fratricide. Fresh effector cells were co-cultured with carboxyfluorescein succinimidyl ester (CFSE)-labeled RPMI 8226 cells either in the presence or absence of DARA at E:T ratios varying from 5:1 to 0:1 for 6 hours. Cells were harvested and stained for viability by Helix viability dye. Target cells were CFSE-gated, and the percentage of dead cells was determined. Alternatively, CFSE-negative NK cells were gated, and the percentage of dead NK cells was determined. Results shown are from 1 representative experiment of 3 performed. *P<0.05, **P<0.005, ***P<0.0005 Student t test, comparison to nearest lower curve. **GDA-601 displays reduced fratricide and enhanced cytotoxicity activity against CD38-expressing MM cells.**

CONCLUSIONS

- GDA-601 NAM-NK cells were genetically modified using CRISPR-Cas9–based gene editing to eliminate ~90% of native CD38 expression.
- CD38-mediated fratricide in GDA-601 was reduced to an undetectable level.
- Co-culture of GDA-601 with MM cells led to increased target cell lysis, elevated degranulation, and increased expression of proinflammatory cytokines compared with controls.
- GDA-601 displays superior antitumoral responses against MM cells and represents a promising adoptive cell therapeutic strategy against MM.

ACKNOWLEDGMENTS

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References

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2. Wang Y, et al. *Front Clin Cancer Res.* 2018;24:4006–4017.