Cytotoxicity of nicotinamide (NAM)-enhanced natural killer (NK) cells (GDA-201) is based on metabolic modulation as demonstrated by artificial intelligence – assisted analysis of NK cell transcriptome and metabolome

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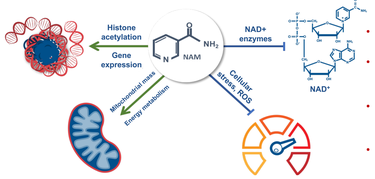
BACKGROUND

- Nicotinamide (NAM), an allosteric inhibitor of nicotinamide adenine dinucleotide (NAD)–dependent enzymes, has been shown to preserve cell function and prevent differentiation in ex vivo cell culture
- GDA-201 is an investigational natural killer (NK) cell immunotherapy derived from allogeneic donors and expanded using interleukin-15 and NAM. In previous preclinical studies, NAM led to increased homing and cytotoxicity, preserved proliferation, and enhanced tumor reduction of NK cells
- In a phase 1 clinical trial, treatment with GDA-201 showed tolerability and clinical responses in patients with refractory non-Hodgkin lymphoma[1]
- While NAM is known to affect cellular metabolism and participate in hundreds of enzymatic reactions as an inhibitor or activator, its mechanism of action and role in GDA-201 cytotoxicity are unknown

NICOTINAMIDE



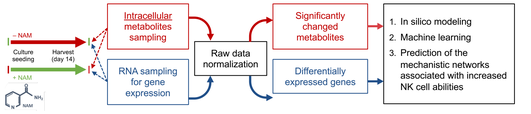
- Plays a key role in **metabolic reprogramming** of cells
- Is a master regulator of **NAD**-related signaling pathways
- Enhances cellular **functionality** and **phenotype**
- Improves **homing** and **retention** to lymphoid tissues

NAD: nicotinamide adenine dinucleotide; NAM: nicotinamide; ROS: reactive oxygen species.

OBJECTIVE

- Identify mechanistic network of interaction between differentially expressed genes and intracellular metabolites associated with enhanced biological functional activity of GDA-201 NK cells

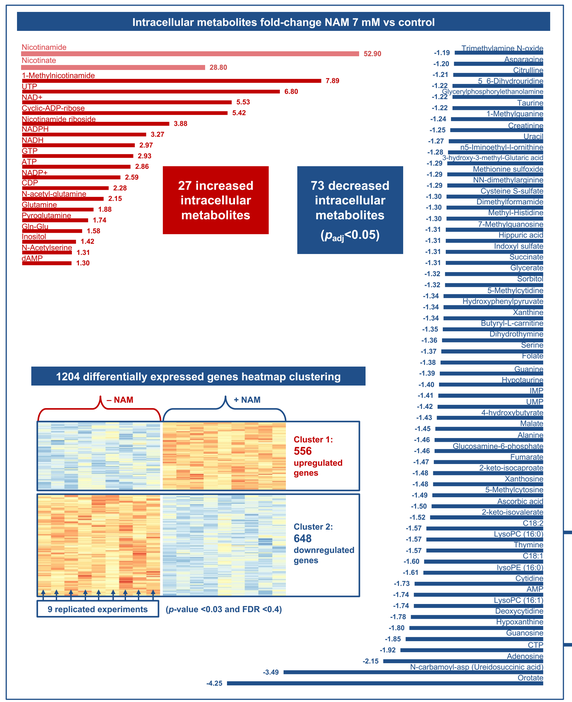
STUDY DESIGN AND METHODS



NAM: nicotinamide; NK: natural killer; RNA: ribonucleic acid.

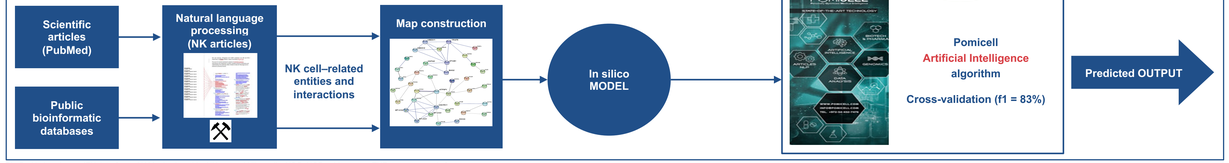
- Apheresis-derived ex vivo–expanded NK cell phenotype, in vitro and in vivo performance were characterized, and transcriptome and metabolome samples were collected
- Next-generation sequencing was performed on Illumina HiSeq 2500 (Cel-SEQ, NGS). Raw sequences were trimmed and filtered using Trim Galore! and carried out using the NeatSeq-Flow platform. Reads were aligned to the human genome using STAR (Ensembl GRCh38.p10). Quality assurance of the process was carried out using FASTQC, MultiQC, and Qualimap. Statistical analysis was carried out using DESeq2 and R SVA/Combat, and Scater packages. Donor-derived batch effect was statistically corrected
- Thermo Q-Exactive Plus mass spectrometer coupled with a Vanquish UHPLC system, using Xbridge BEH Amide column (150 × 2 mm, 2.5 µm particle size; Waters, Milford, MA, USA) was used to quantify the extracted metabolites. All samples were analyzed with a MS1 scan range of m/z 70–1000 with a resolving power of 160,000 at m/z 200. Data analysis was performed using El-Maven and the metabolites identified with an in-house library using authentic standards. Donor-derived batch effect was statistically corrected
- The data from public bioinformatic databases and natural language processed NK cell–related articles were used to create the map of interactions and in silico model for further analysis (Pomicell company)
- Pomicell proprietary machine learning algorithm was used to create a mechanistic network between the differentially expressed gene and significantly changed metabolites
- F1-score cross-validation test was used to filter the most biologically relevant interaction map that could explain the measured in vitro and in vivo results

GDA-201 DISPLAYS DIFFERENTIAL TRANSCRIPTOME AND METABOLOME



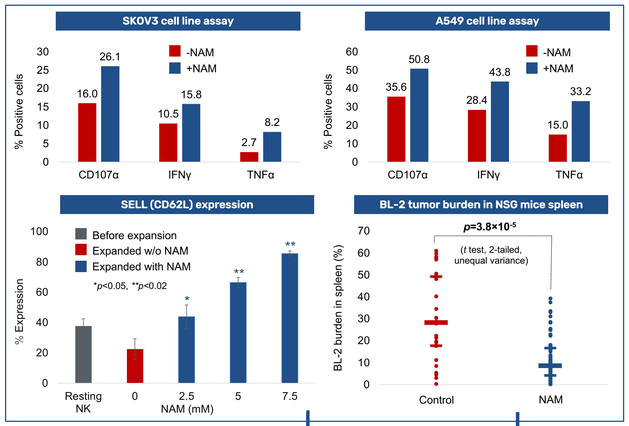
FDR: false detection rate; NAM: nicotinamide.

INTEGRATED IN SILICO MODEL AND ARTIFICIAL INTELLIGENCE ANALYSIS

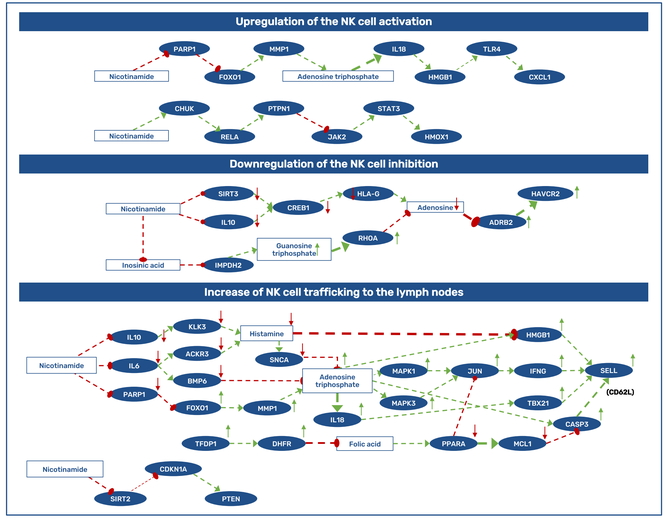


BL: Burkitt lymphoma; IFN: interferon; NAM: nicotinamide; NK: natural killer cells; NSG: NOD scid gamma; TNF: tumor necrosis factor.

THE EFFECT OF NAM ON EX VIVO–EXPANDED NK CELLS (GDA-201)



ARTIFICIAL INTELLIGENCE–PREDICTED MECHANISTIC NETWORK OF INTERACTIONS



NK: natural killer.

SUMMARY

- NAM-mediated reduction of intracellular AMP and inosinic acid levels reduced NK inhibiting ADRB2 signaling
- Upregulated ATP and GTP cytosolic concentrations increase GDA-201 NK cell activation via HMGB1 and HMOX1 activation
- Significant upregulation of CD62L (SELL) membrane expression on GDA-201 NK cells was predicted by multiple different pathways, including TFDP1/DHFR activation, histamine/HMGB1 upregulation, PARP1/FOXO1 activation, and reduced folic acid level PPARa inhibition
- Superior ADCC and cytotoxic GDA-201 activity were followed by an increased expression of a degranulation marker CD107a, and enhanced production of IFNγ and TNFα, that were predicted to be mediated by increased expression of JUN, reduction of intracellular folic acid level, and RELA/PTPN1-mediated upregulation of HMOX1

CONCLUSIONS

- Artificial intelligence–mediated analysis shows GDA-201 NK cells have increased **cytotoxicity**, **ADCC**, **homing**, and **in vivo antitumor** potential

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REFERENCE

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

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