Improved Homing to Bone Marrow, Spleen and Lung of Adoptively Infused NK Cells Expanded *Ex Vivo* with the Small Molecule Nicotinamide Using Feeder-Free Conditions

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National Heart Lung and Blood Institute

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Natural Killer (NK) Cells for Cancer Immunotherapy

Advantages of NK Cells

- NK cells are immune cells that exhibit cytotoxicity without the need for prior sensitization
- Donor NK cells do not increase the risk of GvHD
- Greater interest in the clinical applicability of NK Cells in Adoptive Immunotherapy

Limitations for NK Cell Therapy

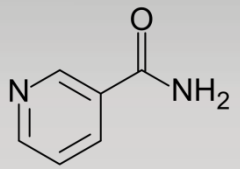
- Insufficient number of NK cells, expansion is necessary
- Impaired homing and short in-patient retention
- Limited *in vivo* proliferation of adoptively transferred NK cells
- Only partial response with NK cells in investigational tumor immunotherapy protocols

Challenges for NK Cell Therapy

- To increase the *in vivo* functionality of NK cells expanded in *ex vivo* cultures

Nicotinamide Modulates the Functionality of *Ex Vivo* Expanded Cells

Focusing on small molecules modulating the activity of NAD-dependent enzymes, we have identified nicotinamide (NAM) as a biological modifier that enhances the functionality, migration, homing to the BM, and engraftment, of cord blood derived $CD34^+$ cells expanded in culture with cytokines



Nicotinamide (NAM)

- The amide form of Vitamin B_3
- A precursor of NAD and NADP (nicotinamide adenine dinucleotide/phosphate)
- A potent inhibitor of enzymes that require NAD^+ for their activities such as:
  - mono and poly ADP ribosyltransferases
  - cyclic ADP ribose/NADase
  - SIRT family of type-III histone deacetylase

NAD^+-dependent enzymes are involved in the regulation of:

- Cell metabolism
- Cell energy
- Redox reaction
- Oxidative stress
- Mitochondrial functions
- Cell motility
- Gene expression
- Post-translational modification

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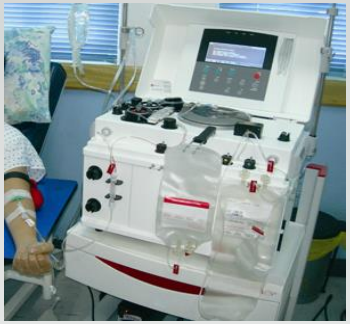
Study Objectives

- To evaluate the effect of NAM on the *in vitro* and *in vivo* functionality of NK cells expanded in culture with IL-2 or IL-15 in feeder-free conditions
- To compare the functionality of NK cells expanded in feeder-free conditions to the functionality of NK cells expanded using SMI-EBV-LCL feeder cells

Feeder-Free NK Expansion Procedure

Cells are collected from donor using apheresis



CD3 cells are depleted

CD3 depleted fraction is cultured in the G-Rex gas permeable cell culture device in medium containing HS, IL2 or IL15, ± NAM (5mM)

2 weeks cell incubation
- No feeder
- No feeding

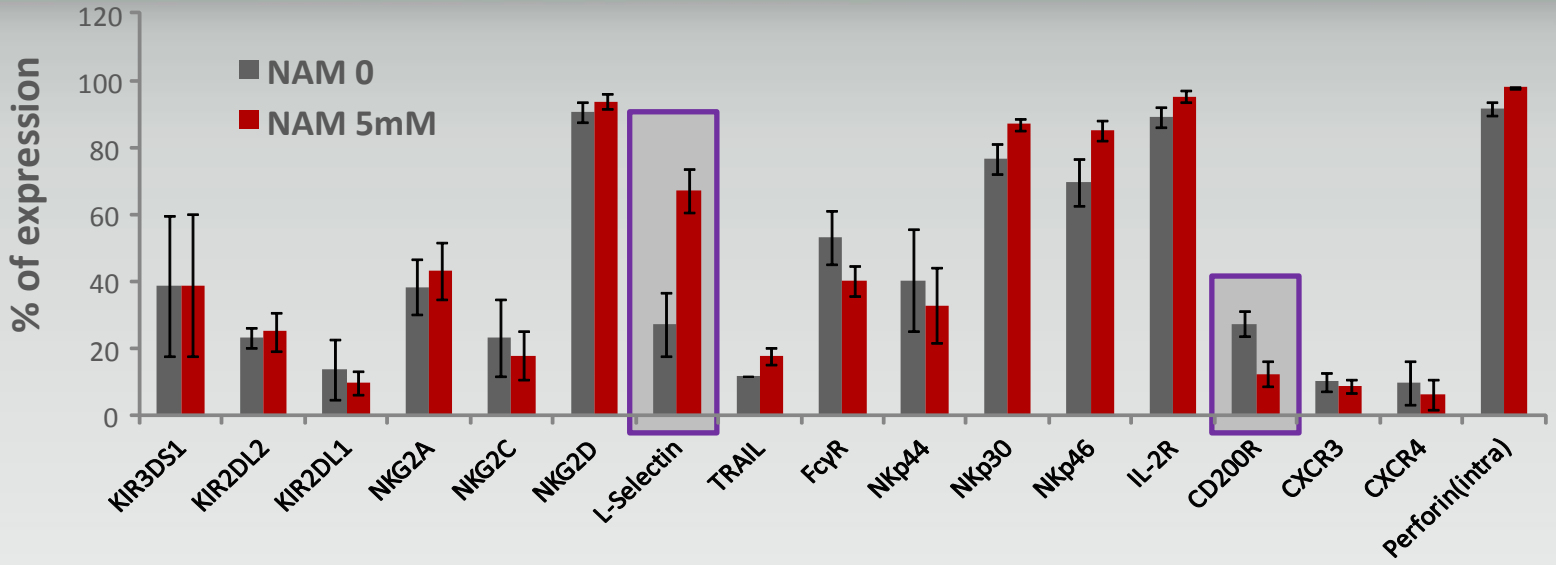
NK expansion **x50 (37-87)**

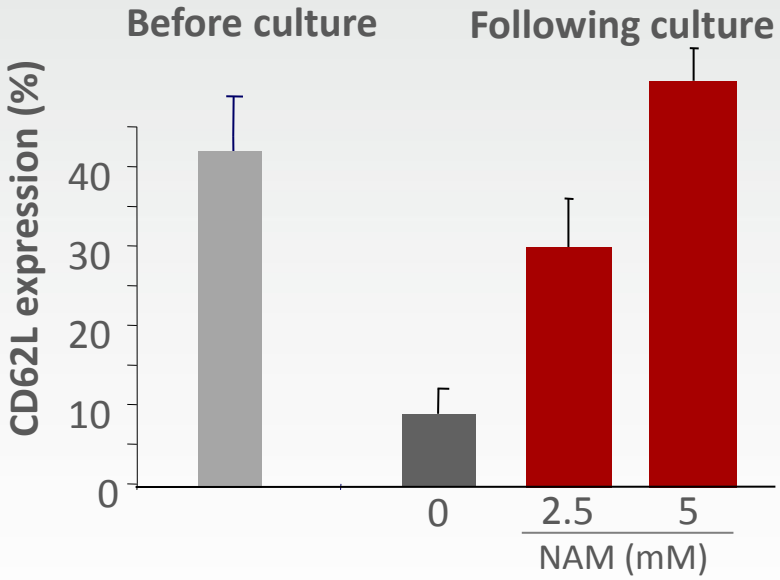
Cell populations	CD3 depleted fraction (%)	After two weeks expansion (%)
NK cells	7.8±0.8%	96.8±1.8%
T cells	0.24±0.1%	0.23±0.16%
B cells	21.3±2.5%	0.77±0.27%
Myeloid cells	39.9±3%	1±0.4%

What We Tested

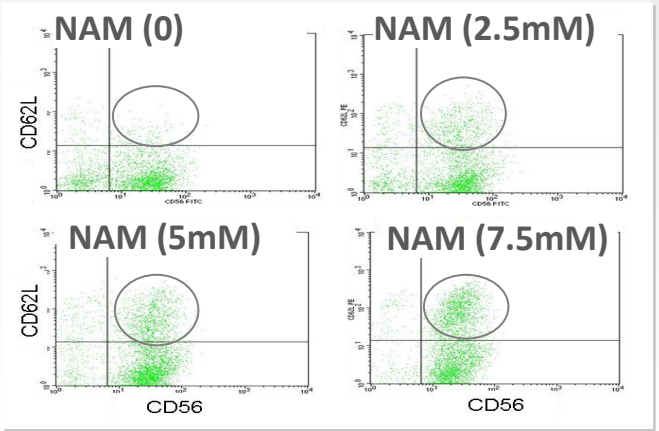
- Cultured cells phenotype
- *In vivo* homing and retention (NSG mice)
- *In vivo* proliferation
- An *in vivo* model of multiple myeloma

NAM Increases the Expression of CD62L/L-selectin and Decreases CD200R





NAM, dose dependent, increases CD62L expression



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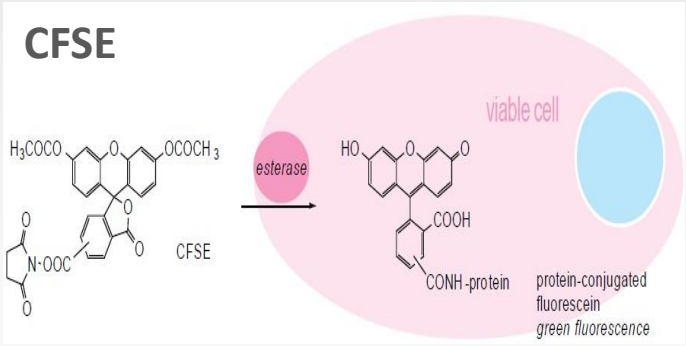
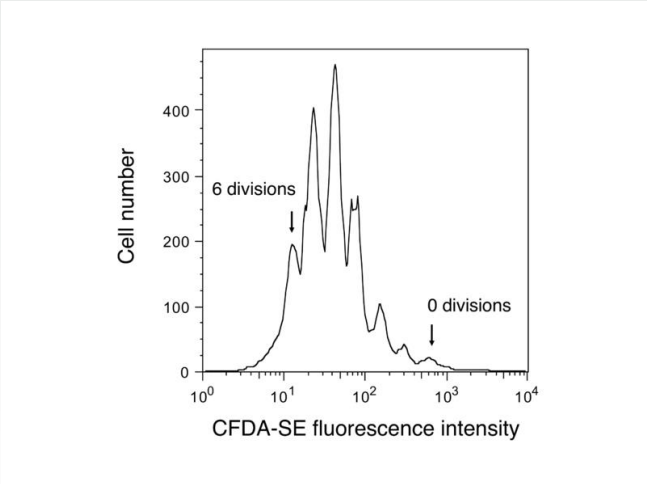
NK Homing and Proliferation in the NSG Mouse Model

***In vivo* model**:

- NSG mice, irradiated at 200-350cGy
- 24hrs later $15x10^6$ NK cells stained with CFSE injected IV
- Mice are injected with IL-2/IL-15 three times a week
- Sacrifice after 4-7 days



Tracking cell proliferation based on CFSE intensity



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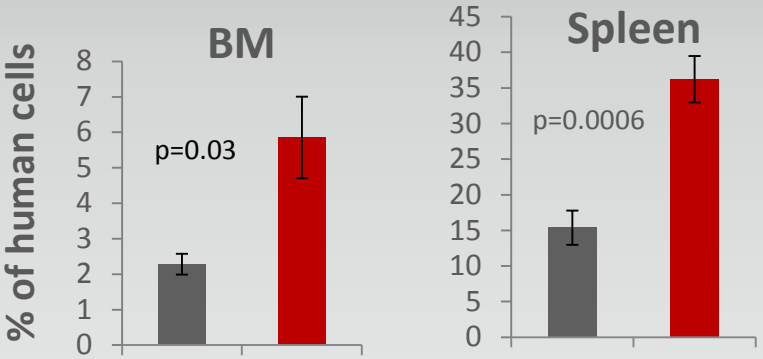
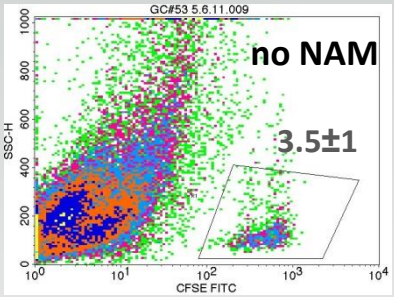
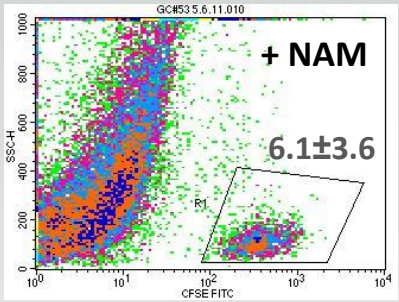
NAM Increases *In Vivo* Homing, Retention and Proliferation

Homing (24 hours)

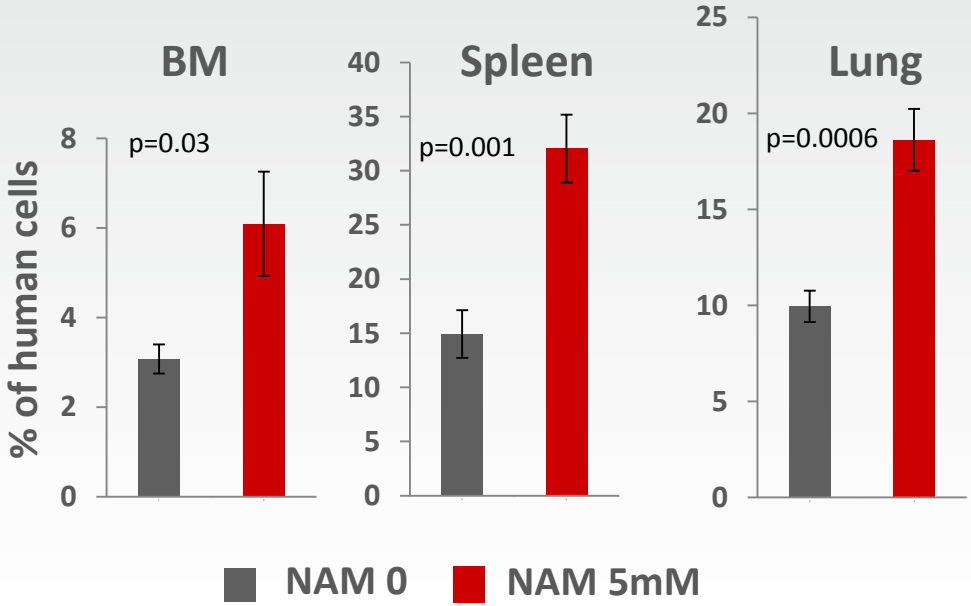


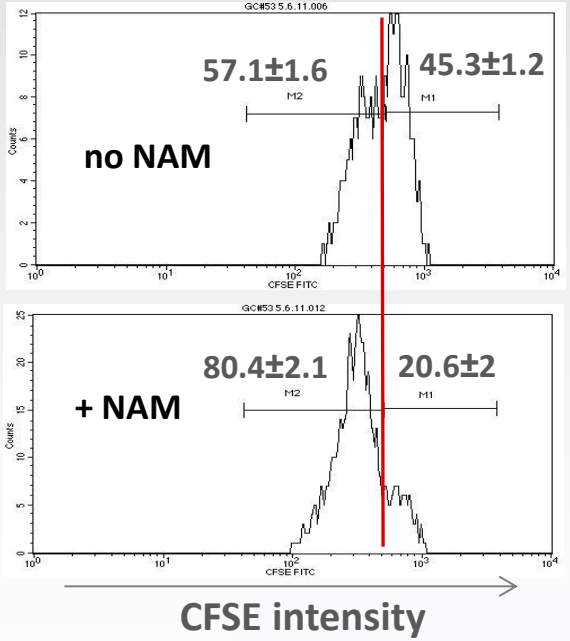
Cells cultured with NAM display increased functionality *in vivo* (BM)





Retention (day 4)

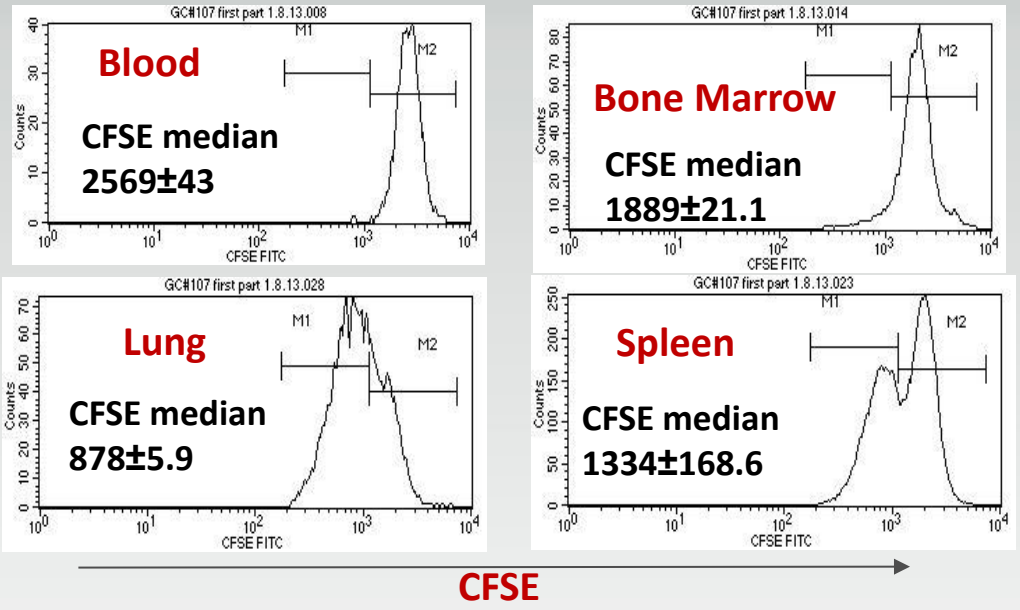




NK Cell Proliferation in NSG Mice

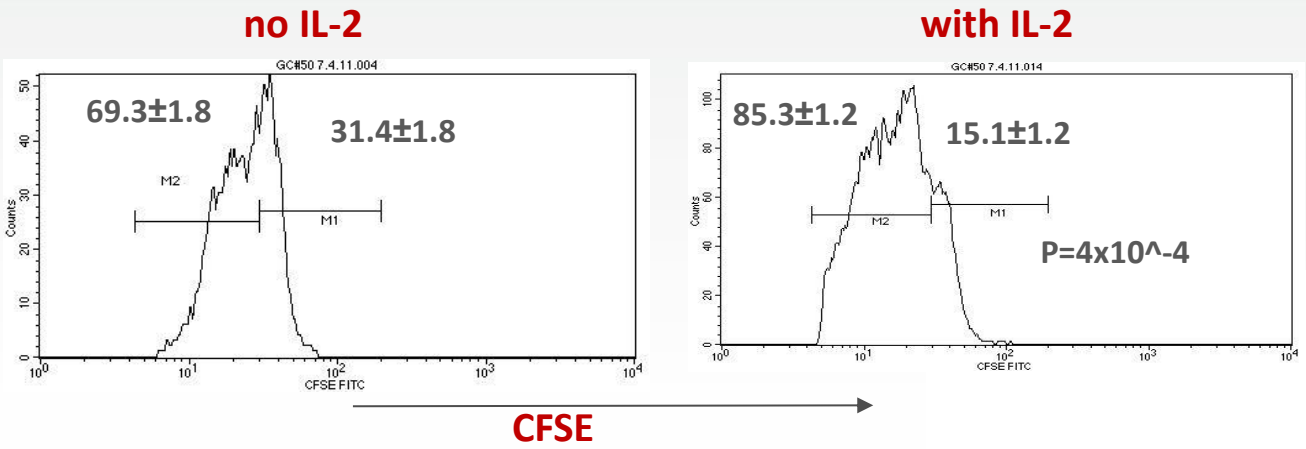
NK cells proliferation *in vivo* without IL-2/IL-15 stimulation

Day 4



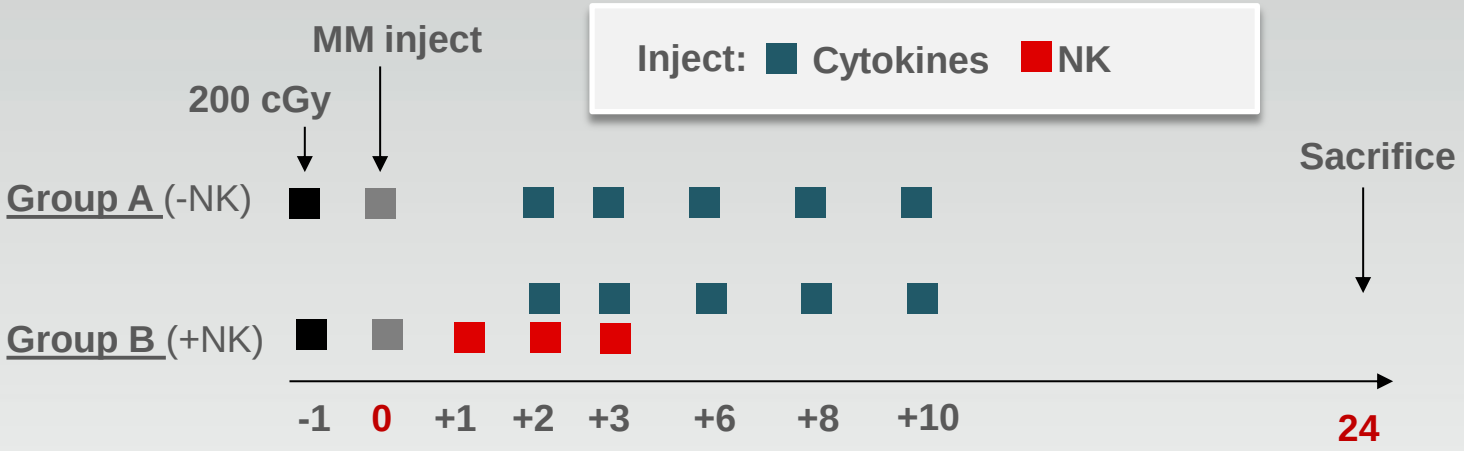
Increased proliferation with IL-2 injection (spleen)

Day 7



Multiple Myeloma (MM) Model in NSG Mice

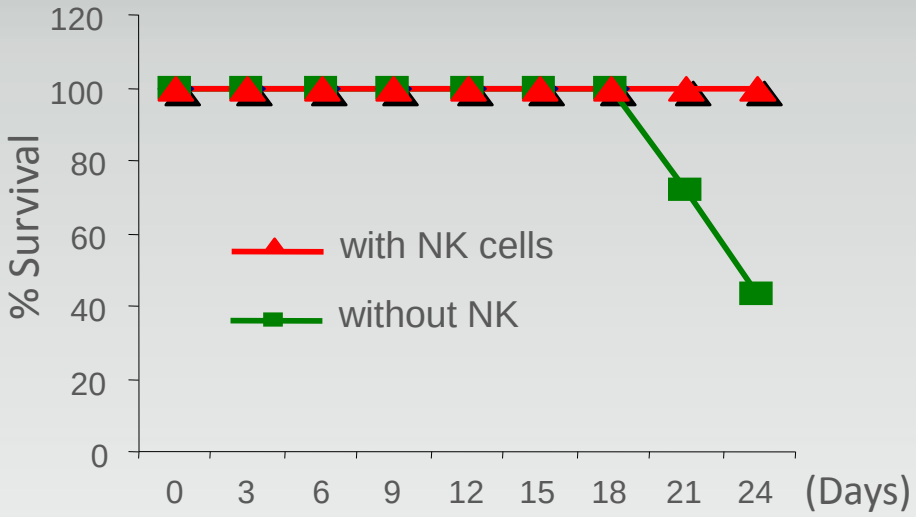
Model Scheme



- NSG mice irradiated at 200cGy (n = 7/group)
- 24hrs later a MM tumor cell line was injected at $5x10^6$ cells/mouse
- NK cells were injected on 3 consecutive days with an average of $13x10^6$ cells/mouse
- IL-2 and IL-15 were injected to the mice every 2 days until day 10
- Mice were sacrificed on day 24
- The potential benefit of NK cell treatment was monitored based on the level of IgG in the blood and % MM in BM

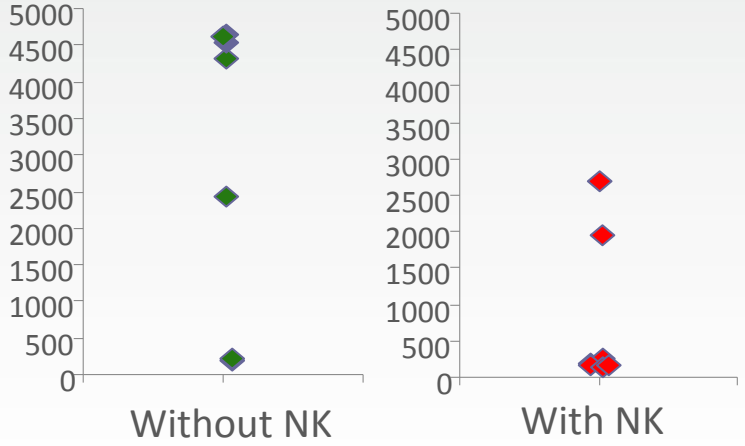
NK Cells Cultured With NAM Eradicated MM in NSG Mice

Survival

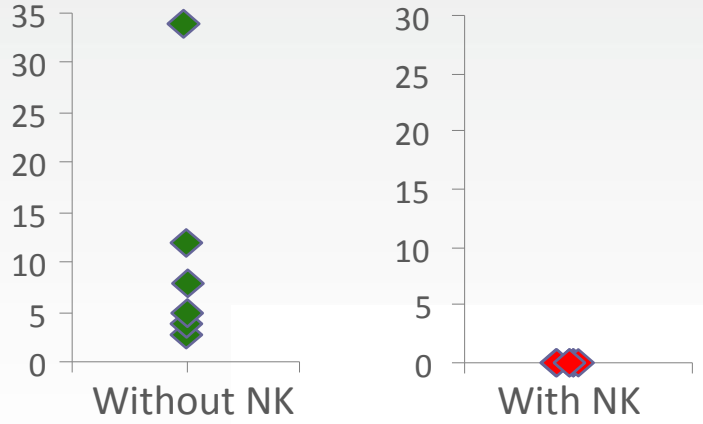


In the group injected with the MM cells without treatment with NK cells the mice became paraplegic.
Therefore, all mice were sacrificed on day 24 and analyzed

Plasma levels of human IgG



MM cells in mouse BM (%)



Summary of NK Expansion on Feeder Free Conditions

- NAM increased CD62L expression
- NAM increased *in vivo* homing, retention and proliferation
- NK cells proliferated *in vivo* without injection of human IL-2/IL-15
- Proliferation was augmented with cytokine injection
- NK cells cultured with NAM abrogate tumor progression in a MM model in NSG mice
- NK expansion on feeder-free conditions is low in comparison to what is currently reported for NK expansion on feeder cells

Number of NK Cells Obtained Following Large Scale Expansion are Sufficient for Their Evaluation in a Clinical Study

Large scale expansion in feeder-free conditions

Leukapheresis

↓

Number of TNC collected - **$1x10^{10}$**

↓

CD3 depletion

↓

$3.5x10^{9}$ cells seeded in culture for 2 weeks

↓

Total cell expansion **x4 (2.5-4.9)**

NK expansion **x50 (37-87)**

↓

A total of **$1.4x10^{10}$ cells** after culture

>96% NK cells

<0.3% T cells

↓

~$2x10^{8}$ cells/kg considering a 70 kg patient

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Study Objectives

- To evaluate the effect of NAM on the *in vitro* and *in vivo* functionality of NK cells expanded in culture with IL-2 or IL-15 in feeder-free conditions
- To compare the functionality of NK cells expanded in feeder-free conditions to the functionality of NK cells expanded using EBV-LCL feeder cells
  - NK cells expanded using SMI-EBV-LCL feeder cells currently being tested in a clinical trial with only minor responses being observed to date
  - *Comparability studies were performed at the NHLBI by Dr. Richard Childs' group*

Gamida Cell & NHLBI NIH Research Collaboration

NK expansion process

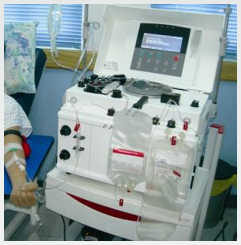
Feeder-free culture conditions

CD3 depletion

2 weeks expansion in culture
- no feeder cells
- no feeding during culture

NK expansion after two weeks
50 (37-87)

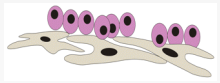
Cells collected from donor apheresis



Feeder cells (EBV-LCL)

CD3 depletion

CD56 selection



CD56⁺ cell culture on SMI-EBV-LCL feeder cell

Cell count and medium refill every 2 days

NK expansion after two weeks
300±40

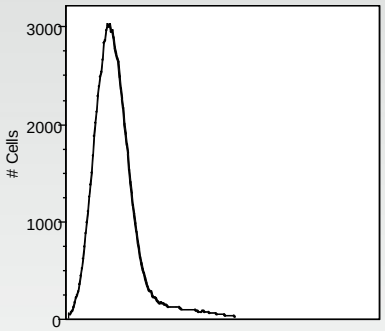
What Was Tested

- Cell phenotype
- Homing/Retention
- Cytotoxic activity assay
- Inflammatory cytokine secretion against tumors

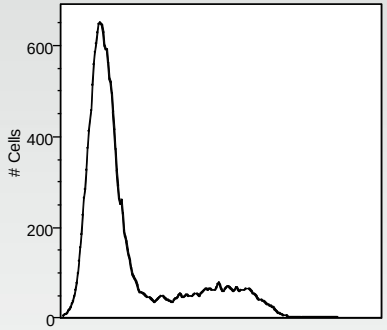
NK Cells Cultured on EBV-LCL Feeder Cells Do Not Express CD62L/ L-selectin

With the exception of CD62L, no consistent differences in the phenotype of NK cells between the various expansion methods were observed

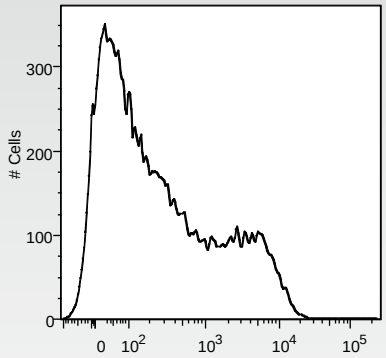
NK cells expanded using EBV-LCL feeder cells



NK cells expanded using feeder-free conditions



NK cells expanded using feeder-free conditions + NAM



CD 62L intensity

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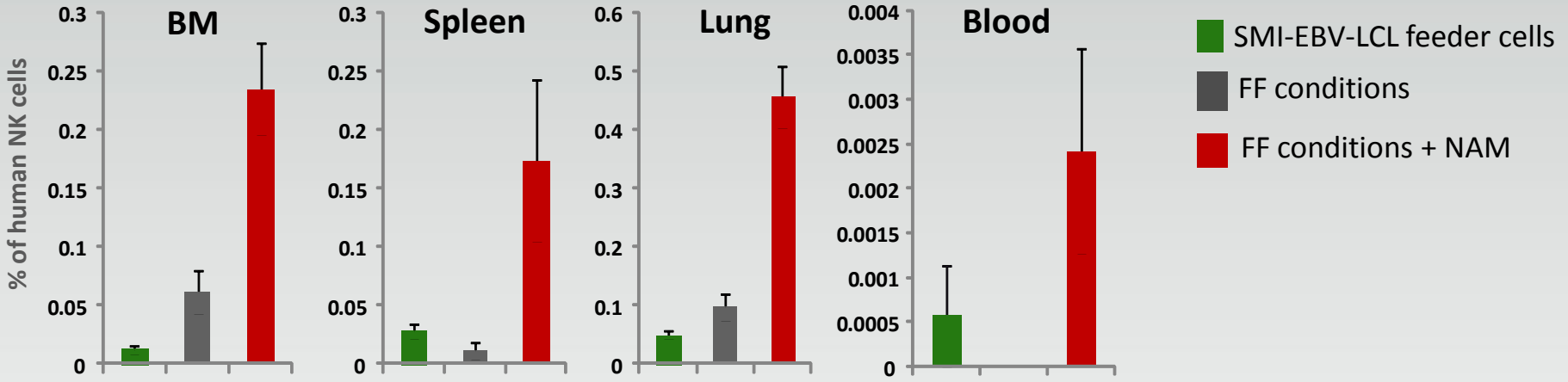
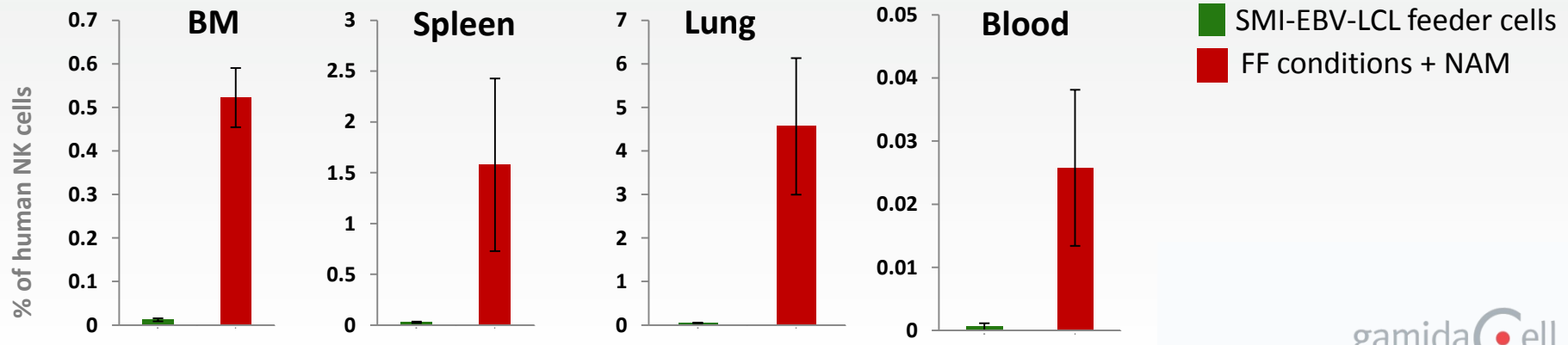
NK Cultured on EBV-LCL Feeders are Hardly Detected *In Vivo*

Detection of human NK cells in NSG mice (4 days post infusion, w/o IL-2 infusion)



IL-2 infusion enhances retention of NK cultured in FF+NAM but without effect on NK cultured on EBV-LCL feeder (4 days post infusion)



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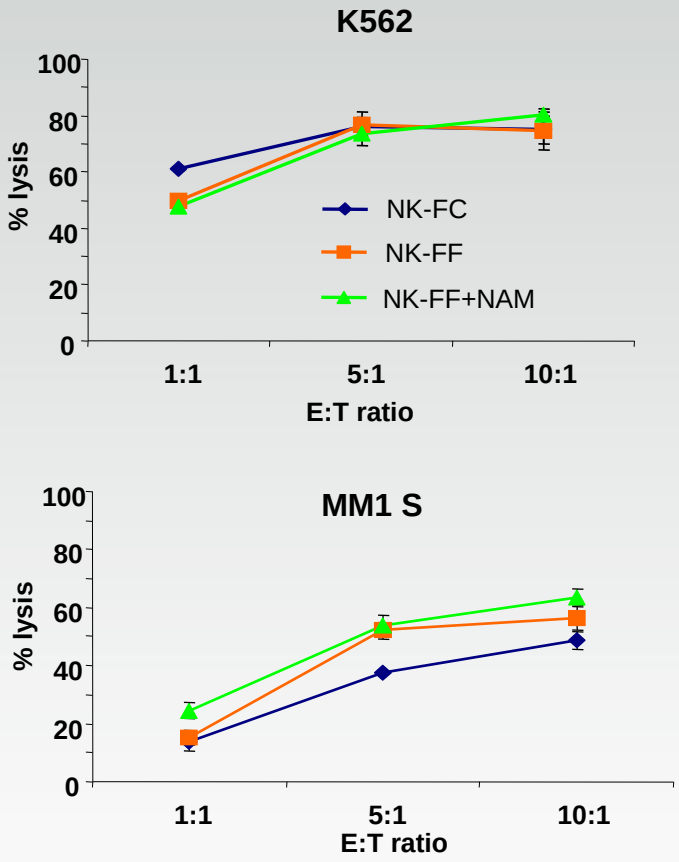
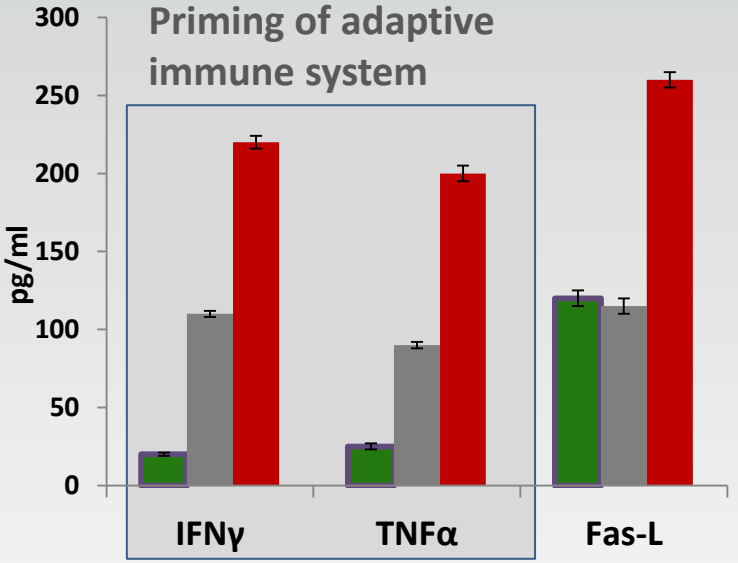
NK Cells Cultured in FF conditions + NAM Display Superior Cytokine Secretion

Cytotoxic activity *in vitro*



Cytokine secretion from killing assay



NK cells expanded using SMI-EBV-LCL feeder cells

NK cells expanded using feeder free conditions

NK cells expanded using feeder free conditions + NAM

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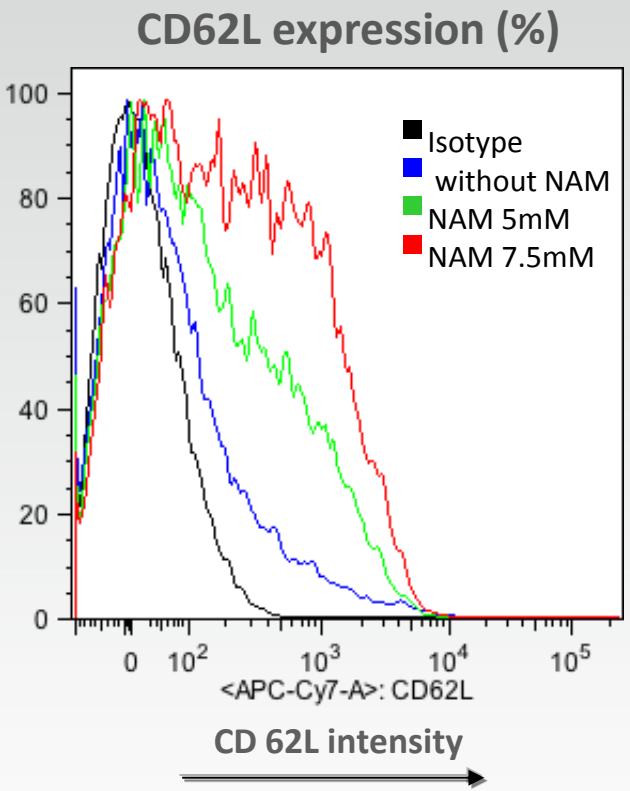
NAM Effect on NK Cells Expanded on EBV-LCL Feeder Cells

With the exception of CD62L, no consistent differences in the phenotype of NK cells were observed between cultures treated with or without NAM

- NAM increases CD62L expression on NK cells cultured on EBV-LCL feeder, albeit not at the level observed on feeder-free cultures
- Ongoing studies aim to determine NAM's effect, *in vitro* and *in vivo* on NK cells cultured with EBV-LCL feeders



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Conclusions and Further Directions

- NK cells expanded with NAM in feeder-free conditions are **highly functional** relative to NK cells expanded without NAM or expanded on SMI-EBV-LCL feeder cells
  - Have enhanced inflammatory cytokine secretion against tumors
  - Substantially up-regulate CD62L
  - Have improved *in vivo* proliferation and homing to multiple organs including the bone marrow
  - Study results suggest NAM expanded NK cells using feeder-free conditions could have superior clinical efficacy compared to EBV-LCL expanded NK cells

A phase I/II clinical trial will be initiated at NHLBI to explore the safety and antitumor efficacy of NK cells expanded *ex vivo* in feeder-free cultures containing NAM, given to patients with hematological malignancies

Thank you

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Multiple myeloma model
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