

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

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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 :

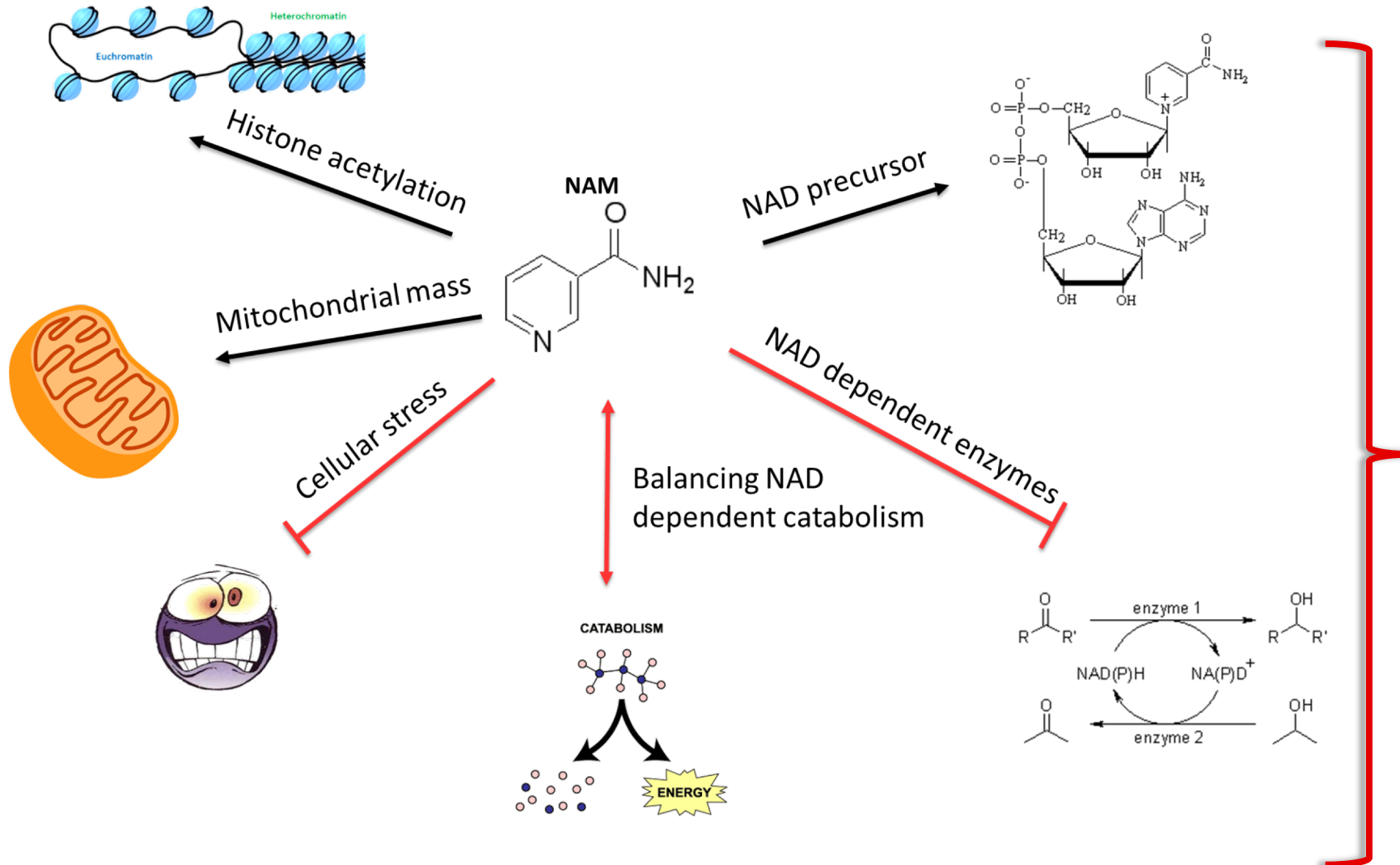
- Impaired migration
- Short in-patient persistence
- Limited *in vivo* proliferation

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 HSC¹
- Overcomes pluripotency deficits and reprogramming barriers in hiPSCs²

¹ *Exp Hematol.*2012:40

² *Stem Cells* 2013:31

NAM Effect on *Ex Vivo* Expanded NK Cells

Expansion Process

Leukapheresis



Day 0

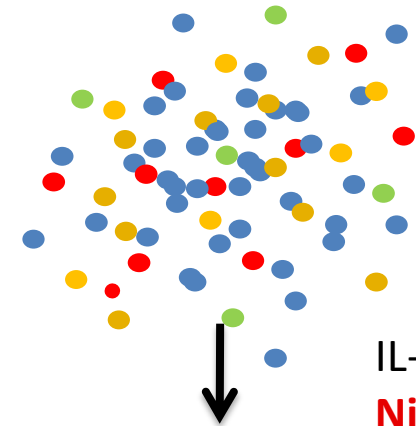
CD3 depletion

At seeding

→ NK cells ~ 30%

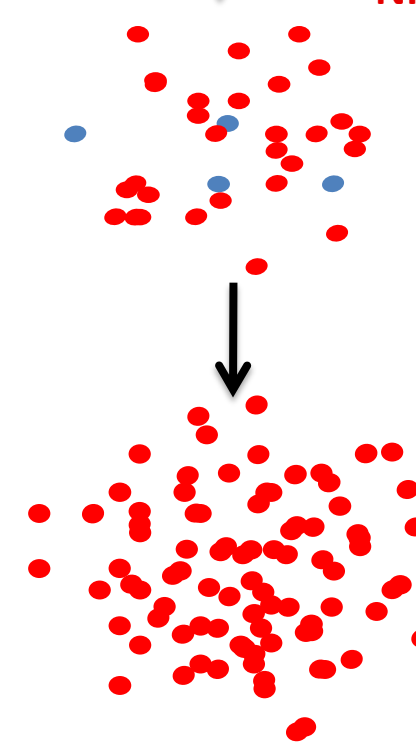


- Gene expression
- *In vivo* migration and persistence
- *In vivo* proliferation
- Cytokine secretion
- Cytotoxicity



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

IL-15 + human serum +
Nicotinamide (NAM)



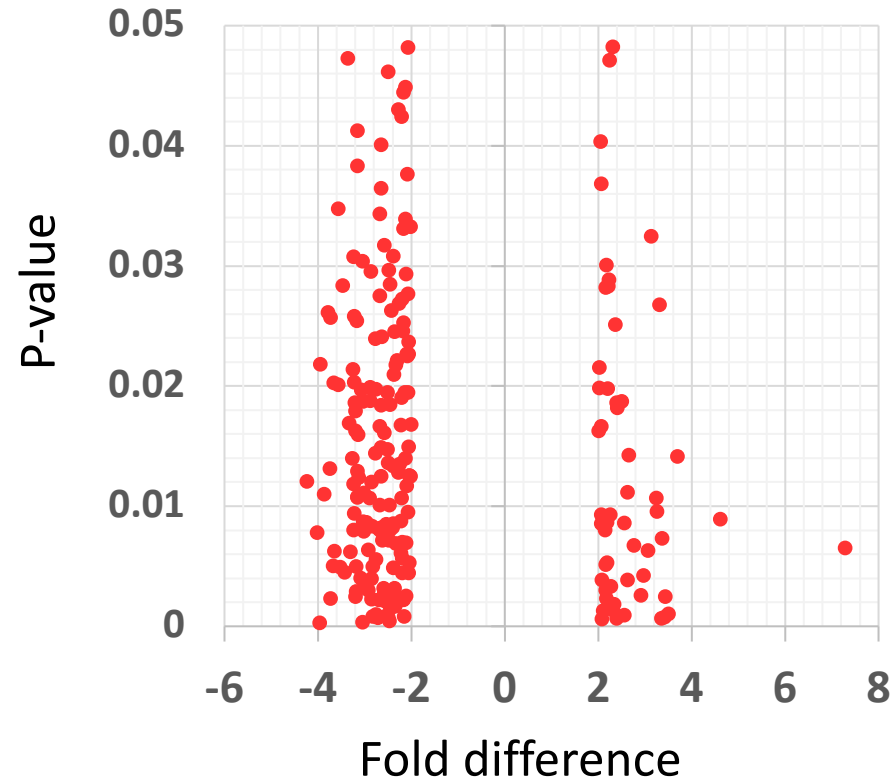
After 14 days in culture:

NK cells > 98%

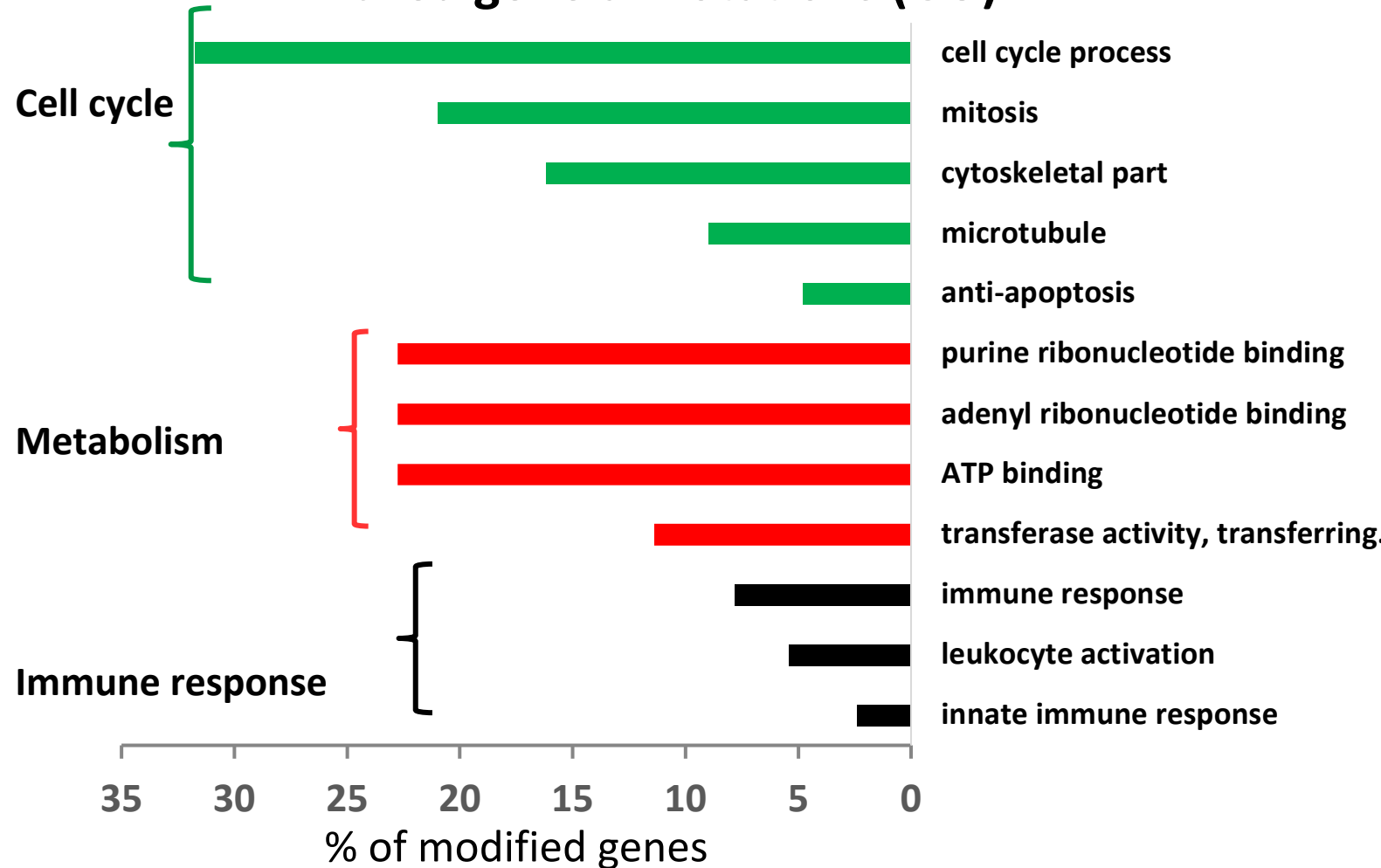
T cells < 0.5%

Gene Chip Analysis of NK Cells Cultured \pm NAM

226 differentially expressed genes



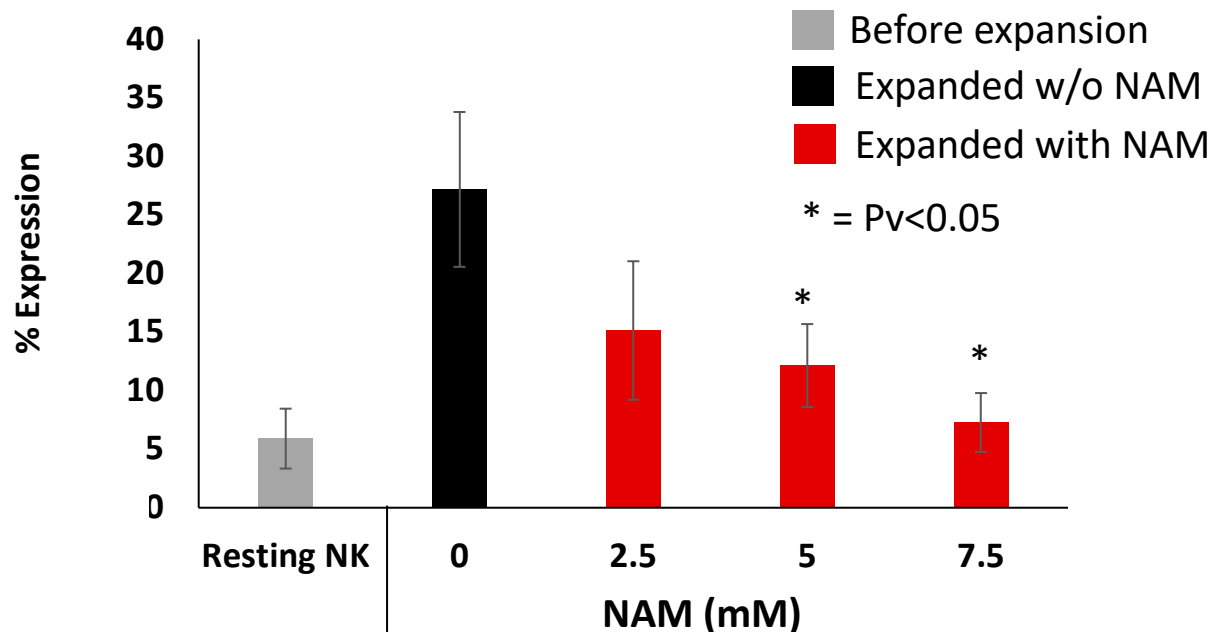
Enriched gene annotations (GO)



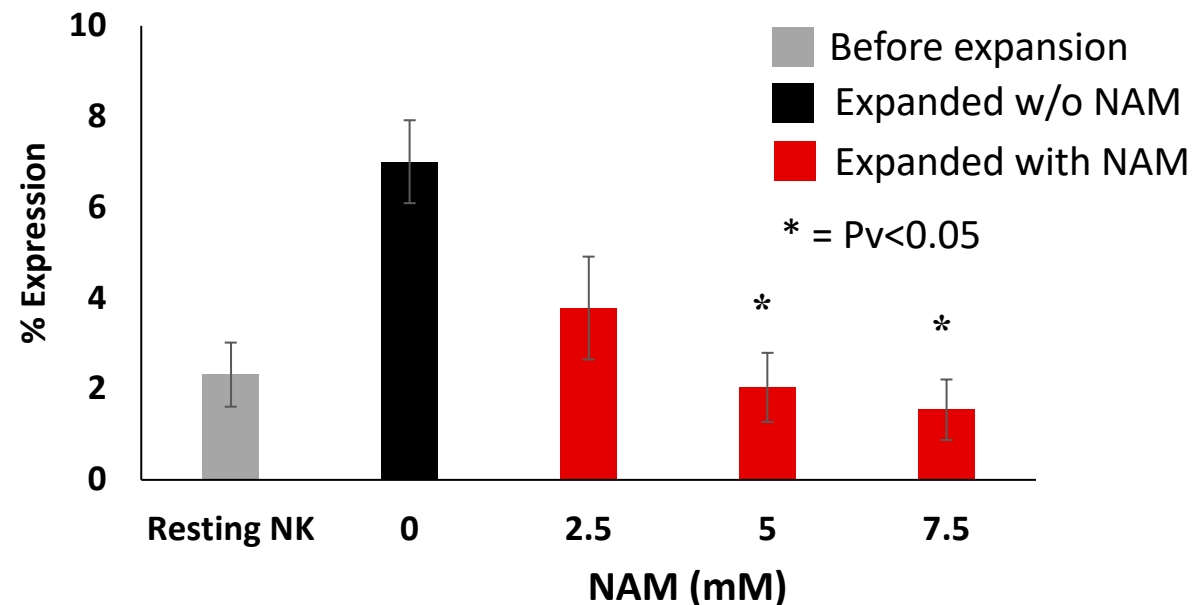
NAM Reduced the Expression of Immune Checkpoint Receptors

Receptors Involved in Tumor Escape (Immuno-evasion)

CD200R

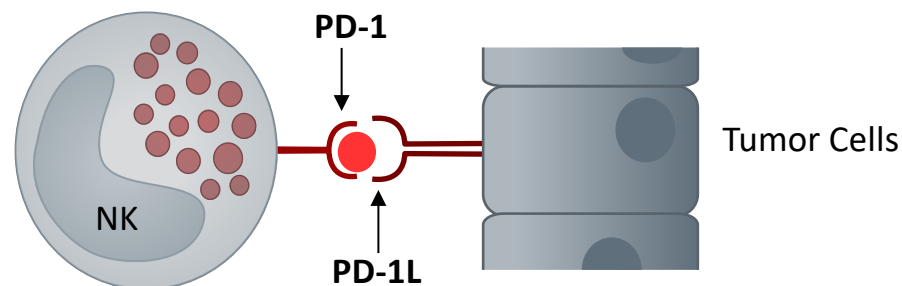


PD-1 (Programmed death receptor1)

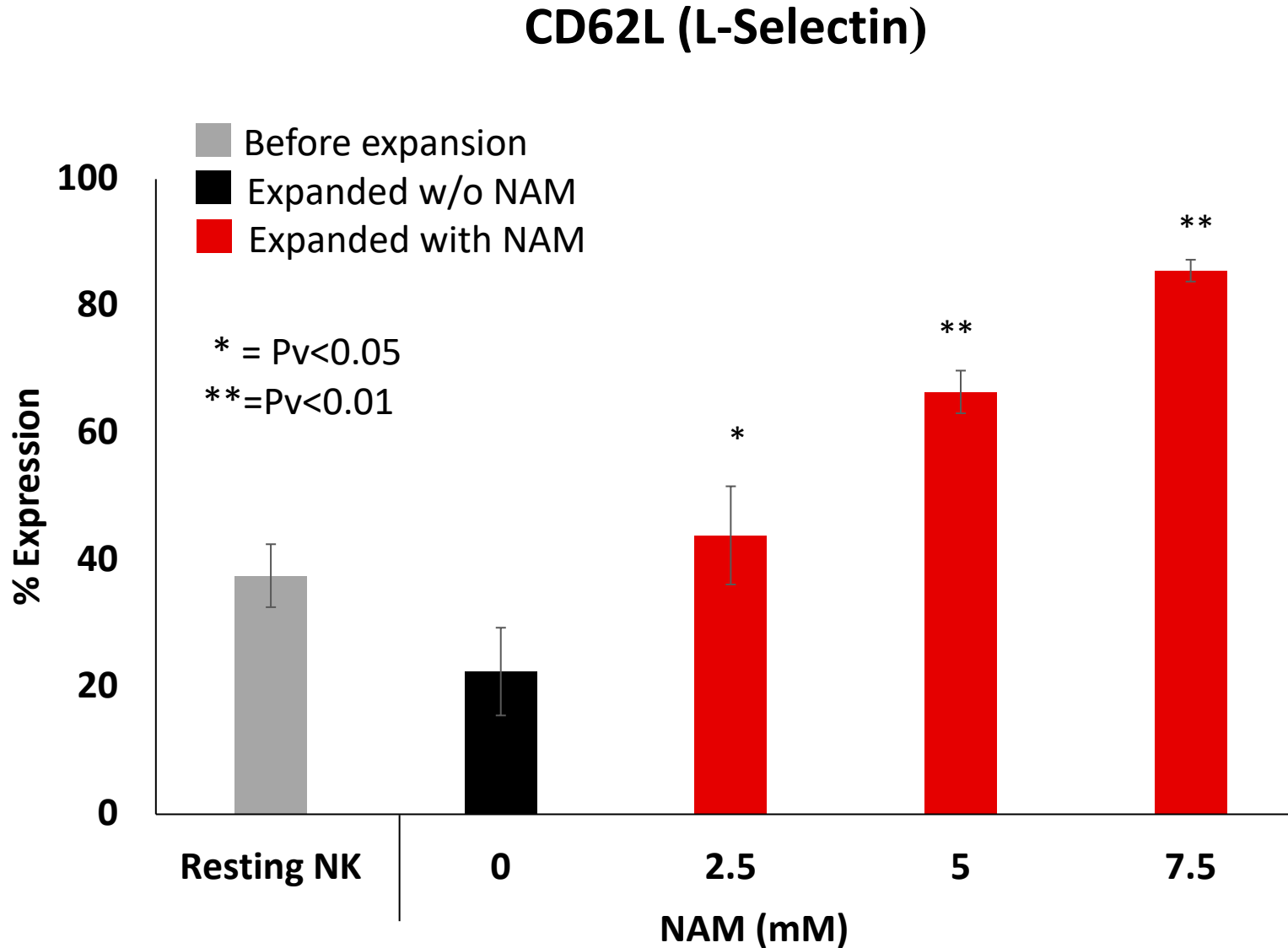


Ligation of CD200 / CD200R or PD-L1 / PD1, suppresses NK cell function and inhibits patient anti-tumor response

Immunosuppression



NAM Increased CD62L (L-selectin) Expression in NK Expansion Cultures

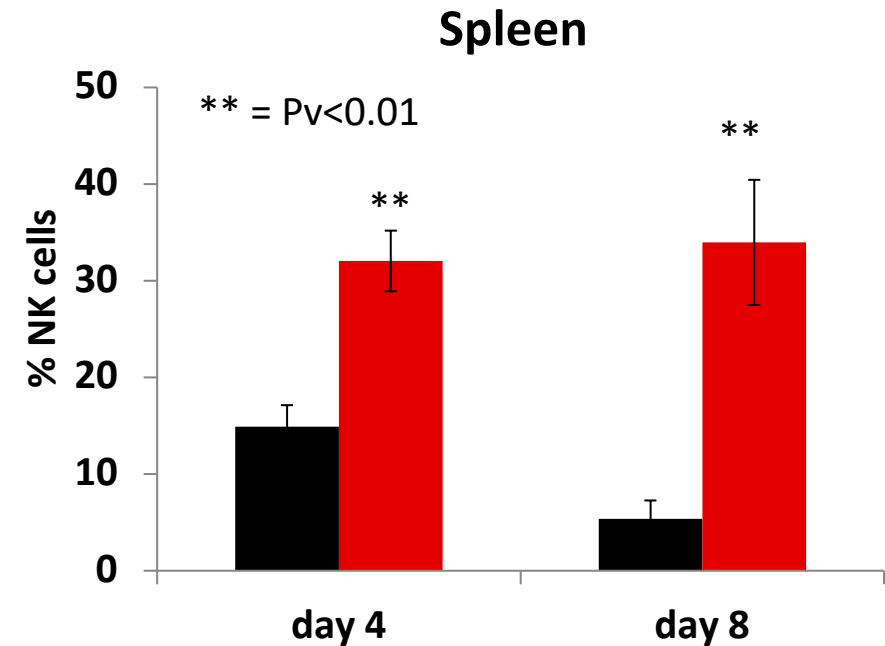
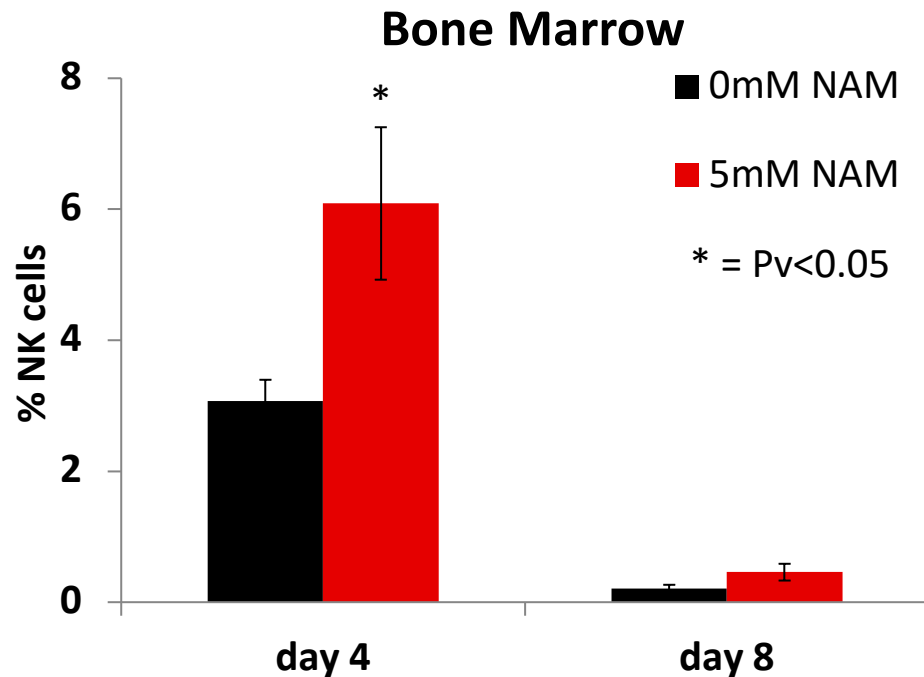
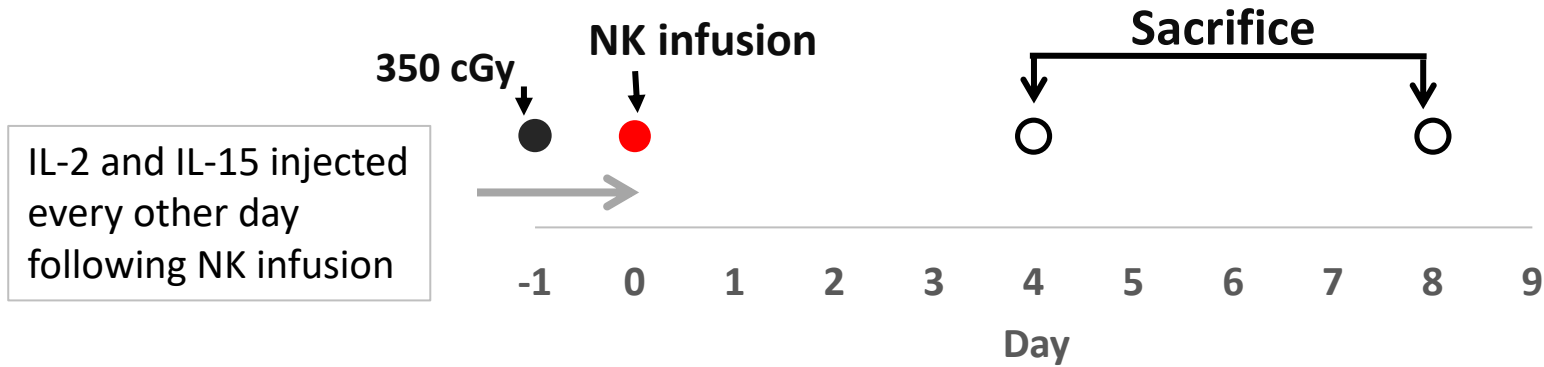


CD62L (L-Selectin) in NK cells was found to affect: ¹

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

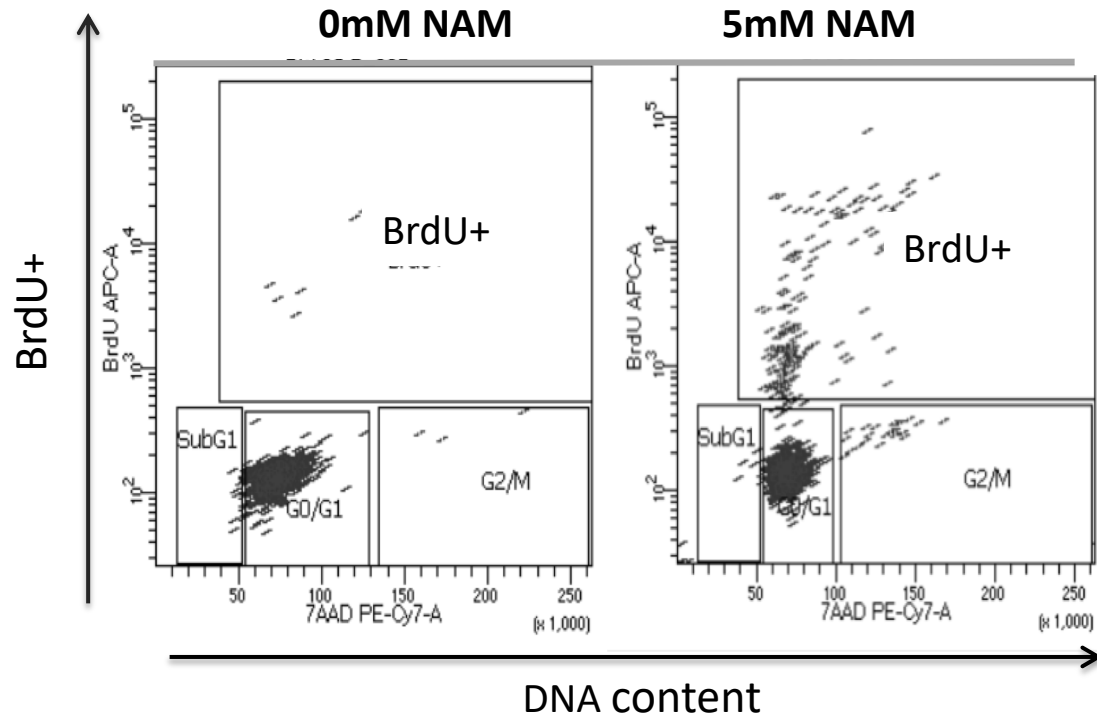
¹Blood. 2010 Aug 26

NAM Leads to Longer NK Persistence in NSG Mice



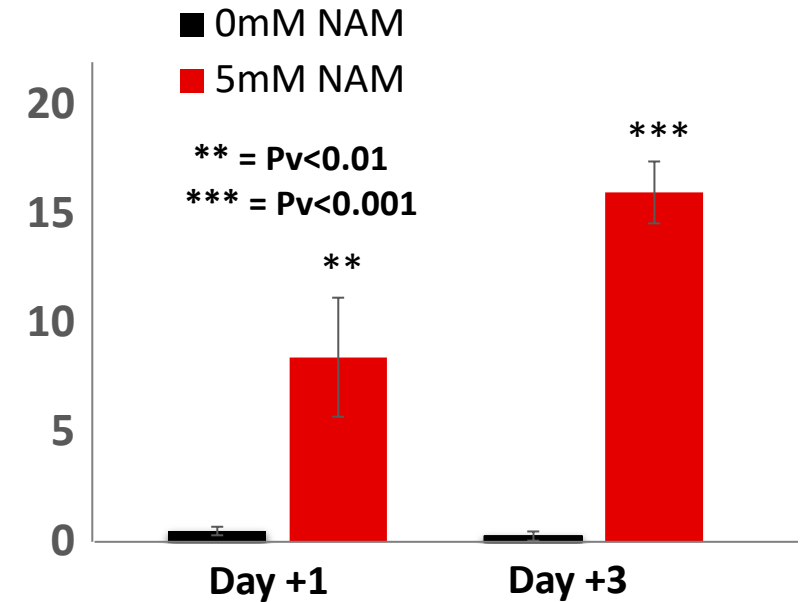
NAM Increased *In Vivo* Proliferation of Infused NK

FACS analysis on gated CD56⁺ cells



%BrdU+ NK cells

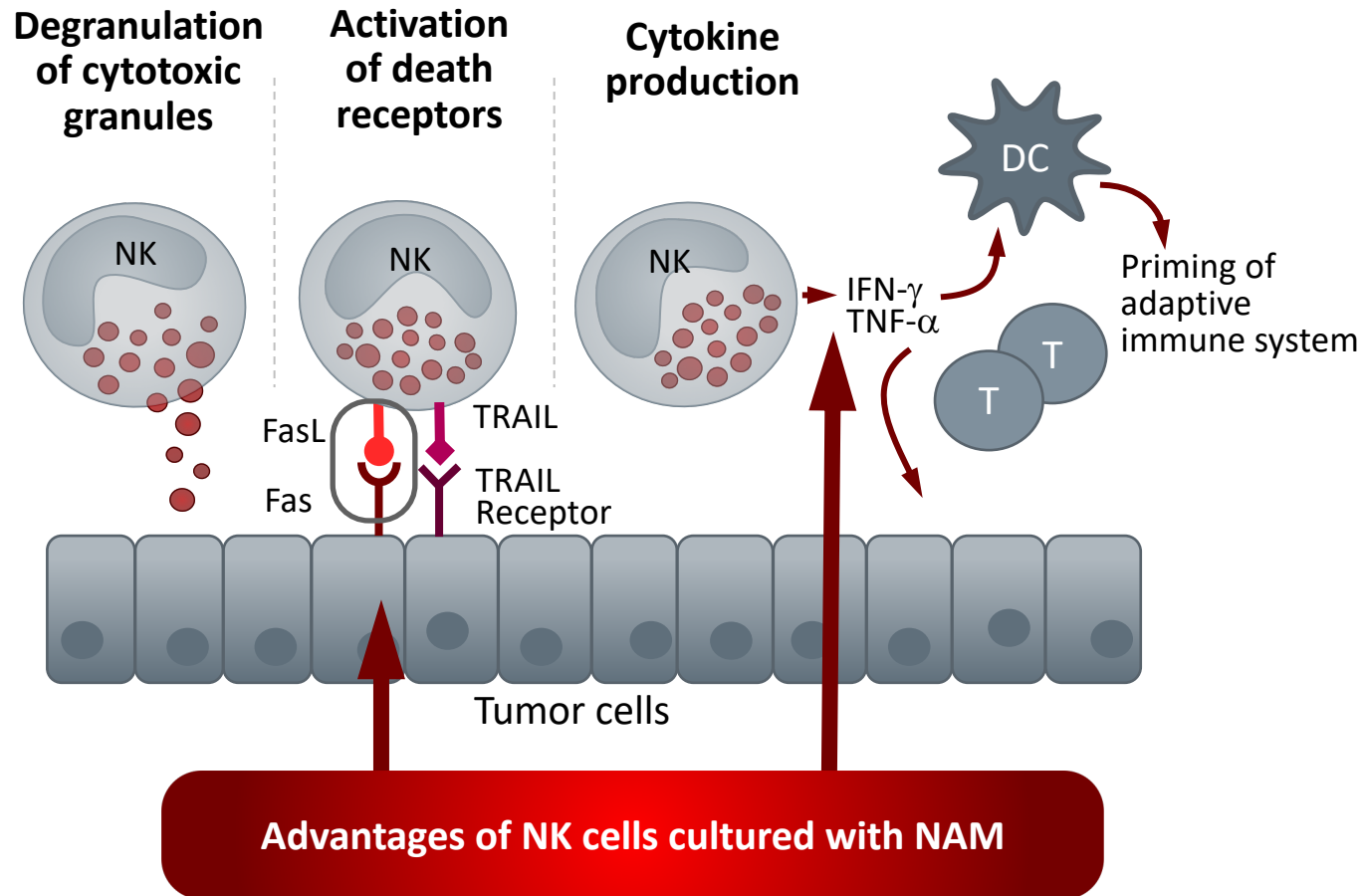
NK *in vivo* proliferation



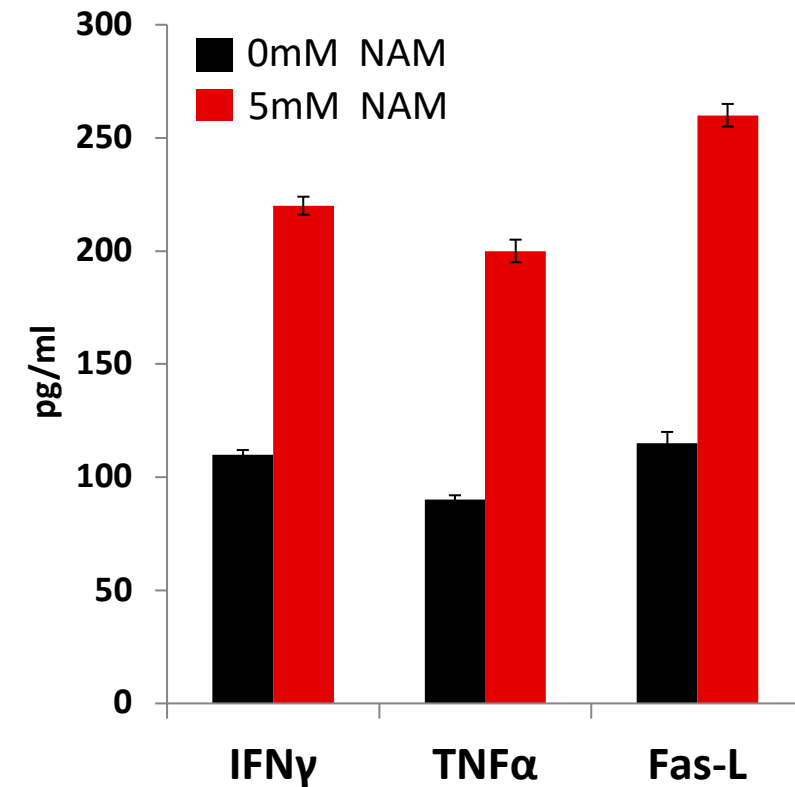
Similar results were obtained with CFSE labeling

Anti-tumor Activity of NK Cells

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



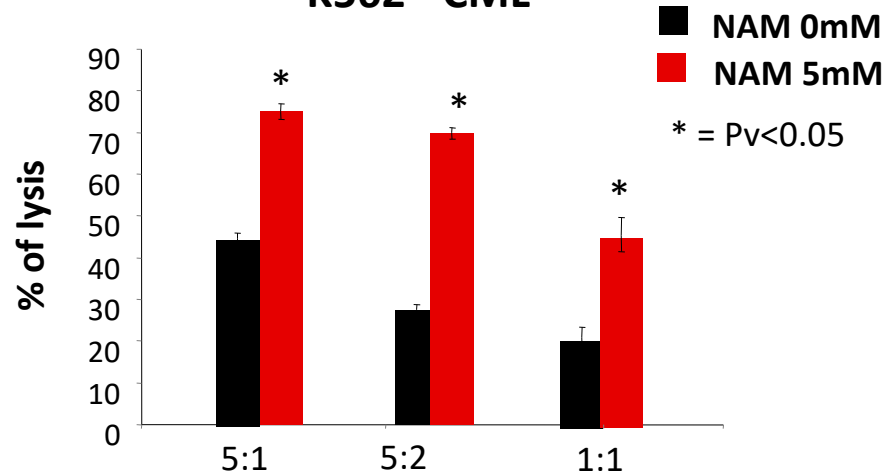
From NK/K562 co-cultures



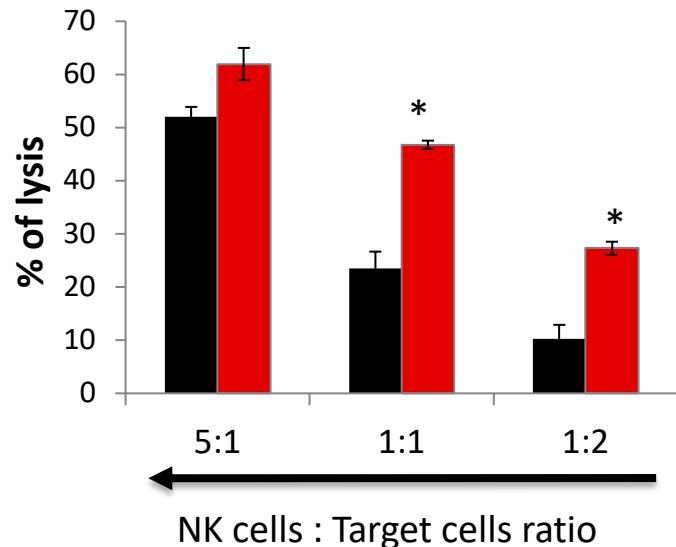
Cytotoxic Potential of NAM-NK

In-vitro

K562 - CML



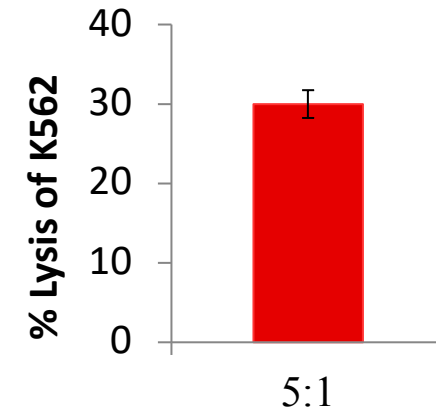
H460 – LCLC carcinoma



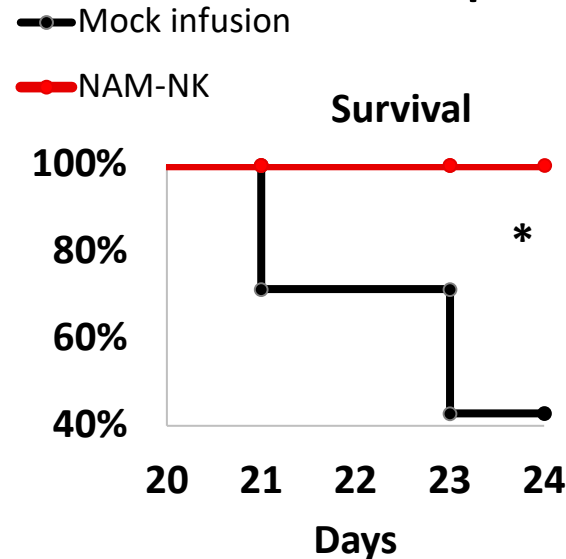
In-vivo

In vivo preservation of cytotoxic potential

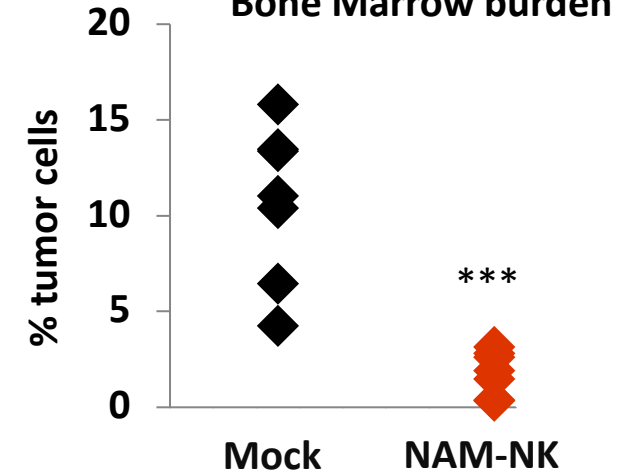
- NSG mice were infused with NAM-NK, IL-2+IL-15
- Following **5 days** NK were purified from spleens
- And assayed for cytotoxicity against K562



Multiple myeloma mouse model

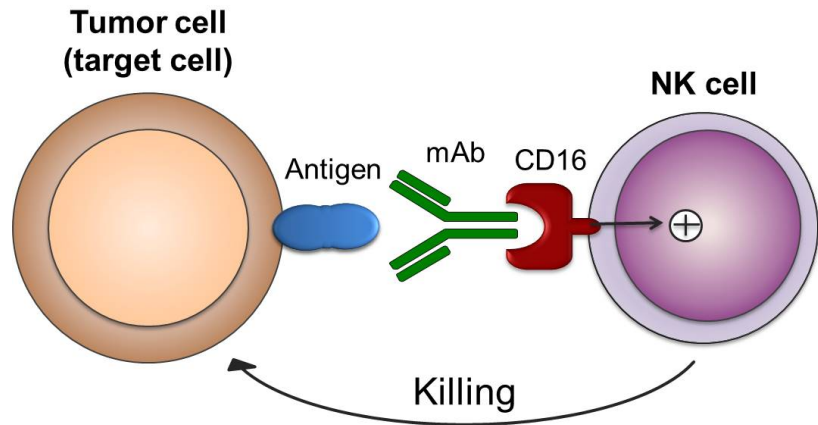


Bone Marrow burden



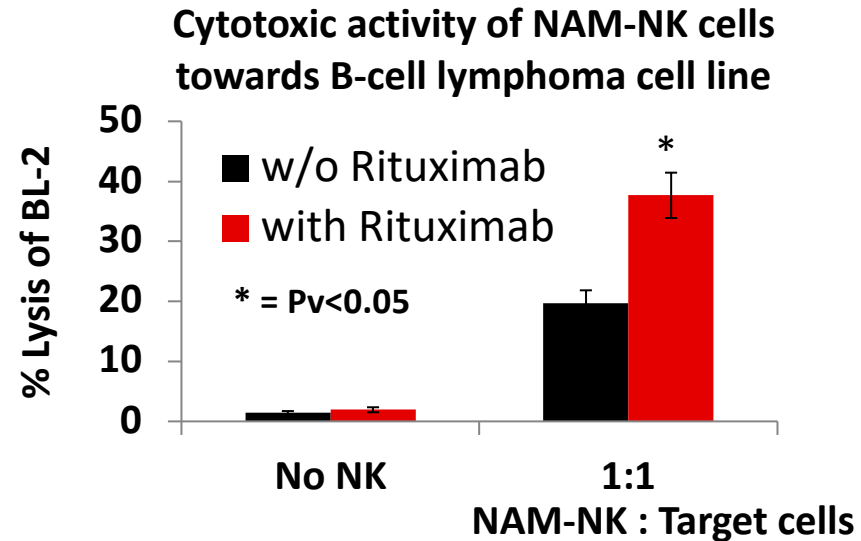
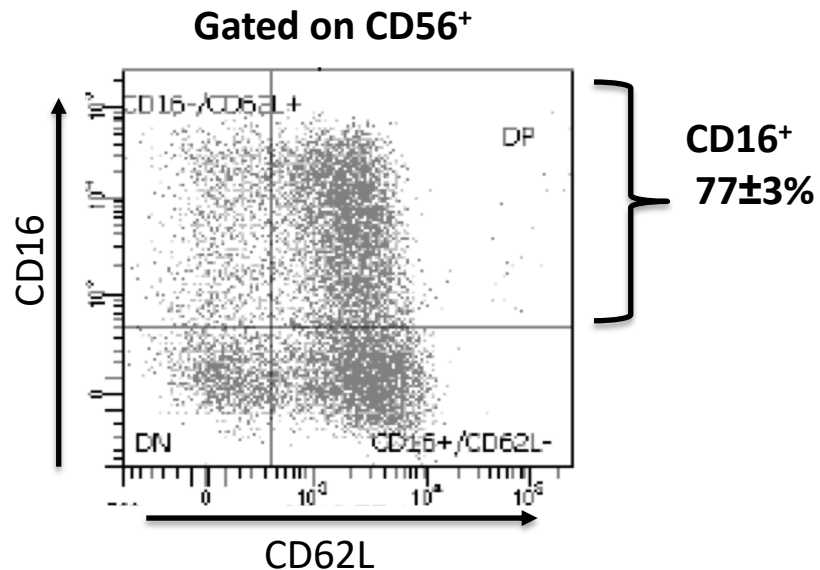
NAM-NK ADCC


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. IFN γ) without priming.

Rituximab enhanced lysis of lymphoma by NAM-NK





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



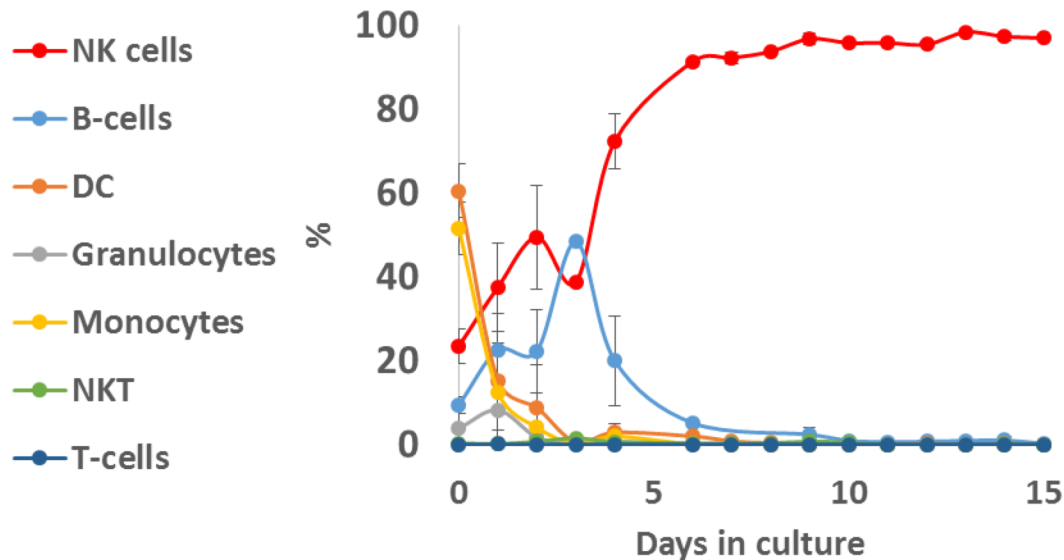
- Characterization
- Functionality

Advantages of CD3 Depletion Over CD56 Selection

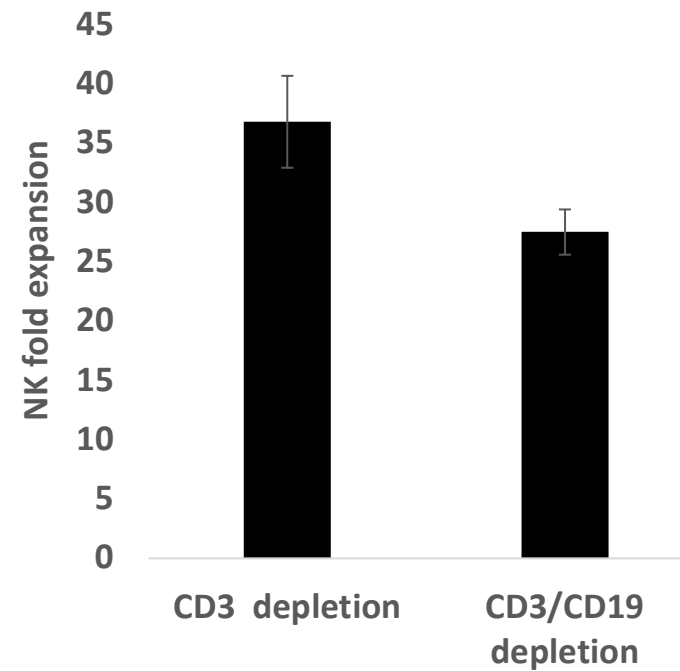
- Comparable expansion following CD3/CD19 depletion
- Myeloid Cells are supporting NK expansion

NK expansion

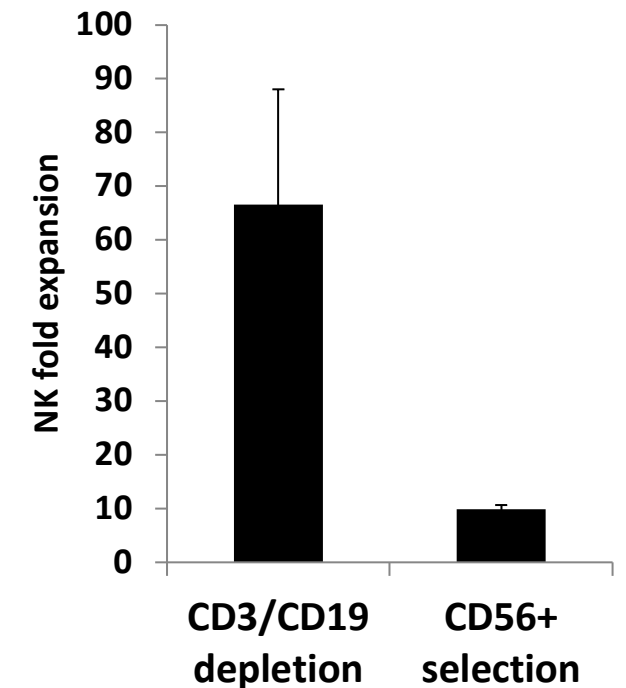
CD3 depleted cultures
Cell composition



CD3 or CD3/CD19 depletion

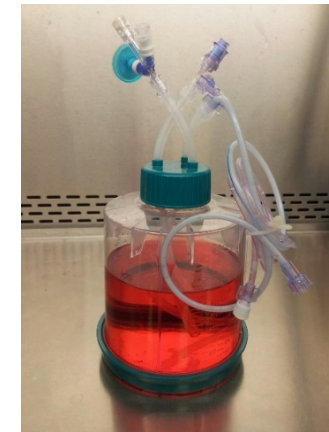
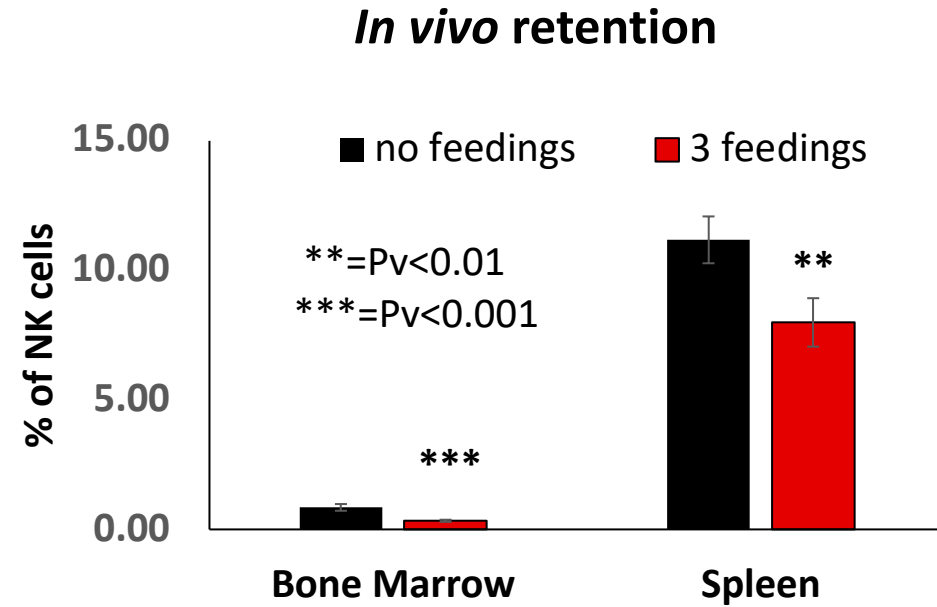
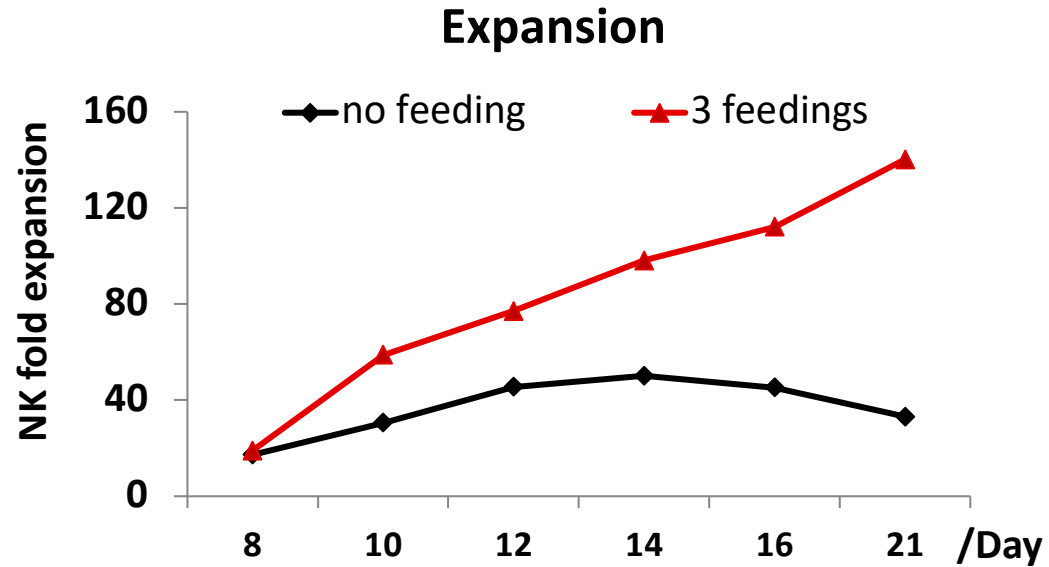


CD3 depletion or CD56 selection



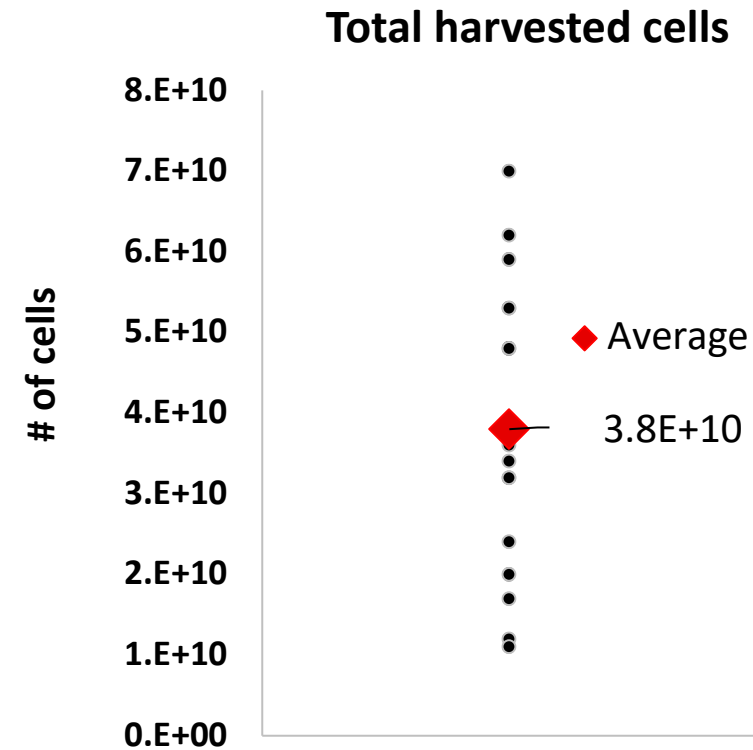
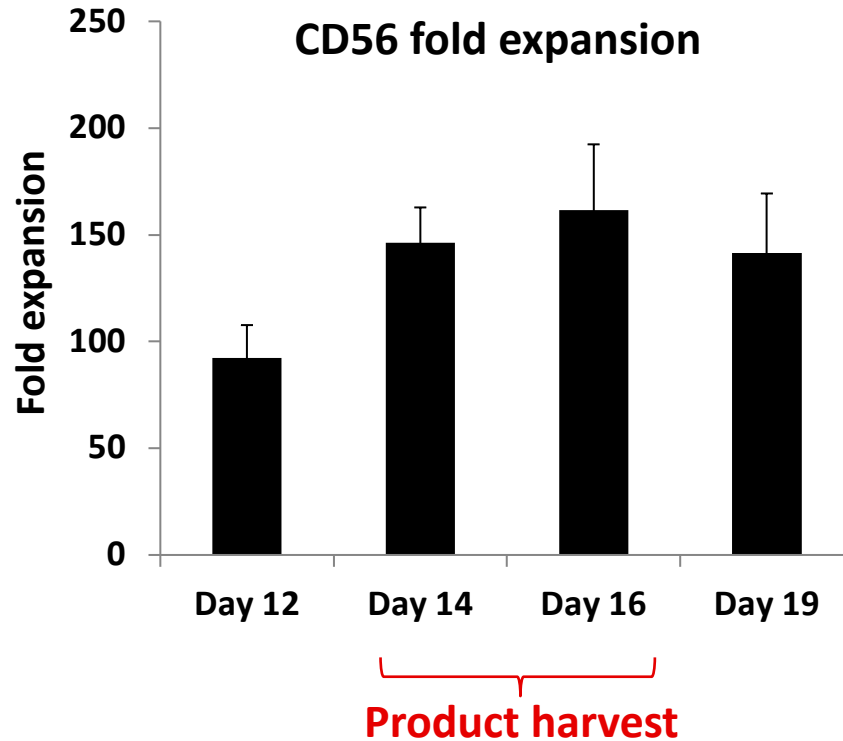
Feeding Regime

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



Harvesting

One feeding during the entire expansion duration



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

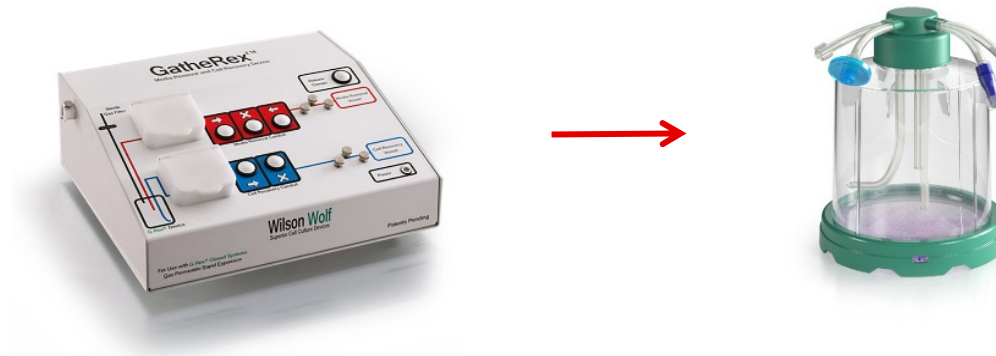
Average final product size 3.8×10^{10}
Median final product size 3.5×10^{10}

GMP Grade Closed Manufacturing of the NAM-NK Cell Product

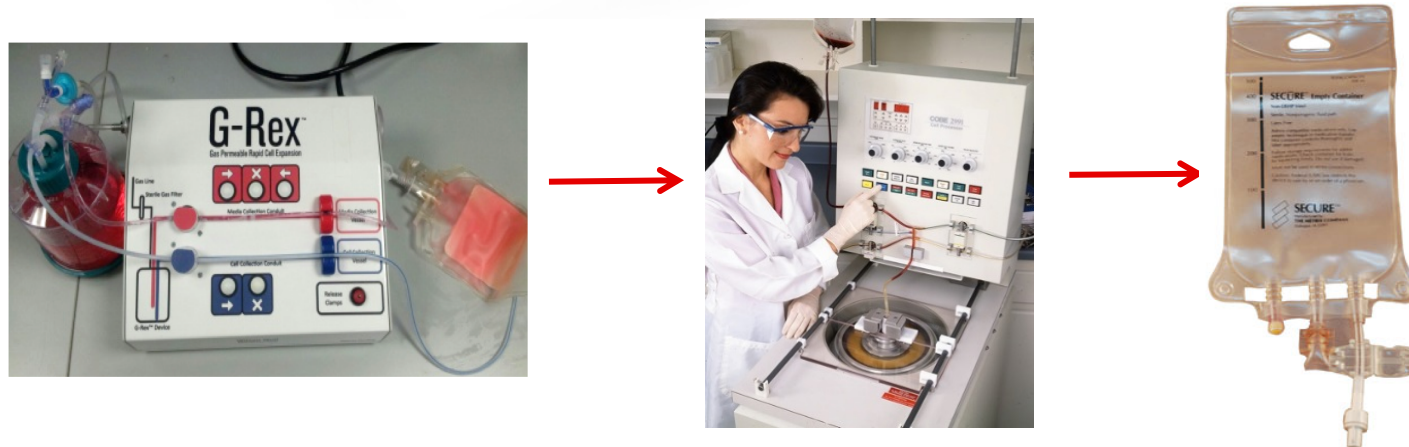
Day 0: seeding of CD3 depleted, apheresis collected cells, in GREX100MCS flasks



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 ($20\text{-}200 \times 10^6$ 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

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