Nicotinamide (NAM) rejuvenates ex vivo–expanded natural killer cells and enhances their tumor killing capacity

Aviad Pato,[1] Orit Berhani-Zipori,[1] Avishay Edri,[1] Julia Rifman,[1] Astar Hailu,[1] Nurit Brycman,[1] Dima Yackoubov,[1] Frank Cichocki,[2] Amnon Peled,[3] Tracey Lodie,[1] Ronit Simantov,[1] Yona Geffen[1]

[1]Gamida-Cell, Jerusalem, Israel, and Boston, MA, USA; [2]Division of Hematology, Oncology and Transplantation, University of Minnesota Medical Center, Minneapolis, MN, USA; [3]School of Medicine-Researchers-Hadassah Medical Center-Gene Therapy, Jerusalem, Israel

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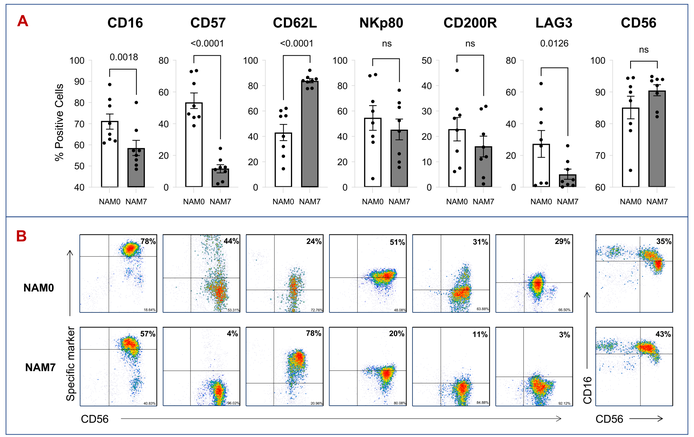
BACKGROUND

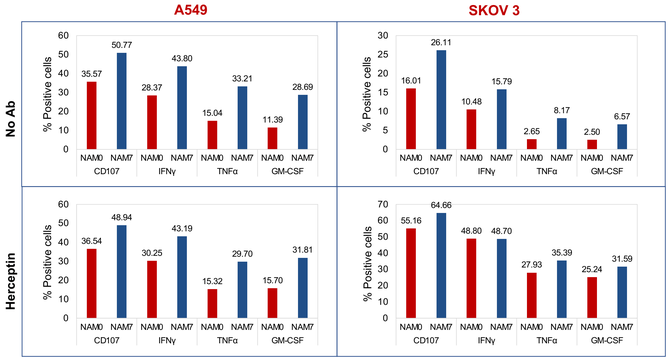
- Adoptive transfer of allogeneic natural killer cells (NKs) is a growing area of innovation in cancer immunotherapy. Peripheral blood NKs are highly functional and cytotoxic; however, they are in their last lineage stage and display elevated markers of exhaustion. Nicotinamide (NAM), an allosteric inhibitor of NAD-dependent enzymes, is a key component in culturing Gamida-Cell NKs (GDA-201). NAM has been shown to preserve cell function and prevent differentiation in ex vivo cultures of NKs and other cells. In this study, we comprehensively characterized the mechanisms underlying the activity of GDA-201 by examining their phenotype, killing capacity, and antitumor activity in both in vitro and in vivo assays. GDA-201 feature a unique phenotype that correlates with NK cell rejuvenation according to classical lineage stages.[1] Furthermore, GDA-201's phenotype is similar to cytokine-induced memory-like (CIML) NKs,[2,3] but remains distinct by downregulating immune checkpoint inhibitors and exhaustion markers, thus suggesting immunotherapeutic advantages. Solid tumors elevate ROS to evade and weaken immune effector cells[4] and, excitingly, GDA-201 showed a strong protective effect against mitochondrial superoxide formation triggered by H_2O_2 oxidative stress. Additionally, when GDA-201 were tested against solid tumor cell lines both in vitro and in vivo they were significantly more potent and cytotoxic as opposed to NKs cultured without NAM. Lastly, clinical responses were already observed in a phase 1 trial of GDA-201 in patients with refractory non-Hodgkin lymphoma.[5] These results strongly suggest the inordinate potential of GDA-201 in becoming a frontline immunotherapy to combat malignant diseases.

GDA-201 DISTINCT PHENOTYPE



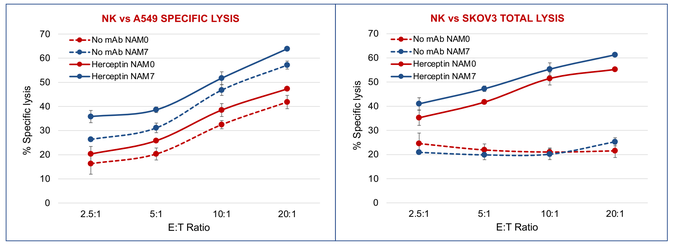
- **GDA-201 Flow Cytometry Phenotype.** NKs were cultured with 7 mM NAM (NAM7) or without NAM (NAM0) **A.** Summary of 8 experiments of NKs with (white bars) and without NAM (gray bars). **B.** Representative dot plots of NAM0 and NAM7 NKs. **GDA-201 phenotype is highly similar to CIML NKs, and correlates with an immature NK lineage, but remains distinct by downregulating immune checkpoint inhibitors and exhaustion markers.**

GDA-201 POTENCY



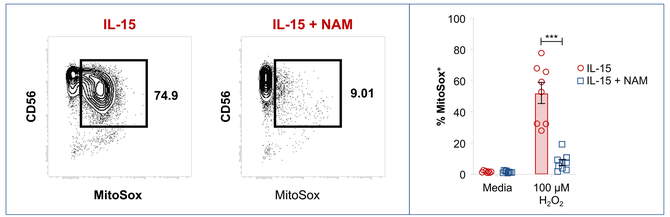
- **Expression levels of activation markers on NAM0 and NAM7 NKs co-cultured with A549 (lung carcinoma) and SKOV3 (ovarian carcinoma) cell lines.** NAM0 and NAM7 NK cells were co-cultured with A549 and SKOV3 cells with and without ADCC-inducing mAb (Herceptin) at an E:T ratio of 1:3 for 5 hours. Cells were then harvested and stained for CD107a, IFNγ, TNFα, and 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. **GDA-201 increases potency via higher expression of proinflammatory cytokines following co-culture with solid tumor cell lines.**

GDA-201 KILLING CAPACITY



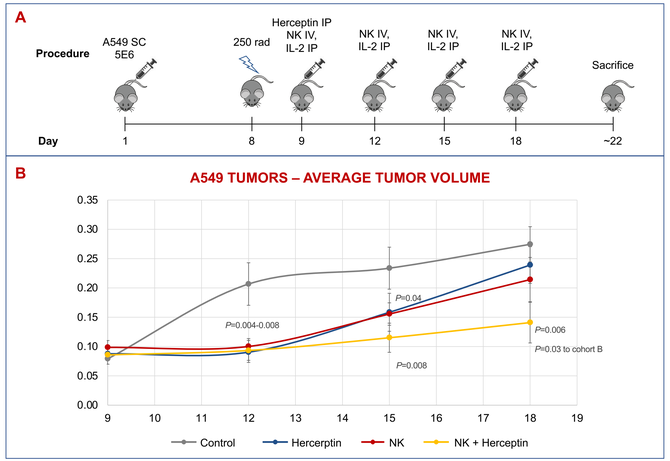
- **Specific Lysis.** NAM0 and NAM7 NKs induced lysis of A549 and SKOV3 target cell lines. NAM0 and NAM7 NK cells were co-cultured with pre-labeled A549 and SKOV3 cells either in the presence or absence of herceptin at E:T ratios varying from 20:1 to 0:1 for 6 hours. Cells were harvested and stained for viability by Helix viability dye. Target cells were CFSE gated, and the percentage of dead cells was determined. Results shown are from 1 representative experiment of 2 performed. **GDA-201 display enhanced cytotoxicity activity against solid tumor cell lines.**

GDA-201 AND OXIDATIVE STRESS



- **MitoSox-Based flow cytometry.** The mitochondria of NAM-expanded NKs produce less lethal superoxides (labeled with a fluorescent marker) when challenged with hydrogen peroxide, thus reducing oxidative stress. **GDA-201 has a significant protective effect against oxidative stress.**

GDA-201 IN A SOLID TUMOR MODEL



- **Antitumoral effect in a solid tumor cancer mouse model. A.** Experimental scheme: 32 NSG mice were divided into 4 groups. Mice were injected subcutaneously with the A549 lung adenocarcinoma solid tumor cell line followed by irritation and submitted to the above-described treatments. **B.** Graph shows average tumor volume measured periodically. **GDA-201 significantly inhibits tumor growth of a solid tumor model in vivo.**

CONCLUSIONS

- NAM-NKs phenotype correlates with CIML NKs and rejuvenation, suggesting a longer life span.
- NAM-NKs are more potent and cytotoxic than NKs cultured without NAM
- NAM-NKs have a strong protective effect against oxidative stress, which favors survivability in the tumor microenvironment
- Preliminary results obtained in a lung adenocarcinoma in vivo model support potent antitumoral activity

Taken together, GDA-201 NKs hold promise in becoming a leading adoptive allogeneic anticancer immunotherapy

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