Nicotinamide (NAM) Modulates Transcriptional Signature of Ex Vivo Cultured UCB CD34+ cells (Omidubicel) and Preserves Their Stemness and Engraftment Potential

Dima Yackoubov[1], Yair Steinhardt[1], Dorit Ashengrau[1], Alina Maliutina[1], Adi Bitansky[1], Sherry Cohen[1], Boaz Buhandler[2], Moshe Shahor[2], Nathan Dinowitz[2], Vered Chalifa-Caspi[3], Amnon Peled[4] Julian Adams[1], Tracey Lodie[1] and Tony Peled[1]

[1]Gamida-Cell, Jerusalem, Israel and Boston, MA. [2]PomiCell, Israel. [3]NIBN, National Institute for Biotechnology in Negev Ltd. [4]Goldyne Savad Institute of Gene Therapy, Jerusalem, Israel

Background

- Historic efforts at expansion of umbilical cord blood, (UBC) derived CD34+ hematopoietic stem cells, (HSCs) *ex-vivo* with cytokines yielded large numbers of progenitors for transplantation but impaired their engraftment.
- *Ex-vivo* HSC expansion resulted in accelerated cell proliferation, elevated levels of reactive oxygen and nitrogen species (ROS and RNS), and upregulation of inflammatory signaling leading to cell differentiation and loss of *in-vivo* functionality.[1]
- We used nicotinamide (NAM), an allosteric inhibitor of NAD-dependent enzymes, to create omidubicel, an investigational cell therapy designed to improve the expansion of CD34+ HSCs for bone marrow transplant.[2]
- A Phase 1/2 trial of omidubicel in patients with high-risk hematologic malignancies showed rapid neutrophil engraftment and a favorable immune reconstitution profile in patients compared to historical controls. We hypothesized that NAM treatment maintains the stemness and engraftment potential of omidubicel, which is associated with clinical benefit.[3]

Nicotinamide (NAM)

Figure 1. A master regulator of NAD-related signaling pathways

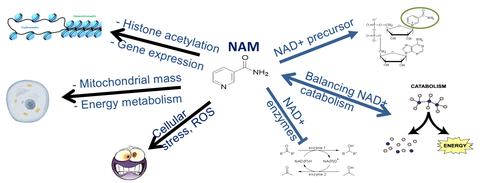
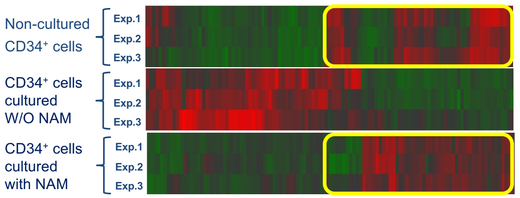


Figure 2. Similarity between CD34+ cells cultured with NAM and non cultured CD34+ cells



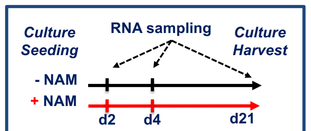
Cluster analysis of differentially expressed genes in CD34+ cells expanded ±NAM for 3-weeks compared to non-cultured CD133+ cells (Affymetrics).

Objective

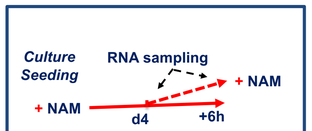
Identify pathways leading to the preservation of engraftment after *ex-vivo* expansion of omidubicel with NAM compared to CD34+ cells grown in the absence of NAM.

Study Design

DATA SET "A"



DATA SET "B"

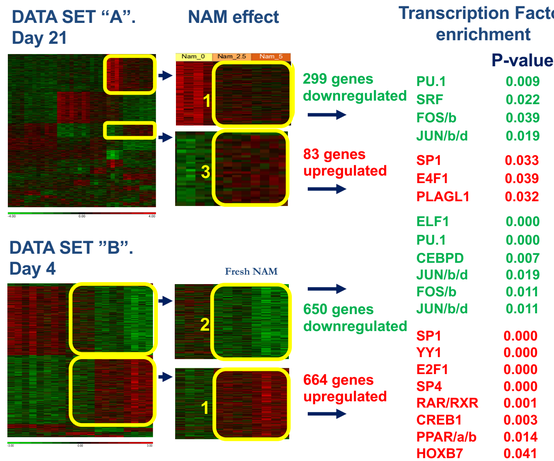


- Next Generation Sequencing (Cel-SEQ, NGS) was performed on HiSeq 2500, rapid v2, Illumina.
- Gene annotation was done based on Human genome GRCh38.p12 version Ensembl/BioMart.
- Total 3,816 Differentially Expressed (DE) genes was defined based on absolute linear fold change of 1.3 and unadjusted p-value <0.01.
- DE-Genes were clustered to "CliCK"-Heatmap. 17 and 5 Clusters were defined in Dataset "A" and "B" respectively.

- **Transcription Factor (TF) enrichment analysis** was done using FunRich software (http://funrich.org/).
- **GO - Biological Process Enrichment** was performed (http://geneontology.org/).
- **INGENUITY (IPA) software was used for Pathway Analysis** (Qiagen).

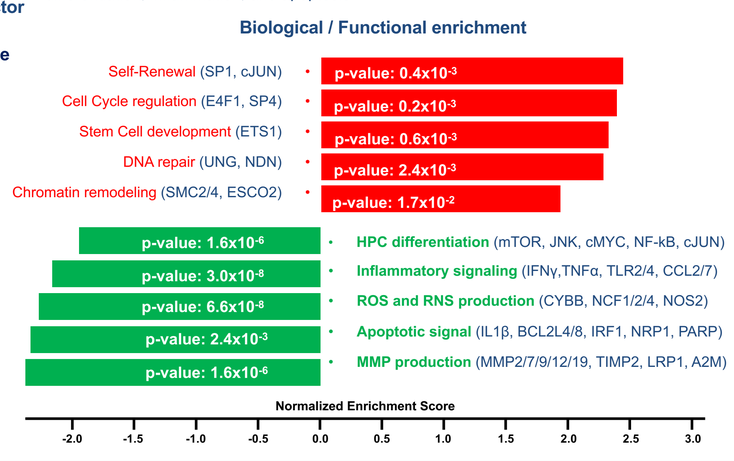
Results

Figure 3. Upregulation and downregulation of families of transcription factors in CD34+ cells expanded with NAM



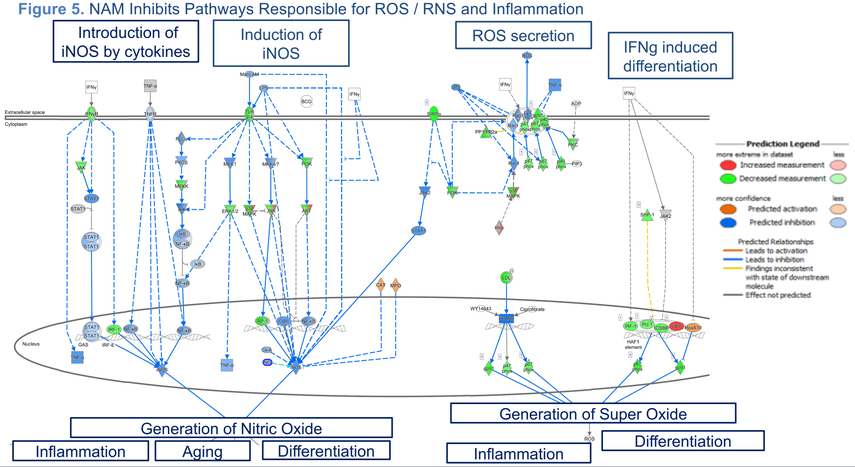
Transcription Factor enrichment	P-value
PU.1	0.009
SRF	0.022
FOS/b	0.039
JUN/b/d	0.019
SP1	0.033
E4F1	0.039
PLAGL1	0.032
ELF1	0.000
PU.1	0.000
CEBPD	0.007
JUN/b/d	0.019
FOS/b	0.011
JUN/b/d	0.011
SP1	0.000
YY1	0.000
E2F1	0.000
SP4	0.000
RAR/RXR	0.001
CREB1	0.003
PPAR/a/b	0.014
HOXB7	0.041

Figure 4. Enrichment analysis showing NAM upregulates transcription factors responsible for stem cell renewal and DNA repair while down-regulating transcription factors that activate cell differentiation, inflammation, and apoptosis



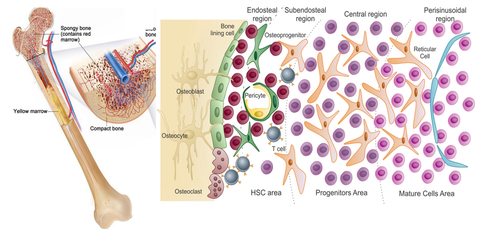
Pathway Inhibition

Figure 5. NAM Inhibits Pathways Responsible for ROS / RNS and Inflammation

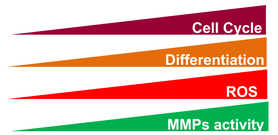


Bone Marrow Niche

Figure 6. Bone marrow niche preserves HSC pool



Quiescent environment
High ROS scavenging
Very low Oxygen (1%)
Dense Extra Cellular Matrix



Conclusions

NAM platform is unique in mimicking the bone marrow niche during *ex vivo* expansion of HSCs.

NAM attenuates genes responsible for:

- Reactive oxygen and nitrogen species production.
- Inflammatory and apoptotic signaling.
- HSC differentiation and maturation.
- Secretion of matrix metalloproteinases (MMP's).

Our gene expression data:

- Reinforce the mechanism of action underlying our NAM technology platform.
- Provide additional insights into the pathways leading to clinically significant engraftment in patients.

The NAM platform has also been used for *ex vivo* expansion of other immune cells (GDA-201 ASH 2019 Abstract #777). Poster #3718.

References

1. Reactive Oxygen Species Regulate Hematopoietic Stem Cell Self-Renewal, Migration and Development, As Well As Their Bone Marrow Microenvironment. Aya Ludin, Shiri Gur-Cohen, Karin Golan, Kerstin B. Kaufmann, Tomer Itkin, Chiara Medaglia, Xin-Jiang Lu, Guy Ledergor, Orit Kollet, Tsvee Lapidot. ANTIOXIDANTS & REDOX SIGNALING. Volume 21, Number 11, 2014. DOI: 10.1089/ars.2014.5941.
2. Nicotinamide, a SIRT1 inhibitor, inhibits differentiation and facilitates expansion of hematopoietic progenitor cells with enhanced bone marrow homing and engraftment. Tony Peled , Hadas Shoham, Dorit Aschengrau , Dima Yackoubov , Gabi Frei , Noga Rosenheimer G , Batya Lerrer , Haim Y. Cohen , Arnon Nagler , Eitan Fibach , and Amnon Peled. Experimental Hematology 2012;40:342–355.
3. Phase I/II Study of Stem-Cell Transplantation Using a Single Cord Blood Unit Expanded Ex Vivo With Nicotinamide. Horwitz M.E., et. al., J Clin Oncol. 2019 Feb 10;37(5):367-374.