Enhanced *In Vivo* Persistence and Proliferation of NK Cells Expanded in Culture with the Small Molecule Nicotinamide: Development of a Clinical-applicable Method for NK Expansion

Tony Peled¹, Guy Brachya¹, Nurit Persi¹, Chana Lador¹, Esti Olesinski¹, Efrat Landau¹, Einat Galamidi¹, Amnon Peled², Jeffrey S. Miller³, Veronika Bachanova³

¹Gamida-Cell, Jerusalem, Israel
²Goldyne Savad Institute of Gene Therapy, Jerusalem, Israel
³Department of Medicine University of Minnesota, Minneapolis, USA

ASH 2017 - Atlanta, Georgia
NK Cell-based Immunotherapy

NK cells are promising for adaptive immune therapy of cancer
- No antigen presentation required
- HLA-matching independent
- Low risk of inducing GvHD
- Synergy with antibodies
- Immune system recruitment

Expansion is required to obtain clinically meaningful doses

Limited functionality of adoptively transferred NK cells:
- Impaired migration
- Short in-patient persistence
- Limited in vivo proliferation

Limited functionality can be attributed to metabolic and stress response induced by extensive activation and expansion in ex vivo cultures

We found Nicotinamide (NAM) modulates characteristics and functions of cells expanded in ex vivo cultures
Epigenetic Regulation by Nicotinamide (NAM)

A master regulator of NAD-related signaling pathways

- In *ex vivo* expansion cultures
- Decrease loss of function in culture expanded HSC
- Overcomes pluripotency deficits and reprogramming barriers in hiPSCs

---

1 Exp Hematol. 2012:40
2 Stem Cells 2013:31
NAM Effect on *Ex Vivo* Expanded NK Cells

Expansion Process

- Leukapheresis
- **Day 0**
  - CD3 depletion
  - **At seeding** → NK cells ~ 30%

IL-15 + human serum + Nicotinamide (NAM)

- One step cell selection
- No “artificial” presenting cells

- Gene expression
- *In vivo* migration and persistence
- *In vivo* proliferation
- Cytokine secretion
- Cytotoxicity

After 14 days in culture:
- NK cells > 98%
- T cells < 0.5%
Gene Chip Analysis of NK Cells Cultured ± NAM

226 differentially expressed genes

Enriched gene annotations (GO)
- Cell cycle
  - cell cycle process
  - mitosis
  - cytoskeletal part
  - microtubule
  - anti-apoptosis
- Metabolism
  - purine ribonucleotide binding
  - adenyl ribonucleotide binding
  - ATP binding
  - transferase activity, transferring
- Immune response
  - immune response
  - leukocyte activation
  - innate immune response

Fold difference vs. P-value

% of modified genes
NAM Reduced the Expression of Immune Checkpoint Receptors
Receptors Involved in Tumor Escape (Immuno-evasion)

Ligation of CD200 / CD200R or PD-L1 / PD1, suppresses NK cell function and inhibits patient anti-tumor response
NAM Increased CD62L (L-selectin) Expression in NK Expansion Cultures

CD62L (L-Selectin) in NK cells was found to affect: ¹

- self-renewal capacity
- trafficking to lymphoid organs
- cytokine responsiveness and secretion

¹Blood. 2010 Aug 26
NAM Leads to Longer NK Persistence in NSG Mice

IL-2 and IL-15 injected every other day following NK infusion

Bone Marrow

- 0mM NAM
- 5mM NAM

* = $P < 0.05$

Spleen

** = $P < 0.01$

% NK cells

Day 4

Day 8

% NK cells

Day 4

Day 8

0

10

20

30

40

50

60

70

80

90

100
NAM Increased \textit{In Vivo} Proliferation of Infused NK

Similar results were obtained with CFSE labeling.
Anti-tumor Activity of NK Cells

- Up-regulation of death receptors
- Increased secretion of inflammatory cytokines: IFN-γ and TNF-α

From NK/K562 co-cultures

<table>
<thead>
<tr>
<th></th>
<th>IFNγ</th>
<th>TNFα</th>
<th>Fas-L</th>
</tr>
</thead>
<tbody>
<tr>
<td>0mM NAM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5mM NAM</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Cytotoxic Potential of NAM-NK

**In-vitro**

- NSG mice were infused with NAM-NK, IL-2+IL-15
- Following 5 days NK were purified from spleens
- And assayed for cytotoxicity against K562

**In-vivo**

In vivo preservation of cytotoxic potential

- NK cells : Target cells ratio

**K562 - CML**

<table>
<thead>
<tr>
<th>NK cells : Target cells ratio</th>
<th>NAM 0mM</th>
<th>NAM 5mM</th>
<th>* = P&lt;0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>5:1</td>
<td></td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>5:2</td>
<td></td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>1:1</td>
<td></td>
<td>*</td>
<td></td>
</tr>
</tbody>
</table>

**H460 – LCLC carcinoma**

<table>
<thead>
<tr>
<th>NK cells : Target cells ratio</th>
<th>NAM 0mM</th>
<th>NAM 5mM</th>
<th>* = P&lt;0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>5:1</td>
<td></td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>1:1</td>
<td></td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>1:2</td>
<td></td>
<td>*</td>
<td></td>
</tr>
</tbody>
</table>

Multiple myeloma mouse model

**Survival**

- Bones Marrow burden

- Mock infusion vs NAM-NK

- % of lysis

- % of tumor cells

- * = P<0.05
NAM-NK ADCC
Antibody-Dependent Cellular Cytotoxicity

NK cells can be activated by antibody bound targets, through their Fc receptors (e.g. CD16). They will then lyse the target cells and recruit immune cells through secretion of cytokines (e.g. IFNγ) without priming.

Rituximab enhanced lysis of lymphoma by NAM-NK

Gated on CD56+

CD16+ 77±3%

Cytotoxic activity of NAM-NK cells towards B-cell lymphoma cell line

* = P<0.05
Development of NAM-NK Cell Product
Steps in Development of NAM-NK Product

**Optimization**
- CD3 depletion vs. CD56 selection
- NAM concentration
- IL-2 or IL-15 for expansion: IL-15 was chosen due to slightly lower T and B cells contamination following expansion

**Up scaling & Additional optimizations**
- GMP-grade reagents
- Seeding concentration
- Feeding regime
- Harvest
- Automation

**Final product**
- Characterization
- Functionality
Advantages of CD3 Depletion Over CD56 Selection
- Comparable expansion following CD3/CD19 depletion
- Myeloid Cells are supporting NK expansion

**NK expansion**

CD3 depleted cultures

- NK cells
- B-cells
- DC
- Granulocytes
- Monocytes
- NKT
- T-cells

Cell composition

Days in culture

NK fold expansion

CD3 or CD3/CD19 depletion

CD3 depletion or CD56 selection

CD3 depletion
CD3/CD19 depletion
CD56+ selection
Feeding Regime

Multiple culture feedings leads to higher NAM-NK expansion at the cost of functionality

**Expansion**

- **no feeding**
- **3 feedings**

![Graph showing NK fold expansion over days with different feeding regimes](image)

**In vivo retention**

- **Bone Marrow**
- **Spleen**

![Bar chart showing % of NK cells in Bone Marrow and Spleen with different feeding regimes](image)

**CD62L expression**

- **no feeding**
- **3 feedings**

![Bar chart showing CD62L expression with different feeding regimes](image)

**Statistical significance**

- **=Pv<0.01**
- *****=Pv<0.001**
Harvesting

One feeding during the entire expansion duration

- Percent of NK at harvest: >98%
- Percent of T cells at harvest: <0.5%

Average final product size $3.8 \times 10^{10}$
Median final product size $3.5 \times 10^{10}$
GMP Grade Closed Manufacturing of the NAM-NK Cell Product

**Day 0**: seeding of CD3 depleted, apheresis collected cells, in GREX100MCS flasks

**Day 8-10**: Feeding  
Fresh medium is added with GatheRex

**Day 14-16**: Harvesting by disposal of top medium. Transfer cells to a collection bag. After washing, cells are ready for infusion
NAM-NK Summary

Quality over extensive expansion:
- Improved *in vivo* migration, survival and proliferation
- Lower PD-1 and CD200R expression
- Higher inflammatory cytokines secretion
- Highly cytotoxic
- High CD16 expression allowing ADCC

Simple and closed manufacturing system:
- No genetically modified APC
- Only one cell feeding step
Phase I Study of NAM-NK

NCT03019666, recruiting
University of Minnesota Cancer Center, Minneapolis (UMN)

Study objectives
- Determine MTD (20-200x10^6 cells/Kg)
- Disease progression

Study design
- 24 patients with multiple myeloma or non-Hodgkin lymphoma
- Mismatched related donor derived NK cells
- In combination with monoclonal antibody (Elotuzumab or Rituximab)
Acknowledgments

Gamida-Cell Ltd.
Cell Therapy Technologies
Jerusalem, Israel
Tony Peled
Nurit Persi
Chana Landor
Esti Olesinski
Efrat Landau
Einat Galamidi

Hadassah Medical Center
Goldyne Savad Institute of Gene Therapy
Jerusalem, Israel
Amnon Peled
Devorah Olam
Lola Weiss
Katia Beider

Molecular & Cellular Therapeutics (MCT)
University of Minnesota
Minneapolis
Veronika Bachanova
Jeffrey S. Miller
David McKenna
Diane Kadidlo
Darin Sumstad