

CYTOTOXICITY OF NICOTINAMIDE ENHANCED NATURAL KILLER CELLS GDA201 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 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 IL-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 I clinical trial, treatment with GDA-201 showed tolerability and clinical responses in patients with refractory non-Hodgkin lymphoma (NHL) (Bachanova, et. al., Blood 134:777, 2019). While NAM is known to affect cellular metabolism and participate in 510 enzymatic reactions –in 66 as an inhibitor or activator– its mechanism of action and role in GDA-201 cytotoxicity is unknown.

Methods In order to define the network of intracellular interactions that leads to the GDA-201 phenotype, flow-cytometry, next generation sequencing (NGS), and liquid chromatography–mass spectrometry (LC-MS)-based metabolite quantification were performed on NK cells cultured for 14 days with IL-15 and human serum in the presence or absence of NAM (7 mM). Artificial Intelligence (AI) machine learning analysis was applied by Pomicell in order to analyze the data using the Pomicell databases supporting data extracted from multiple origins including scientific articles organized using natural language processing tools. AI training was done using a combined algorithm designed to blindly explain and predict the transcriptomic and metabolomic (omics) profile.

Results Omics analyses defined 1,204 differentially expressed genes, and 100 significantly modified metabolites in the presence of NAM. An in silico model was created that successfully predicted the experimental data in 83% of the cases. Upregulation of TIM-3 expression in GDA-201 was predicted to be mediated by inhibition of IL-10 and SIRT3, via CREB1/HLA-G signaling and adrenoceptor beta 2 (ADRB2) upregulation. Adenosine metabolite reduction supports this and suggests dopaminergic activation of NK cytotoxicity. Upregulation of CD62L in the presence of NAM was predicted to be mediated by transcription factor Dp-1 (TFDP1) via dihydrofolate reductase (DHFR) activation and intracellular folic acid reduction. Interferon-gamma and CASP3 modulation (via JUN and MCL1, respectively), via PPARa inhibition, support that finding.

Conclusions In conclusion, AI machine learning of transcriptome and metabolome data revealed multiple pleiotropic metabolic pathways modulated by NAM. These data serve to further elucidate the mechanism by which NAM enhances cell function, leading to the observed cytotoxicity and potency of GDA-201.

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-TLV

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