NICOTINAMIDE REJUVENATES EX-VIVO EXPANDED NATURAL KILLER CELLS AND ENHANCES THEIR TUMOR KILLING CAPACITY

¹Aviad Pato*, ¹Astar Hailu, ¹Nurit Brickman, ¹Dima Yackoubov, ²Frank Cichocki, ³Amnon Peled, ¹Ronit Simantov, ¹Tracey Lodie, ¹Julia Rifman, ¹Yona Geffen, ¹Orit Behani-Zipori, ¹Avishay Edri. ¹Gamida-Cell, Jerusalem, Israel; ²University of Minnesota, Minneapolis, MN, USA; ³Hadassah Medical Center, Jerusalem, Israel

Background Adoptive transfer of Natural Killer cells (NKs) is a growing area of innovation in cancer immunotherapy. Nicotinamide (NAM), an allosteric inhibitor of NAD-dependent enzymes, has been shown to preserve cell function and prevent differentiation in ex vivo culture of NK (NAM-NK) and other cells. Clinical responses were observed in a Phase 1 trial of NAM-NK (GDA-201) in patients with refractory non-Hodgkin lymphoma (Bachanova, et. al., Blood 134:777, 2019). We now characterize the mechanisms underlying the activity of NAM-NK by exploring their phenotype, functionality, and antitumor activity.

Methods CD56 positive cells obtained from healthy donors were cultured for 14 days with IL-15 in the presence or absence of NAM (7 mM). Cell-surface antigens were characterized using flow cytometry (FACS). Mitochondrial reactive oxygen species (ROS) were measured using MitoSox-based FACS. In vitro killing of tumor cells was evaluated by FACS after co-culture of NAM-NK with HER-2-positive A549 (lung adenocarcinoma) or SKOV-3 (ovarian cancer) cells in the presence or absence of trastuzumab; NK cell CD107a and intracellular IFN γ , TNF α , and GM-CSF were also measured by FACS. In vivo activity was determined using a subcutaneous tumor model in NSG mice injected with A549 cells (5x10⁶ sc) and treated with NAM-NK cells (20x10⁶ cells ip/day) on Day 9–12 with IL-2 (300 ug ip), with or without trastuzumab (100ug ip).

Results Characterization of cell surface markers revealed elevated CD56, CD62L, and CD49a and decreased CD16, CD57, and Nkp80 in NAM-NK compared to NK cultured without NAM. CD200R and LAG3 were decreased. NAM-NKs also demonstrated decreased mitochondrial superoxide formation triggered by H₂O₂ oxidative stress. NAM-NK co-cultured with A549 and SKOV-3 cells had increased expression of the degranulation marker CD107a and the proinflammatory cytokines INF- γ , TNF- α , and GM-CSF compared to NK without NAM, and demonstrated increased cytotoxicity in the presence and absence of trastuzumab. Finally, NAM-NK inhibited A549 tumor growth in vivo; tumor growth inhibition was potentiated in the presence of trastuzumab and greater than with trastuzumab alone.

Conclusions These data suggest that NAM rejuvenates cultured NK, generating activated and potent NK cells. NAM-NK (GDA-201) display a distinct phenotype similar to cytokine-induced memory like (CIML) NK, but with downregulation of immune checkpoint inhibitors such as CD200R and LAG3. In addition, NAM-NK are greatly resistant to ROS—a known mechanism of tumor resistance—and highly cytotoxic in in vitro and in vivo assays. The promising potential of GDA-201 as an anticancer immunotherapy is being explored further in clinical trials

Ethics Approval We hereby declare that the collection of the Apheresis units in the three participating institutes (sites) has been done under an approved clinical study that meets the following requirements: 1. Ethics approval has been obtained from the local EC at each of the sites, prior to any study related activities. 2. The working procedures of the EC at the

sites for conduct of clinical studies are in due compliance with local regulations (Israeli Ministry of Health) and provisions of Harmonized International Guidelines for Good Clinical Practice, namely: ICH-GCP3. Sites follow EC conditions & requirements in terms of submissions, notifications, and approval renewals. 4. Participants gave Informed Consent (approved by the EC) before taking part in the study. 5. Informed Consent has been approved by the ECs. The Israeli template of Informed Consent is in used and it includes study specific information (e.g. study goal, design, method, duration, risks, etc.). Name of the Institute Name of the EC/IRB EC Study No. Hadassah Medical Center Helsinki Committee 0483-16-HMORambam Health Care Campus Helsinki Committee 0641-18-RMBIchilov Sourasky Medical Center Tel-Aviv Helsinki Committee 0025-17-TLVAnimal study- All experiments were approved by the Animal Care and Use Committee of the Hebrew University. MD 19-15815-5.

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