GDA-201: A CRYOPRESERVED, READILY AVAILABLE FORMULATION OF NICOTINAMIDE-ENABLED NATURAL KILLER CELLS, SHOWS HIGH POTENCY AND CYTOTOXICITY IN VITRO

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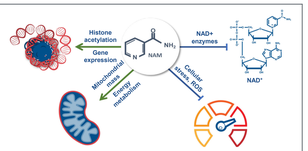
BACKGROUND

- Natural killer (NK) cells are cytotoxic lymphocytes that have drawn considerable attention in recent years as a promising immunotherapy for cancer. However, limited NK persistence in vivo has been a barrier to clinical success
- GDA-201 is an allogeneic NK cell immunotherapy candidate derived from healthy donors
- Clinical responses were demonstrated in a Phase 1 trial of a fresh formulation of GDA-201 in patients with refractory non-Hodgkin lymphoma[1]
- We now report the development and characterization of cryopreserved GDA-201, expanded using the nicotinamide (NAM) platform

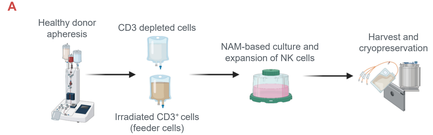
FIGURE 1. NAM ADVANTAGE IN NK CELL CULTURE

Nicotinamide:
- Plays a key role in the **metabolic reprogramming** of cells
- Master regulator of **NAD**-related signaling pathways
- Enhances cellular **functionality** and **phenotype**
- Improves **homing** and **retention** of lymphoid tissues
- Increases **metabolic fitness**
- Preserves a **non-exhausted phenotype**
- Protects against **oxidative stress**



NAD, nicotinamide adenine dinucleotide; NAM, nicotinamide; NK, natural killer; ROS, reactive oxygen species.

FIGURE 2. GDA-201 MANUFACTURING PROCESS



(**A**) GDA-201 manufacturing flow. Apheresis units from healthy donors were CD3-depleted and cultured for 14 days with NAM and interleukin-15 in the presence of irradiated CD3+ feeder cells from the same donor. On harvest day, cells were split into several cryobags, frozen, and maintained in liquid nitrogen.

(**B**) The GDA-201 irradiation protocol eliminates the proliferative capacity of CD3+ T cells. Irradiated (right) or non-irradiated (left) CD3+ cells were labeled with CellTrace Violet and cultured for 13 days in GDA-201 medium. The intensity of the CellTrace Violet was followed by flow cytometry throughout the duration of the culture to determine the proliferation capacity of the cells.

NAM, nicotinamide; NK, natural killer.

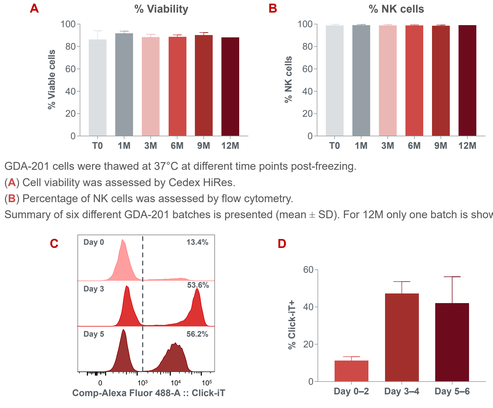
METHODS

Each batch of GDA-201 was frozen in several cryobags and stored in liquid nitrogen. For long-term stability studies, bags were pulled at different time points post-freezing, thawed at 37ºC, and cells were tested for viability, phenotyping, function and potency. The data from different batches was summarized

- Cell number and viability were detected by Trypan Blue exclusion method using Cedex HiRes analyzer
- Cell purity was detected by staining cells with anti-CD56 and anti-CD3 antibodies (NK cells were CD56+CD3-)
- Cell function was evaluated by a fluorescence activated cell sorting-based cytotoxicity assay. GDA-201 cells were co-cultured with carboxyfluorescein diacetate succinimidyl ester (CSFE)-labeled human cancer cell lines at an effector to target (E:T) ratio of 10:1. Target cell killing was determined by staining of viability dye. Specific lysis was calculated using control of staining target cells without GDA-201
- Cell potency was determined by flow cytometry assay for degranulation and intra-cellular staining of cytokines. GDA-201 cells were co-cultured with human cancer cell lines. Brefeldin and Golgi-stop were added before co-culture, and after 3.5 hours the cells were fixed and stained with anti-tumor necrosis factor (TNF)-α and anti-interferon (IFN)-γ antibodies
- The Click-iT® EdU Flow Cytometry Assay Kit (ThermoFisher) was used for cell proliferation detection. In this application, the alkyne is found in the ethynyl moiety of EdU, which is incorporated into DNA during active DNA synthesis, whereas the azide is coupled to Alexa Fluor® 488 dye

RESULTS

FIGURE 3. GDA-201 EXHIBITS HIGH VIABILITY AND HIGH PURITY UP TO 12 MONTHS POST-MANUFACTURING, AND PRESERVES THE ABILITY TO PROLIFERATE POST-THAW



GDA-201 cells were thawed at 37°C at different time points post-freezing.

(**A**) Cell viability was assessed by Cedex HiRes.

(**B**) Percentage of NK cells was assessed by flow cytometry.

Summary of six different GDA-201 batches is presented (mean ± SD). For 12M only one batch is shown.

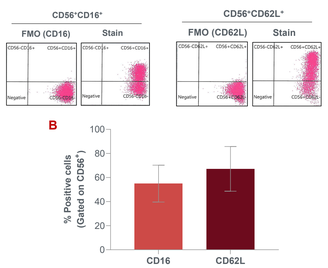
GDA-201 cells were thawed followed by plating in IL-2 containing medium. Cell samples were stained every 2 days for viability, CD56, and EdU using the Click-iT assay kit.

(**C**) Representative histograms from one experiment showing percentage of proliferating NK cells for up to 6 days post-thaw.

(**D**) Percentage of proliferating NK cells. Data in graph summarizes 2 experiments (mean ± SEM).

IL, interleukin; M, month; NK, natural killer; SD, standard deviation; SEM, standard error of the mean; T, time.

FIGURE 4. GDA-201 MAINTAINS EXPRESSION OF CD16 AND CD62L



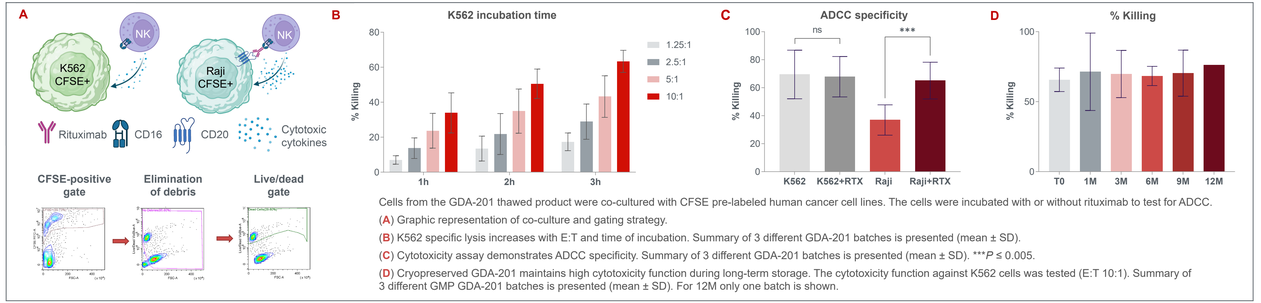
GDA-201 cells were stained immediately after thawing for CD16 and CD62L. CD16 mediates antibody-dependent cellular cytotoxicity and CD62L is a bone marrow homing and retention marker.

(**A**) Representative plots for CD16 (left panel) and CD62L (right panel), each with the relative FMO control.

(**B**) Percentage of CD16 and CD62L (mean ± SD) from different GDA-201 batches. Data summarize seven batches.

FMO, fluorescence minus one; SD, standard deviation; SEM, standard error of the mean.

FIGURE 5. GDA-201 MAINTAINS HIGH CYTOTOXICITY AND ADCC FUNCTION UP TO 12 MONTHS



Cells from the GDA-201 thawed product were co-cultured with CFSE pre-labeled human cancer cell lines. The cells were incubated with or without rituximab to test for ADCC.

(**A**) Graphic representation of co-culture and gating strategy.

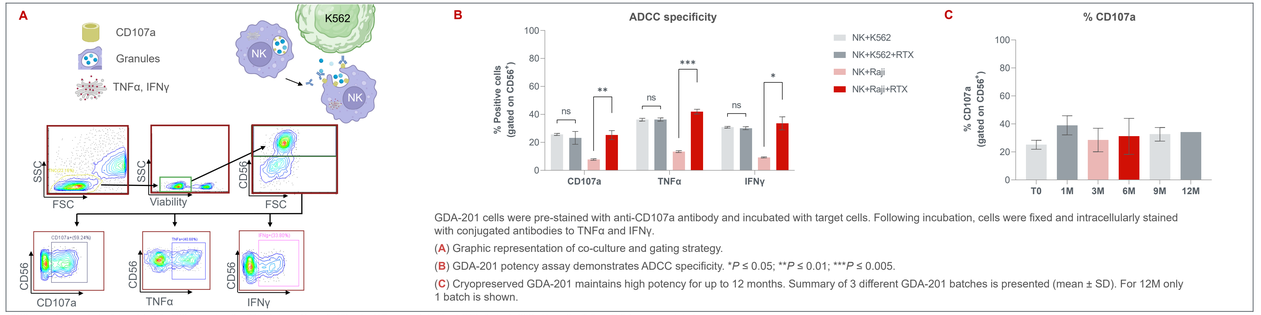
(**B**) K562 specific lysis increases with E:T and time of incubation. Summary of 3 different GDA-201 batches is presented (mean ± SD).

(**C**) Cytotoxicity assay demonstrates ADCC specificity. Summary of 3 different GDA-201 batches is presented (mean ± SD). ***$P \leq 0.005$.

(**D**) Cryopreserved GDA-201 maintains high cytotoxicity function during long-term storage. The cytotoxicity function against K562 cells was tested (E:T 10:1). Summary of 3 different GMP GDA-201 batches is presented (mean ± SD). For 12M only one batch is shown.

ADCC, antibody-dependent cellular cytotoxicity; CFSE, carboxyfluorescein diacetate succinimidyl ester; E:T, effector:target; GMP, Good Manufacturing Practice; M, month; NK, natural killer; ns, not significant; RTX, rituximab; SD, standard deviation; T, time.

FIGURE 6. GDA-201 MAINTAINS HIGH POTENCY: INTRACELLULAR SECRETION OF TNF-α AND IFN-γ AND EXTRACELLULAR DEGRANULATION MARKER CD107A



GDA-201 cells were pre-stained with anti-CD107a antibody and incubated with target cells. Following incubation, cells were fixed and intracellularly stained with conjugated antibodies to TNFα and IFNγ.

(**A**) Graphic representation of co-culture and gating strategy.

(**B**) GDA-201 potency assay demonstrates ADCC specificity. *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.005$.

(**C**) Cryopreserved GDA-201 maintains high potency for up to 12 months. Summary of 3 different GDA-201 batches is presented (mean ± SD). For 12M only 1 batch is shown.

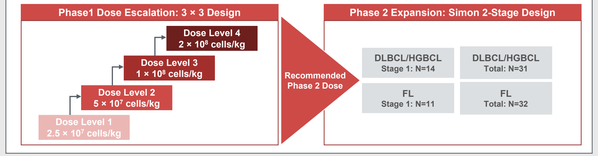
ADCC, antibody-dependent cellular cytotoxicity; FSC, forward scatter; IFN, interferon; M, month; NK, natural killer; ns, not significant; RTX, rituximab; SSC, side scatter; T, time; TNF, tumor necrosis factor.

SUMMARY AND CONCLUSIONS

- A robust and scalable cryopreserved GDA-201 formulation was developed based on NAM technology.
- GDA-201 previous characterization showed that it expresses high levels of CD56, CD16, CD49a, CD62L, low levels of CD57, and low level of immune checkpoints such as LAG3 and CD200R
- GDA-201 are donor-derived, expanded, and enhanced NK cells that can mediate ADCC and have potent antitumor activity
- The safety and efficacy of GDA-201 are being evaluated in an ongoing clinical study in patients with non-Hodgkin lymphoma

FIGURE 7. PHASE 1/2 MULTICENTER STUDY EVALUATING THE SAFETY AND EFFICACY OF GDA-201 IN PATIENTS WITH NON-HODGKIN LYMPHOMA IS ONGOING

Patients with relapsed or refractory B cell follicular lymphoma (FL) or high grade B cell lymphoma (HGBCL)/diffuse large B cell lymphoma (DLBCL) (NCT05296525)



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1. Bachanova V, et al. *Blood* 2019; 134(Suppl 1):777.

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