

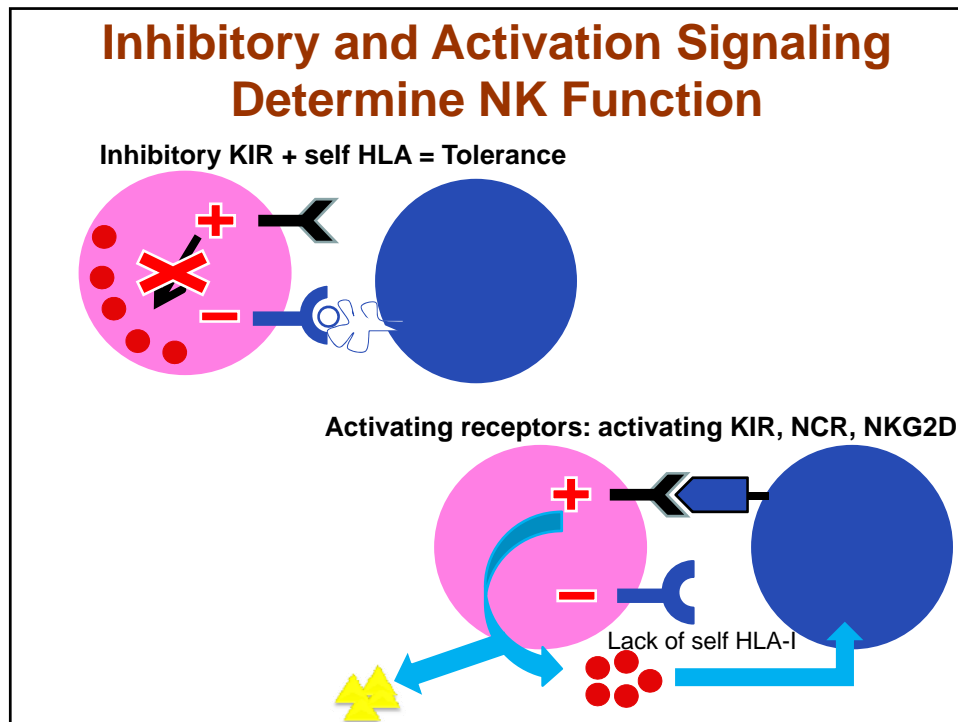
NK Cells for Therapy

Natasha Lapteva, Ph.D.

Center for Cell and Gene Therapy
Baylor College of Medicine
Houston, TX

Natural Killer (NK) cells

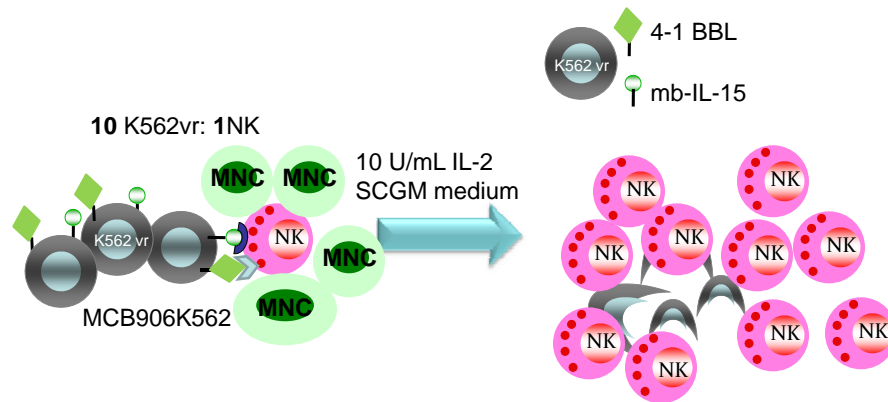
- Cytotoxic lymphocytes of innate immune system
- Mediate innate responses to viruses and intracellular pathogens
- Recognize alterations in MHC-I and ligands expressed in stressed/malignant cells
- Tumor rejection: destroy cancer cells resistant to chemotherapy with little or no toxicity to the host
- Used for treatment of malignancies, e.g. AML, melanoma, multiple myeloma



Protocols for Manufacture NK Products

- NK clinical trials require high doses of cells
- Starting material are apheresis cells, depletion of CD3⁺/CD19⁺ cells
- Expansion protocols with cytokines (without feeder cells) require prolonged cultures
- Expansion protocols with feeder cells generate high yields and high potency cells in short time

Expansion of NK Cells *Ex-vivo*



Described by Dario Campana et al

Questions for Manufacturing Transfer

- Can we grow NK cells in G-Rexes ?
- Shipment of cryopreserved or fresh NK cells ?
- Can we generate more potent NKs?

Growth in Bags

- Low ratio of medium to surface area for optimal gas exchange
- Requires large numbers of bags
- Frequent feeding and culture manipulations
- Large harvest volume

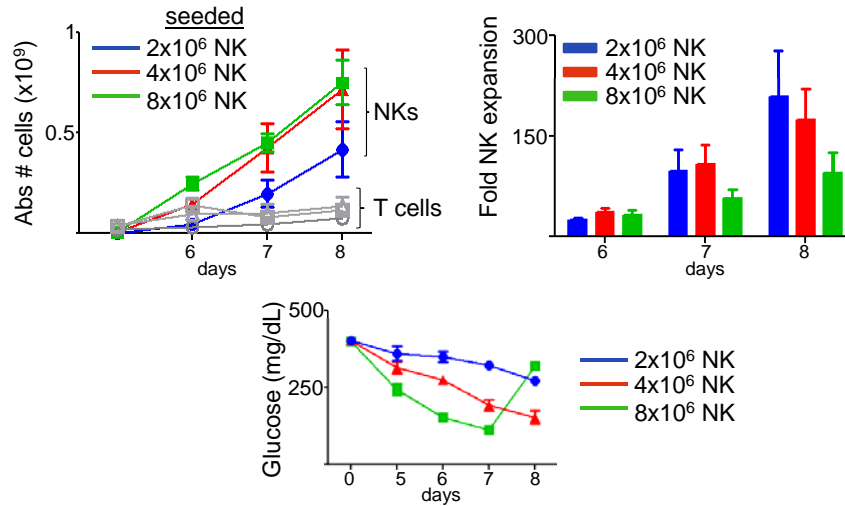
Gas Permeable Rapid Expansion Device (Wilson Wolf - G-Rex)

- Gas permeable membrane allows optimal exchange
- Supports cell growth with large volumes of media on a small surface area
- Waste dilution
- Minimizes manipulation
- Low harvest volume

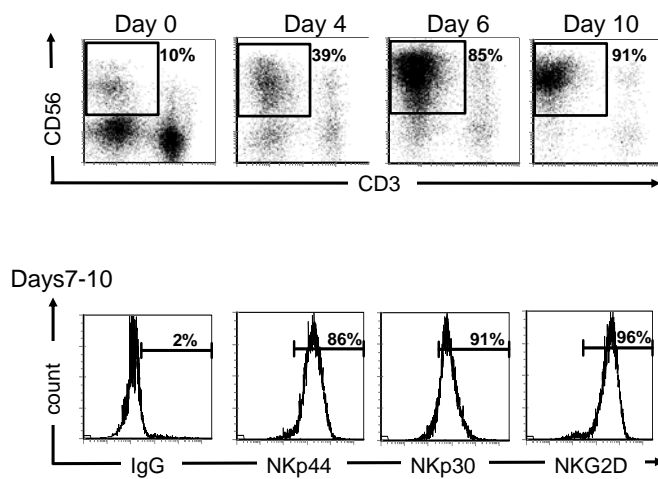


Optimization of NK Expansion in G-Rex

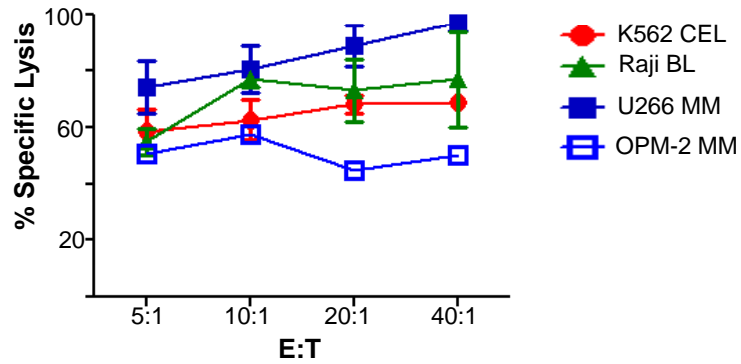
>200-fold expansion in CCD100 up to 1.2×10^9 cells/G-Rex100



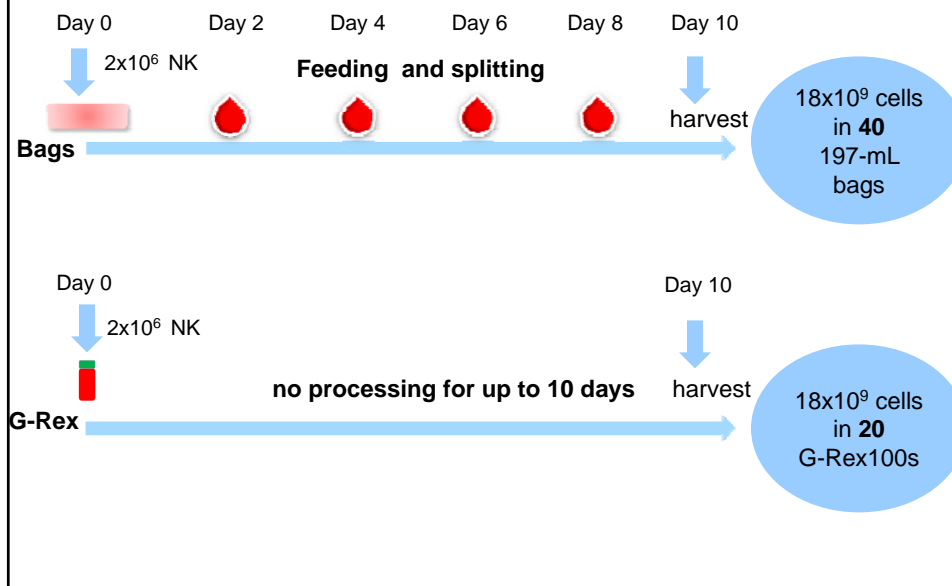
Expression of NK Activation Markers

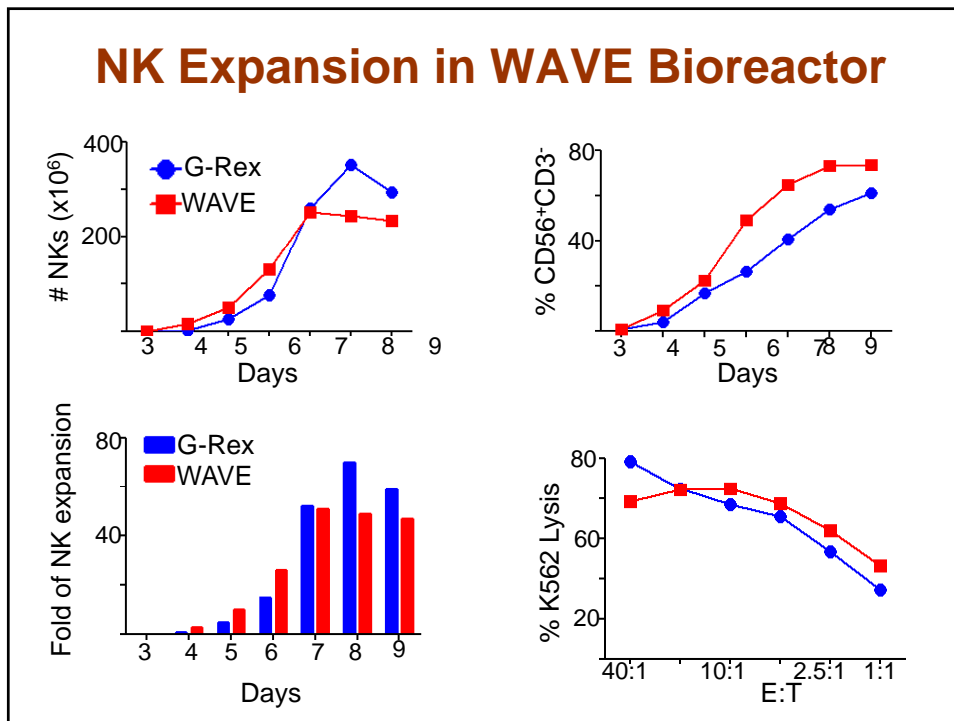


Cytotoxicity of Expanded NK cells



NK Expansion in Bags and G-Rexes

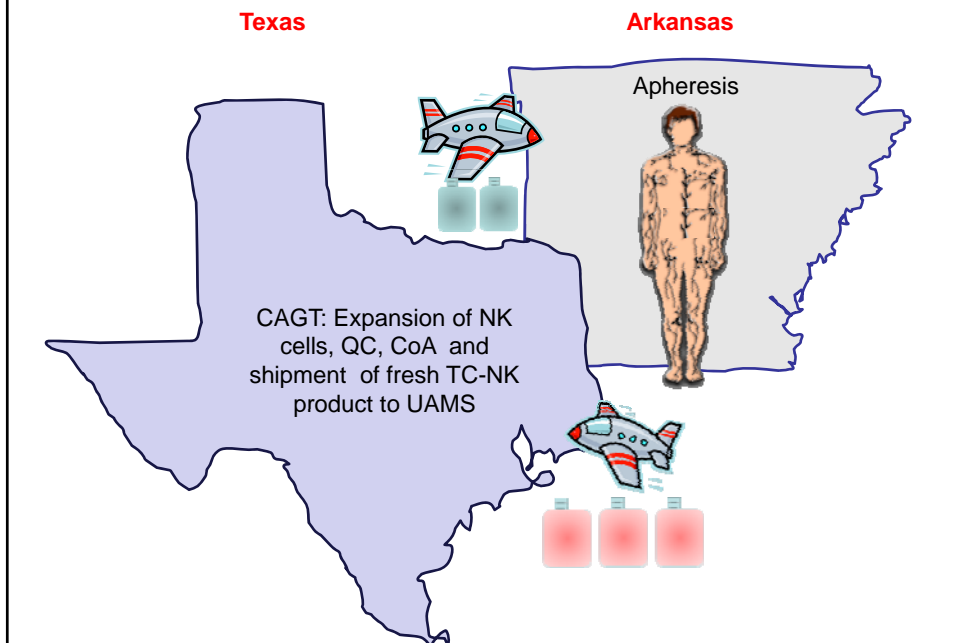


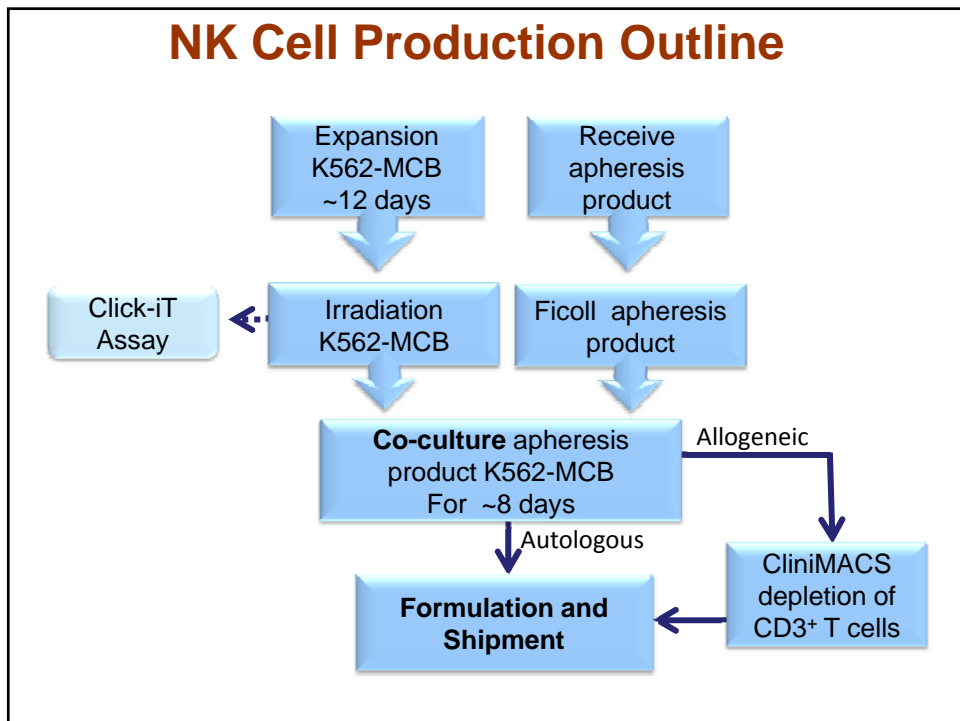
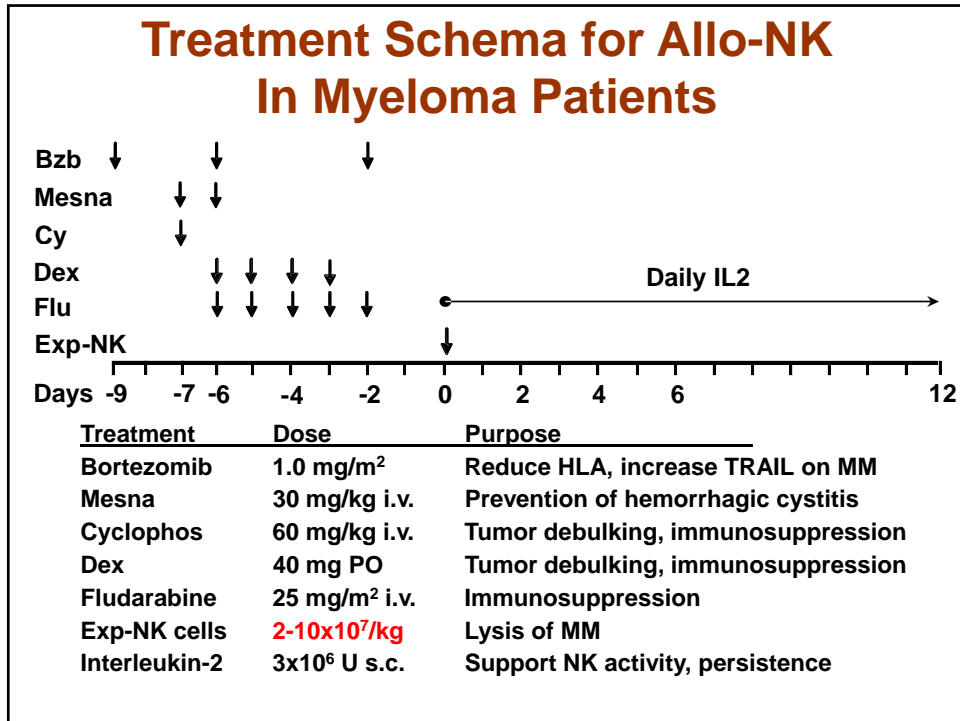



WAVE Evaluation: Final Product Release Specifications

	Release specification	G-Rex	WAVE
%CD56 ⁺ CD3 ⁻	>50%	61.3%	73.5%
Viability (7-AAD)	>70%	86.5%	84.2%
%GFP ⁺	<0.1%	0%	0%
%CD56 ⁺ CD3 ⁻ NKG2D ⁺	n/a	95%	91%
%CD56 ⁺ CD3 ⁻ NKp30 ⁺	n/a	56%	77%
%CD56 ⁺ CD3 ⁻ NKp44 ⁺	n/a	53%	47%
Cytotoxicity to K562 at 20:1 of E:T	>20%	84.8%	85%

Clinical Protocol Logistics







CERTIFICATE OF ANALYSIS
Center for Cell & Gene Therapy, GMP Cell Processing Facility
Baylor College of Medicine, Houston, Texas 77030
TC-NK CELLS

Overnight release criteria for shipped fresh NK cells

Test	Specification
Viability	>70%
%CD56 ⁺ CD3 ⁻	>50% for auto- >70% for allo-
# CD3 ⁺ CD56 ⁻	<5x10 ⁵ cell/kg for allo-
%GFP ⁺ K562	<0.1%
Gram stain	negative
Endotoxin	<5.0 EU/mL
Potency	>20% K562 lysis at 20:1 E:T
HLA-A,B	Matching donor

NK Cell Expansion is Variable

Subject	Donor Type	Start NK #	End NK #	NK Expansion
Validation 1	Healthy Donor	9x10 ⁷	1x10 ¹⁰	114
Validation 2	Healthy Donor	9x10 ⁷	9x10 ⁹	100
Subject 1	Myeloma Pt	9x10 ⁷	6x10 ⁹	67
Subject 2A ²	Healthy Donor	1.25x10 ⁸	3.9x10 ⁹	31
Subject 2B ²	Healthy Donor	1.5x10 ⁸	4.5x10 ⁹	30
Subject 3	Healthy Donor	1.5x10 ⁸	2.4x10 ¹⁰	160
Subject 4	Myeloma Pt	1.5x10 ⁸	2.6x10 ⁹	26
Subject 5	Myeloma Pt	1.5x10 ⁸	3.8x10 ⁹	25
Subject 6	Healthy Donor	1.5x10 ⁸	1.33x10 ¹⁰	89
Subject 7	Myeloma Pt	1.5x10 ⁸	1.1x10 ¹⁰	73

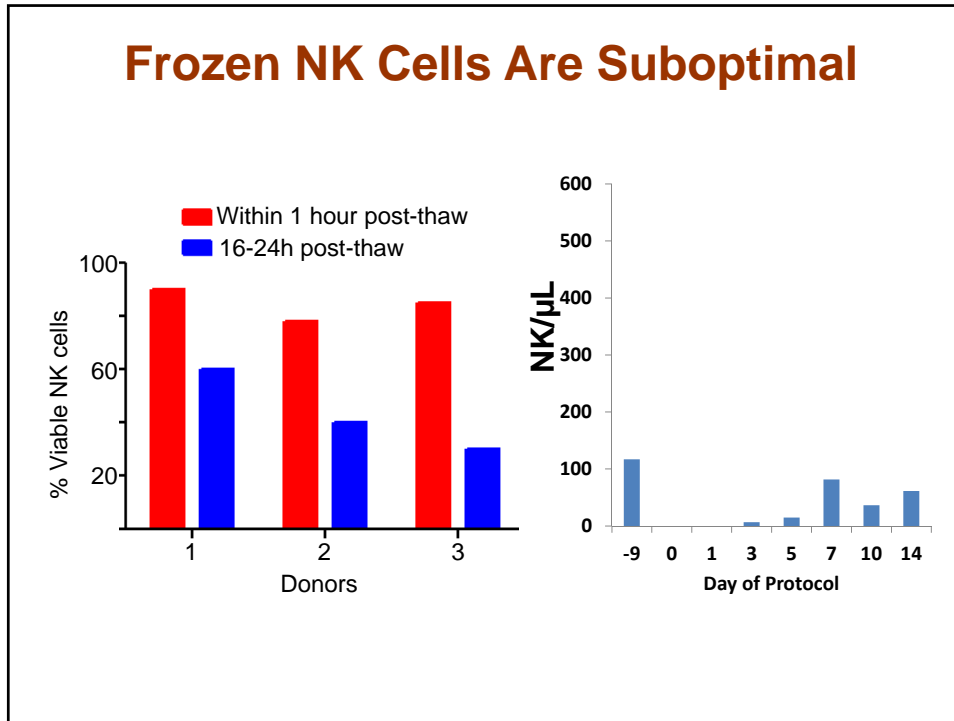
NK Purity, Potency and Viability

		Subject	NK %	T %	Viability %	Potency
Non CD3 Depleted products	autologous donors	Val1	52	34	72	63.4
		Val2	69	19	86	61.9
		1	88	4	83	65
		4	84	6	92	39
		Subject	NK %	T %	Viability %	Potency
CD3 Depleted products	allogeneic donors	Val 1	93	0.1	91	92
		Val 2	93	0.04	97	79
		2A	86	0.21	96	74
		2B	89	1.02	89	74
		3	85	0.09	91	65

Potency = % lysis of K562 at 20:1 E:T ratio

Recovery After NK Cryopreservation Was Suboptimal

Subject	Cryopreserved NK cell dose for infusion	Recovery (%)	Actual infused NK cell Dose	Viability By flow	Potency
Val 1	4.7x10 ⁷ /kg	61	2.9x10 ⁷ /kg	78%	77%
Val 2	4.9x10 ⁷ /kg	100	4.9x10 ⁷ /kg	99%	90%
1	4.9x10 ⁷ /kg	65	3.4x10 ⁷ /kg	94%	94%
2A	2.1x10 ⁷ /kg	100		75%	79%
2B	1.3x10 ⁷ /kg	100		83%	
3	5.0x10 ⁷ /kg	100	5.0x10 ⁷ /kg	77%	70%
4	3.4x10 ⁷ /kg	65	2.2x10 ⁷ /kg	94%	ND



Can we ship fresh NK products?

Frozen or Fresh NK Products

Frozen

One product for multiple infusions

40% HBSS
10% DMSO
12.5% HSA

Post- thaw

- Good viability right after thaw
- Recovery low after 24h
- Not cytotoxic right after thaw
- Do not expand well *in vivo*

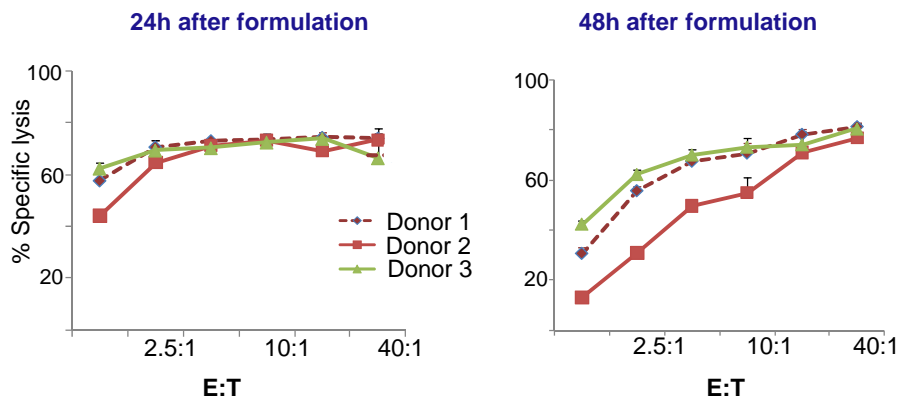
Fresh

One product for one infusion

5% HSA

- Good viability ?
- Cytotoxic ?
- Expand well *in vivo* ?
- Continue to expand during shipment ?

Fresh NK Potency is Retained After Shipping



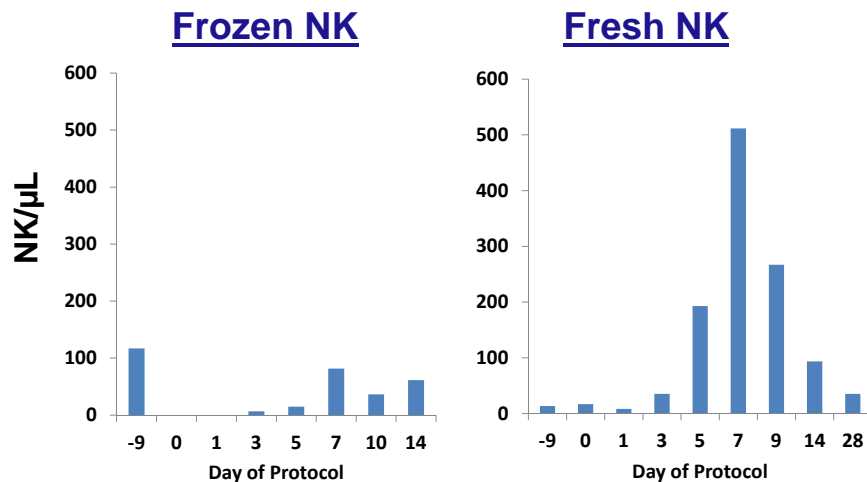
Fresh NK Continue to Expand During Shipping

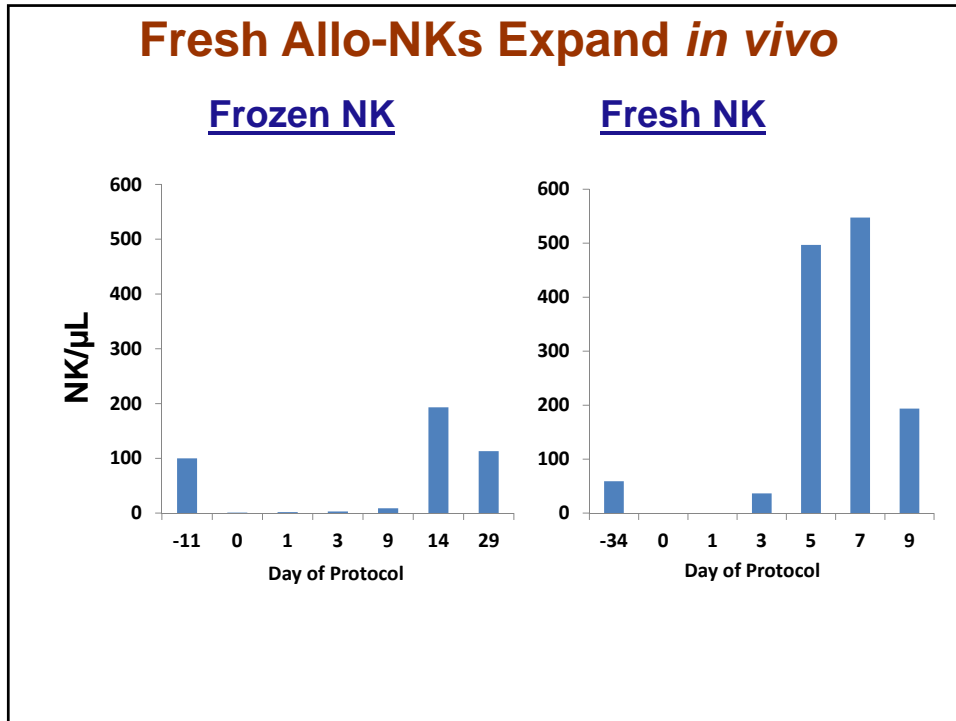
Time post formulation (h)	0	24	48	0	24	48
Sample	Manual Count (M/ml)	Manual Count (M/ml)/ Recovery (%)	Manual Count (M/ml)/ Recovery (%)	Viability Trypan/7AAD (%)	Viability Trypan/7AAD (%)	Viability Trypan/7AAD (%)
Donor 1	10	12.1/121%	13.7/137%	NA/85.8	81/97.8	87.8/98.3
Donor 2	10	15.0/150%	9.7/97%	NA/80.8	85/98.9	75.8/97.9
Donor 3	10	18.8/188%	17.1/171%	NA/86.9	87/99.3	89.5/98.6

Fresh NK Purity and T cell Content

Time post formulation (h)	0	24	48	0	24	48
Sample	CD3-CD56+ (%)	CD3-CD56+ (%)	CD3-CD56+ (%)	CD3+CD56- (%)	CD3+CD56- (%)	CD3+CD56- (%)
Donor 1	71.1	68.9	70.7	19.7	16.7	15.9
Donor 2	62.8	65	67.1	24.7	21.8	18.6
Donor 3	86.2	85.5	87.3	8	7.8	6.1

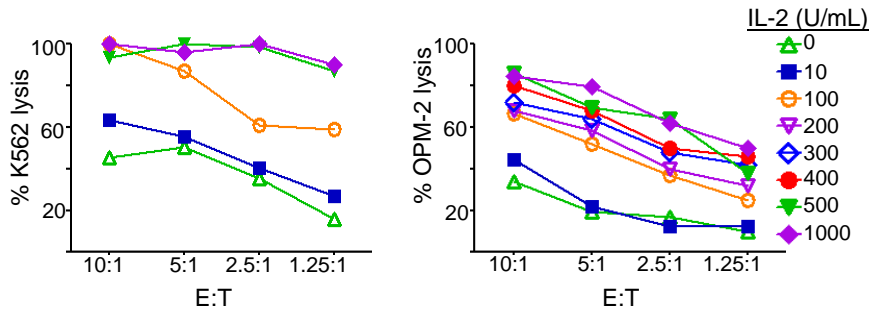
Fresh Auto-NKs Expand *in vivo*



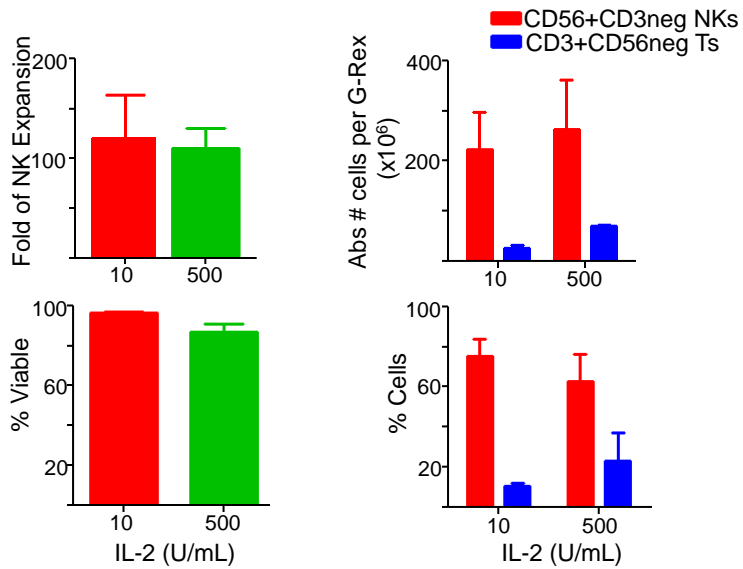


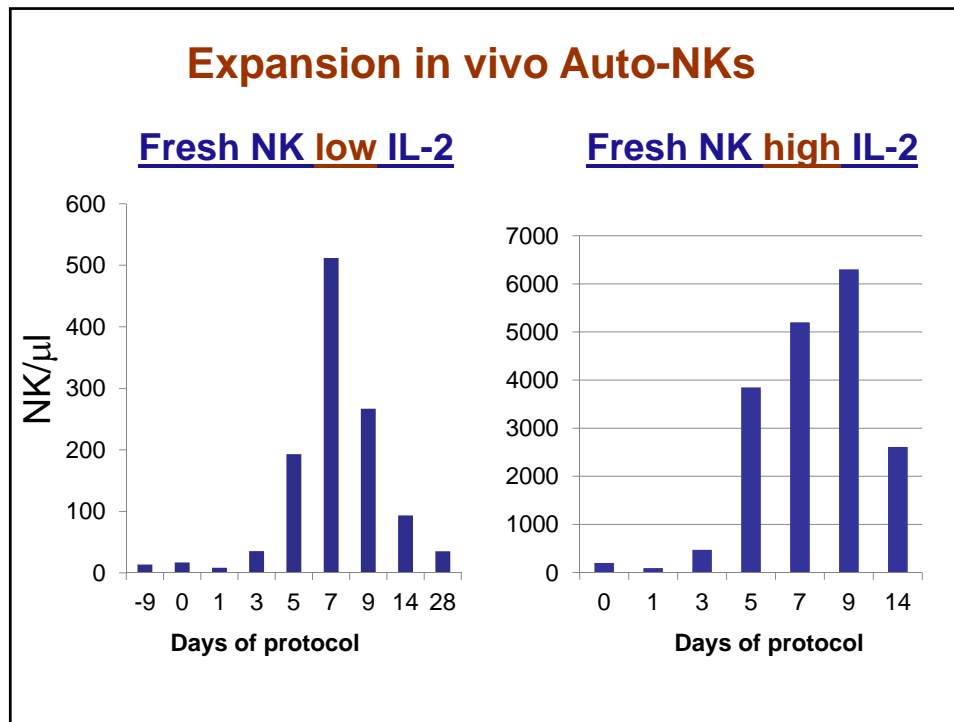
Can we grow more potent NK cells?

Improved Potency of NKs Generated with High IL-2 Doses



Comparable Expansion for 10 U/mL and 500 U/mL IL-2 Protocols





Conclusions

- **NK cells efficiently expand in G-Rexes within 10 days w/o manipulations**
 - **Fold expansion is donor dependent (25 to 160-fold)**
- **Shipment fresh NK cells:**
 - **Retain viability and potency after 48h in 5% HSA at RT and frozen ice-packs**
 - **Allow for higher infused NK dose**
 - **Expand further after infusion *in vivo***
- **Potency *in vitro* is improved with higher IL-2 during manufacturing**

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