GDA-501: ENGINEERED NAM-NK CELLS WITH HER2-CAR EXPRESSION DEMONSTRATE INCREASED CYTOTOXICITY AGAINST HER2-EXPRESSING SOLID TUMORS

Poster 273

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INTRODUCTION

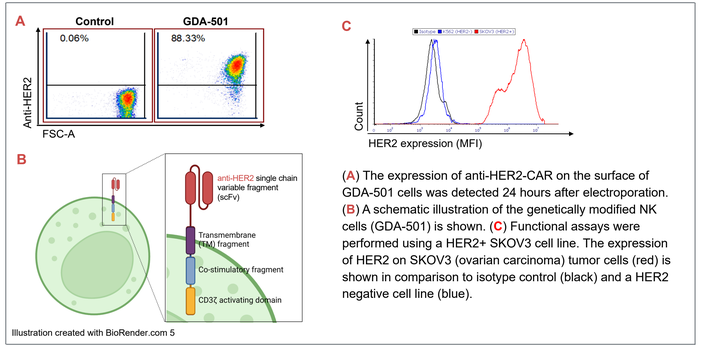
- Genetic modification strategies of allogeneic natural killer (NK) cells provide a promising next-generation immunotherapeutic tool. Ex vivo expansion of allogeneic NK cells using the nicotinamide (NAM) platform enhances NK cell functionality by preventing cell exhaustion, enhancing cytotoxic activity, generating a protective effect against oxidative stress, and exhibiting improved homing to lymphoid tissues
- The success of immunotherapy in solid tumors has been limited due to several barriers, including the immunosuppressive tumor microenvironment, inefficient trafficking, and heterogeneity of tumor antigens. Several therapeutic approaches to overcome these limitations have emerged
- Gene modification of NK cells may enhance their functionality and provide a promising next-generation immunotherapeutic tool. Chimeric antigen receptors (CAR) can target specific antigens on tumors. Human epidermal growth factor receptor 2 (HER2)-CAR may target HER2+ solid tumors, such as breast, gastric, and ovarian carcinomas

METHODS AND WORKFLOW

- Fresh apheresis samples from healthy donors were depleted of CD3 cells and co-cultured with irradiated feeder cells (CD3+ fraction). NAM-NK cells were cultured for 12-14 days, followed by electroporation with mRNA encoding an anti-HER2-CAR. The expression of the CAR was evaluated by flow cytometry
- In vitro functional analyses of potency and cytotoxicity of modified NAM-NK cells were assessed by intracellular expression of proinflammatory cytokines and killing activity when co-cultured with tumor cell lines
- In vivo anti-tumor effects were evaluated using the HER2-expressing solid tumor (SKOV3) model in NOD SCID gamma (NSG) mice

RESULTS

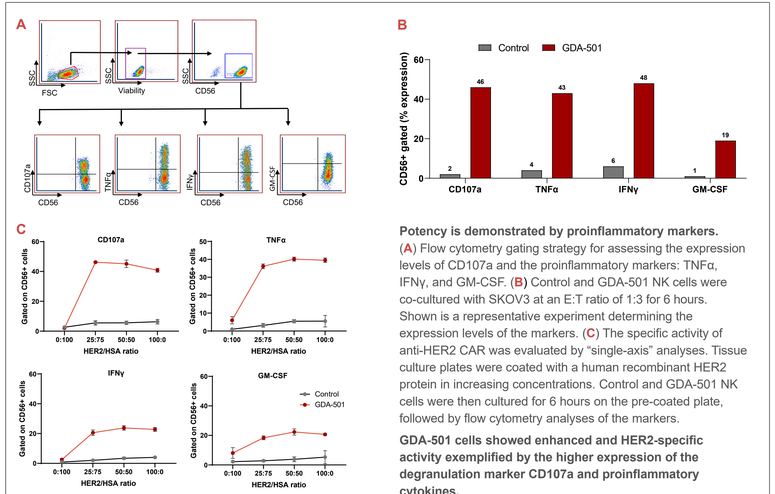
FIGURE 1. DETECTION OF αHER2-CAR ON GDA-501 AND IDENTIFICATION OF TARGET CELLS



(A) The expression of anti-HER2-CAR on the surface of GDA-501 cells was detected 24 hours after electroporation. (B) A schematic illustration of the genetically modified NK cells (GDA-501) is shown. (C) Functional assays were performed using a HER2+ SKOV3 cell line. The expression of HER2 on SKOV3 (ovarian carcinoma) tumor cells (red) is shown in comparison to isotype control (black) and a HER2 negative cell line (blue).

CAR: chimeric antigen receptor; FSC-A: forward scatter area; HER2: human epidermal growth factor receptor 2; MFI: mean fluorescence intensity; NK: natural killer.

FIGURE 2. GDA-501 DISPLAYS ENHANCED PROINFLAMMATORY ACTIVITY

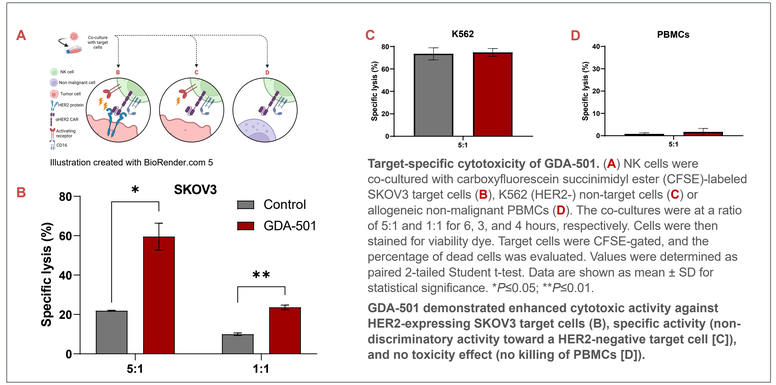


Potency is demonstrated by proinflammatory markers. (A) Flow cytometry gating strategy for assessing the expression levels of CD107a and the proinflammatory markers: TNFα, IFNγ, and GM-CSF. (B) Control and GDA-501 NK cells were co-cultured with SKOV3 at an E:T ratio of 1:3 for 6 hours. Shown is a representative experiment determining the expression levels of the markers. (C) The specific activity of anti-HER2 CAR was evaluated by "single-axis" analyses. Tissue culture plates were coated with a human recombinant HER2 protein in increasing concentrations. Control and GDA-501 NK cells were then cultured for 6 hours on the pre-coated plate, followed by flow cytometry analyses of the markers.

GDA-501 cells showed enhanced and HER2-specific activity exemplified by the higher expression of the degranulation marker CD107a and proinflammatory cytokines.

FSC: forward scatter; GM-CSF, granulocyte-macrophage colony-stimulating factor; HER2: human epidermal growth factor receptor 2; HSA: human serum albumin; IFNγ, interferon gamma; NK: natural killer; SSC, side scatter; TNFα, tumor necrosis factor-alpha. Results shown are from 1 representative experiment of 3 performed.

FIGURE 3. GDA-501 DEMONSTRATES INCREASED KILLING CAPABILITIES

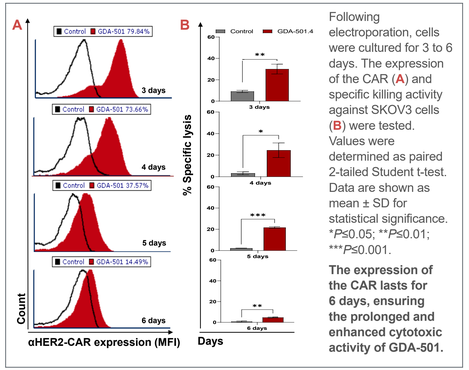


Target-specific cytotoxicity of GDA-501. (A) NK cells were co-cultured with carboxyfluorescein succinimidyl ester (CFSE)-labeled SKOV3 target cells (B), K562 (HER2-) non-target cells (C) or allogeneic non-malignant PBMCs (D). The co-cultures were at a ratio of 5:1 and 1:1 for 6, 3, and 4 hours, respectively. Cells were then stained for viability dye. Target cells were CFSE-gated, and the percentage of dead cells was evaluated. Values were determined as paired 2-tailed Student t-test. Data are shown as mean ± SD for statistical significance. *$P \leq 0.05$; **$P \leq 0.01$.

GDA-501 demonstrated enhanced cytotoxic activity against HER2-expressing SKOV3 target cells (B), specific activity (non-discriminatory activity toward a HER2-negative target cell [C]), and no toxicity effect (no killing of PBMCs [D]).

CAR: chimeric antigen receptor; HER2: human epidermal growth factor receptor 2; NK: natural killer; PBMC: peripheral blood mononuclear cell; SD: standard deviation.

FIGURE 4. GDA-501 SHOWS PROLONGED AND ENHANCED CYTOTOXICITY

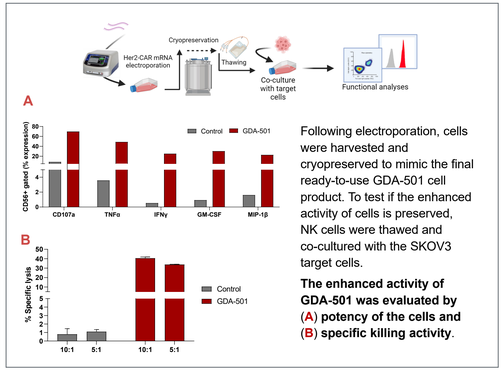


Following electroporation, cells were cultured for 3 to 6 days. The expression of the CAR (A) and specific killing activity against SKOV3 cells (B) were tested. Values were determined as paired 2-tailed Student t-test. Data are shown as mean ± SD for statistical significance. *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.

The expression of the CAR lasts for 6 days, ensuring the prolonged and enhanced cytotoxic activity of GDA-501.

CAR: chimeric antigen receptor; HER2: human epidermal growth factor receptor 2; MFI: mean fluorescence intensity; SD: standard deviation.

FIGURE 5. GDA-501 PRESERVES ENHANCED ACTIVITY FOLLOWING CRYOPRESERVATION

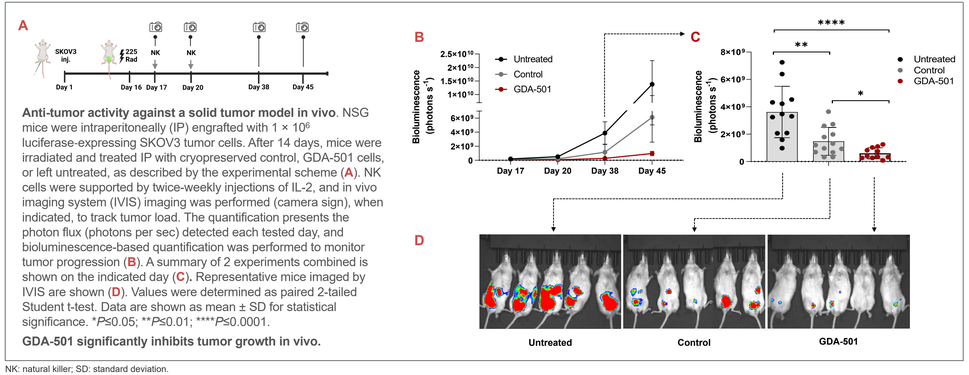


Following electroporation, cells were harvested and cryopreserved to mimic the final ready-to-use GDA-501 cell product. To test if the enhanced activity of cells is preserved, NK cells were thawed and co-cultured with the SKOV3 target cells.

The enhanced activity of GDA-501 was evaluated by (A) potency of the cells and (B) specific killing activity.

CAR: chimeric antigen receptor; GM-CSF, granulocyte-macrophage colony-stimulating factor; HER2: human epidermal growth factor receptor 2; IFNγ, interferon gamma; MIP-1β: macrophage inflammatory protein-1 beta; TNFα, tumor necrosis factor-alpha.

FIGURE 6. GDA-501 INHIBITS TUMOR GROWTH OF A HER2-SOLID TUMOR MODEL IN VIVO



Anti-tumor activity against a solid tumor model in vivo. NSG mice were intraperitoneally (IP) engrafted with 1×10^6 luciferase-expressing SKOV3 tumor cells. After 14 days, mice were irradiated and treated IP with cryopreserved control, GDA-501 cells, or left untreated, as described by the experimental scheme (A). NK cells were supported by twice-weekly injections of IL-2, and in vivo imaging system (IVIS) imaging was performed (camera sign), when indicated, to track tumor load. The quantification presents the photon flux (photons per sec) detected each tested day, and bioluminescence-based quantification was performed to monitor tumor progression (B). A summary of 2 experiments combined is shown on the indicated day (C). Representative mice imaged by IVIS are shown (D). Values were determined as paired 2-tailed Student t-test. Data are shown as mean ± SD for statistical significance. *$P \leq 0.05$; **$P \leq 0.01$; ****$P \leq 0.0001$.

GDA-501 significantly inhibits tumor growth in vivo.

NK: natural killer; SD: standard deviation.

CONCLUSIONS

- GDA-501 significantly enhances the potency and killing effect of NAM-NK cells, with a target-specific activity in vitro and in vivo
- GDA-501 represents a unique allogeneic cell therapy potentially targeting HER2+ solid tumors

ACKNOWLEDGMENTS

Editorial support was provided by Evidence Scientific Solutions and was funded by Gamida Cell.

PRESENTED AT THE SOCIETY FOR IMMUNOTHERAPY OF CANCER (SITC) ANNUAL MEETING; NOVEMBER 08–12, 2022; BOSTON, MA, USA