Manufacture of Natural Killer Cells as Treatment for Multiple Myeloma

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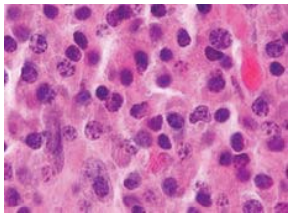
Multiple Myeloma (MM)



- ❑ Plasma cell malignancy
- ❑ Myeloma cells accumulate in bone and bone marrow
- ❑ Diagnosed in 1-4 per 100,000
- ❑ Prognosis is 5-7 years with advanced treatment

Natural Killer (NK) cells

- ❑ Cytotoxic lymphocytes of innate immune system
  $CD56^{+}CD3^{-}$ cells

- ❑ Immediate responders to viruses and intracellular pathogens

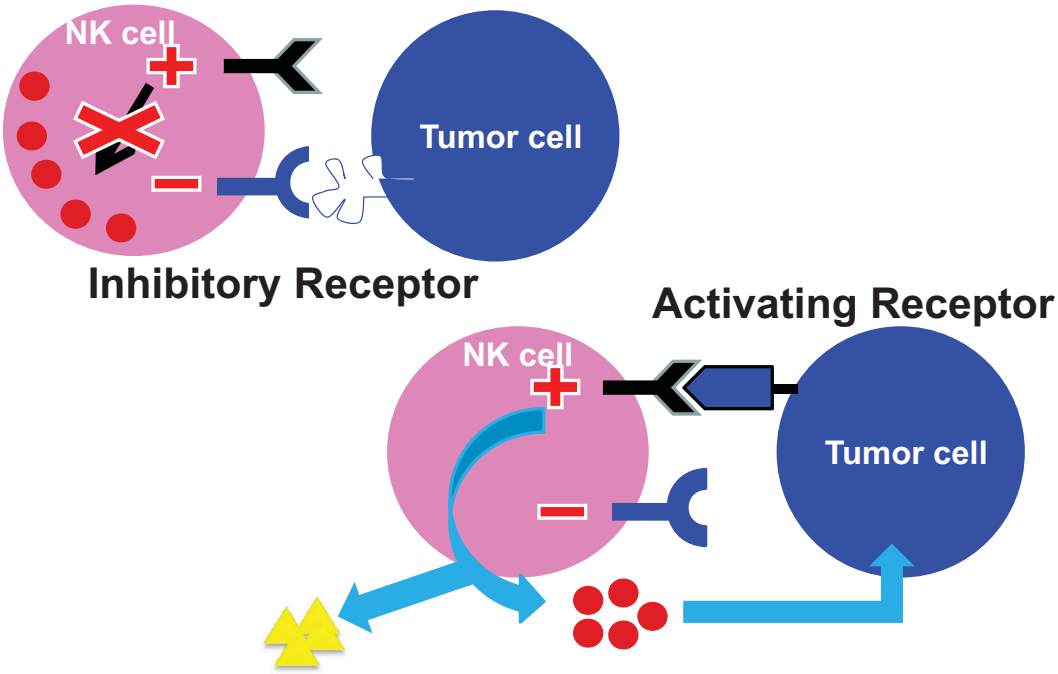
- ❑ Alerted by
  - ❑Ligands of stressed/malignant/infected cells
    - ❑Including lack of MHC class
  - ❑Cytokines produced by activated DCs and MΦs

- ❑ Recognize and kill a range of tumor types including MM

NK Cells

- Homeostatic cells
  - – Constitutively express TGF-β
- When alerted secrete
  1. MIP1α, MIP1β and Rantes
  2. IFN-γ, TNF-α, GM-CSF
  3. IL-5, IL-10 and IL-13
- Direct effector function
- Activated and guide adaptive immunity

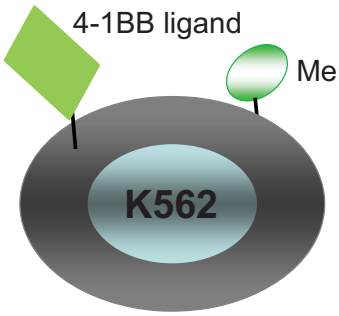
Inhibitory and Activation Signaling Determine NK Function



Protocols for Manufacture NKs

- NK clinical trials require high doses
- For fresh or overnight activated NK cells
  - Use apheresis cells,
  - Deplete of $CD3^+/CD19^+$ cells
  - Purity >50%
- Expansion using cytokines only
  - requires prolonged cultures
- Expansion with feeder cells/ cytokines
  - Rapid expansion
  - high potency cells

Campana Method for NK Cell Expansion

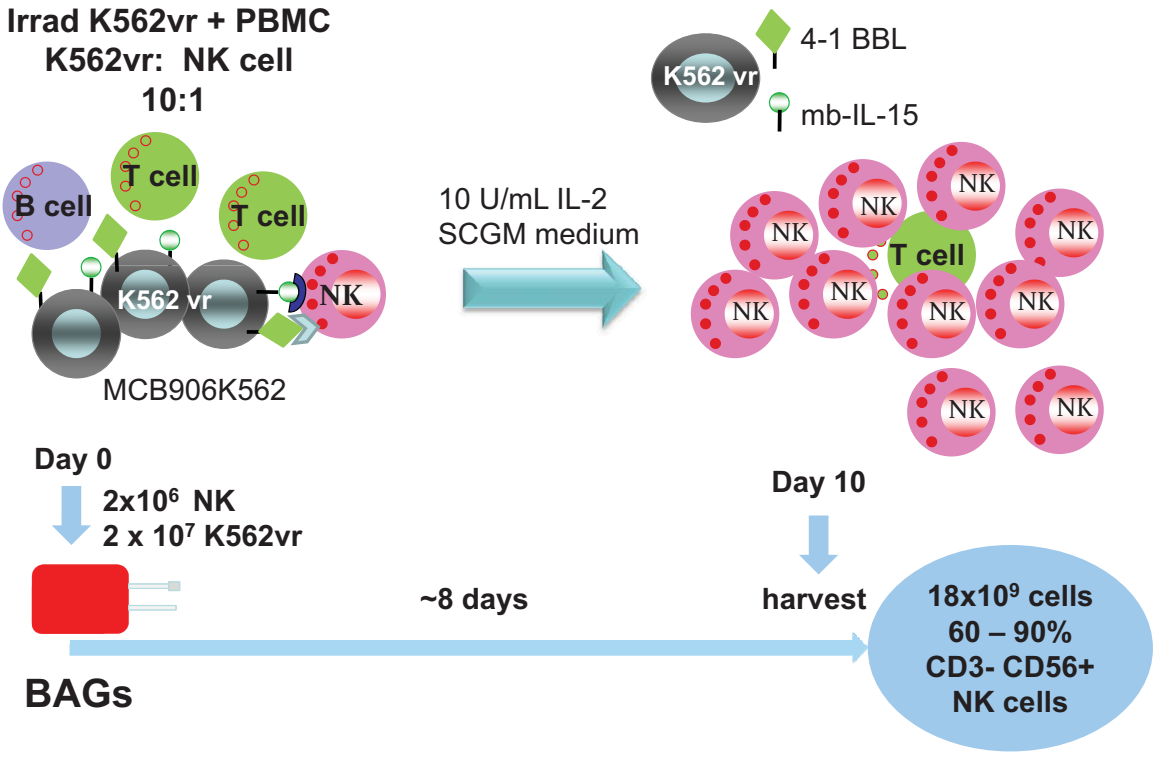


K562vr

- K562vr
  - HLA I and II negative
    - Activated NK cells
  - IL-15 growth factor
  - 4-1BB ligand costimulatory
- Soluble IL-2

- Master/working cell bank generated
  - PACT (part I)
  - MCB906K562

Campana Method for NK Cell Expansion



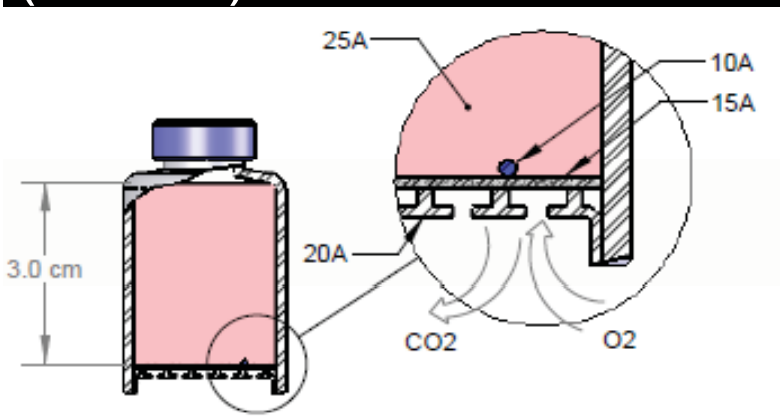
Questions for Manufacturing Transfer

- Can we replace bags with G-Rex?
- 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

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

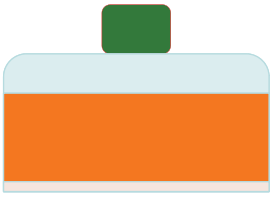


Gas permeable membrane allows optimal exchange of CO2 and O2
Supports cell growth with large volumes of media
No rocking or stirring

Juan Vera, (

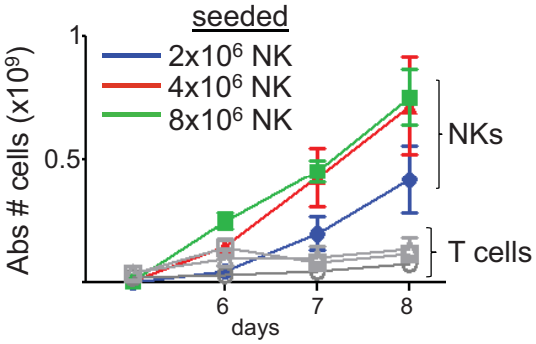
Gas Permeable Rapid Expansion Device (Wilson Wolf - G-Rex)

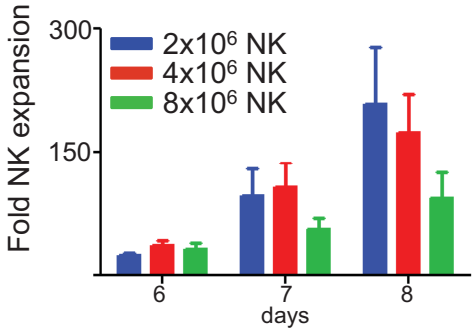
- Dilution of waste
  - Does not become acidic
- Minimizes manipulation
- Low harvest volume

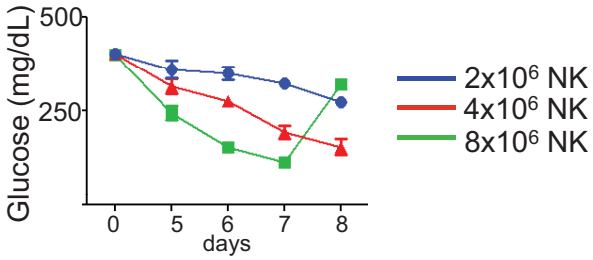


Optimization of NK Expansion in G-Rex

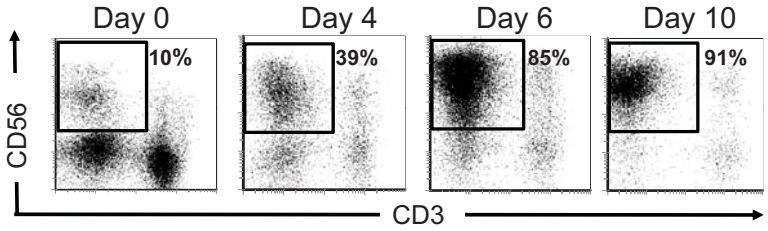
>200-fold expansion in GRex100 up to 2.0 x10^9 cells/G-Rex100

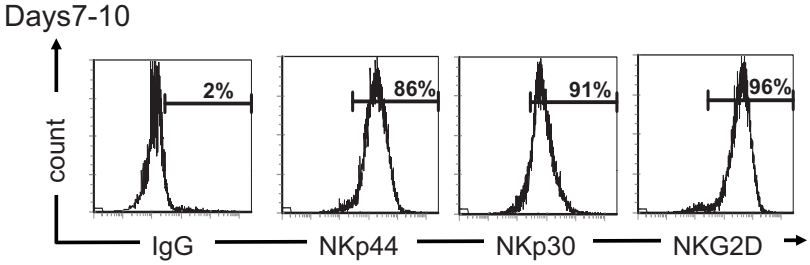




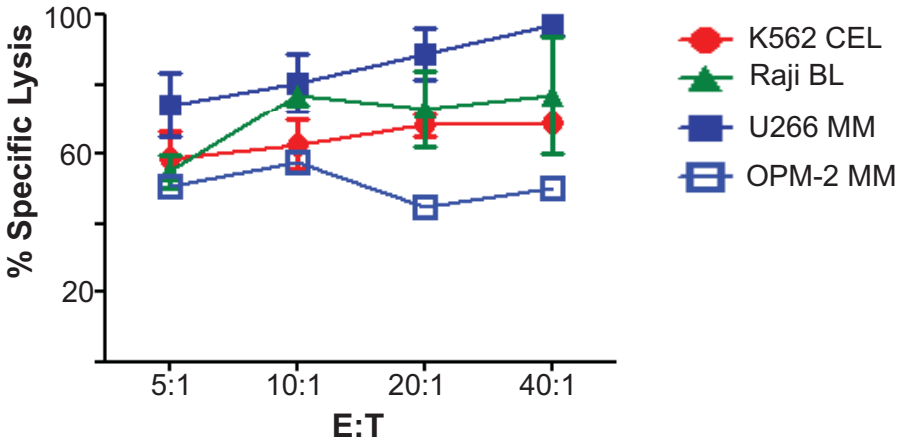


Expression of NK Activation Markers

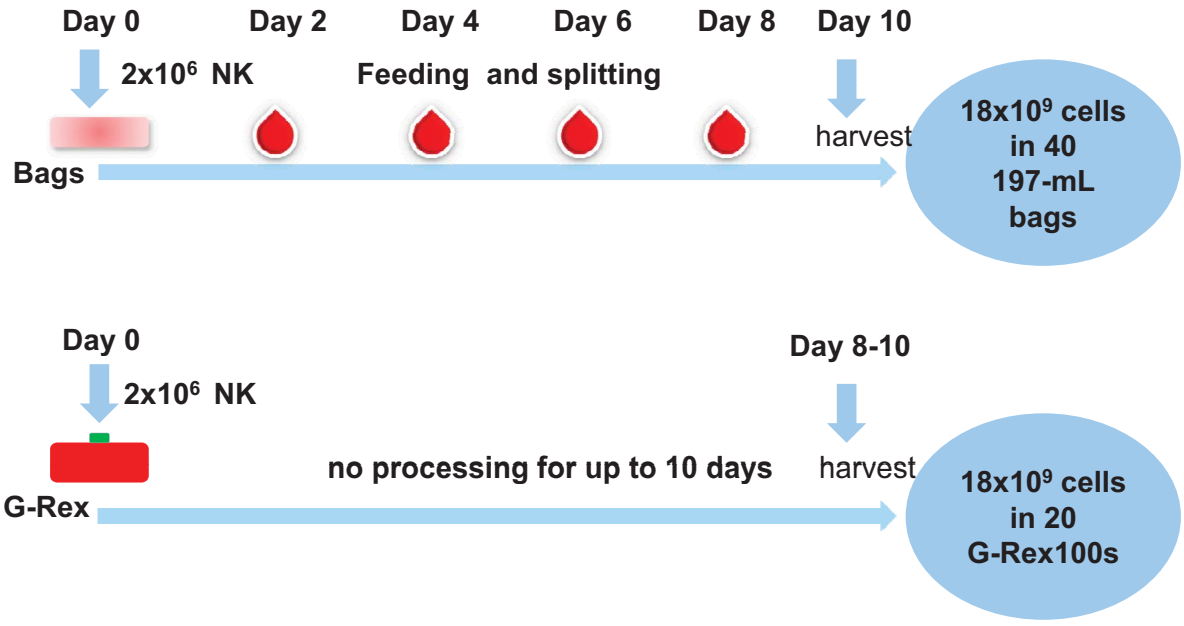




Cytotoxicity of Expanded NK cells



NK Expansion in Bags and G-Rexes



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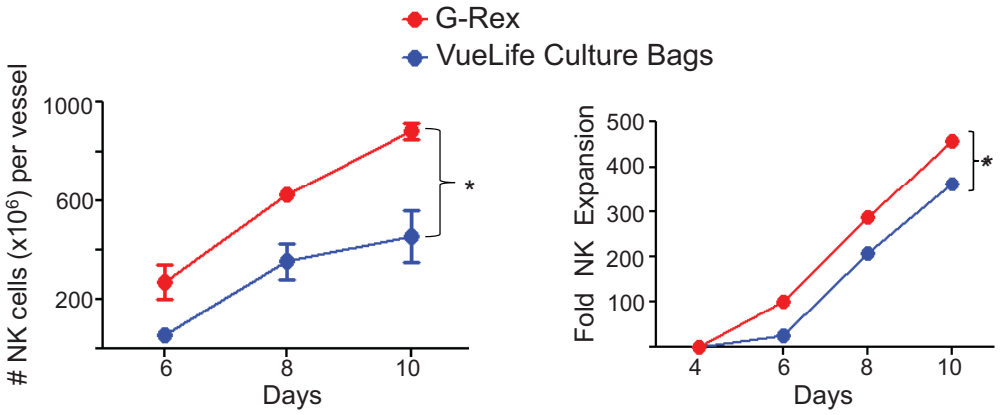
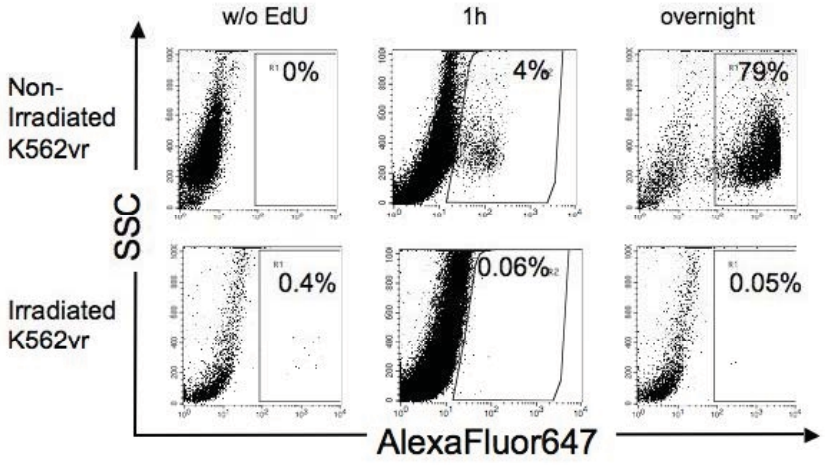


In-Process Assay Validation
Click-It Assay

5-7 days post-irradiation

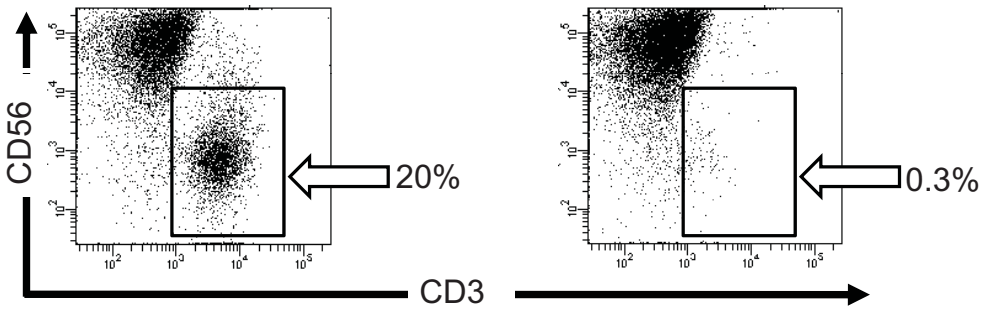


Irradiated K562vr feeder cells do not proliferate
Based on uptake of EdU by 0.05% of cells

In-Process Procedure Validation

Allogeneic Products: Depletion of $CD3^+$ T cells using CliniMACS (depletion 2.1 program)

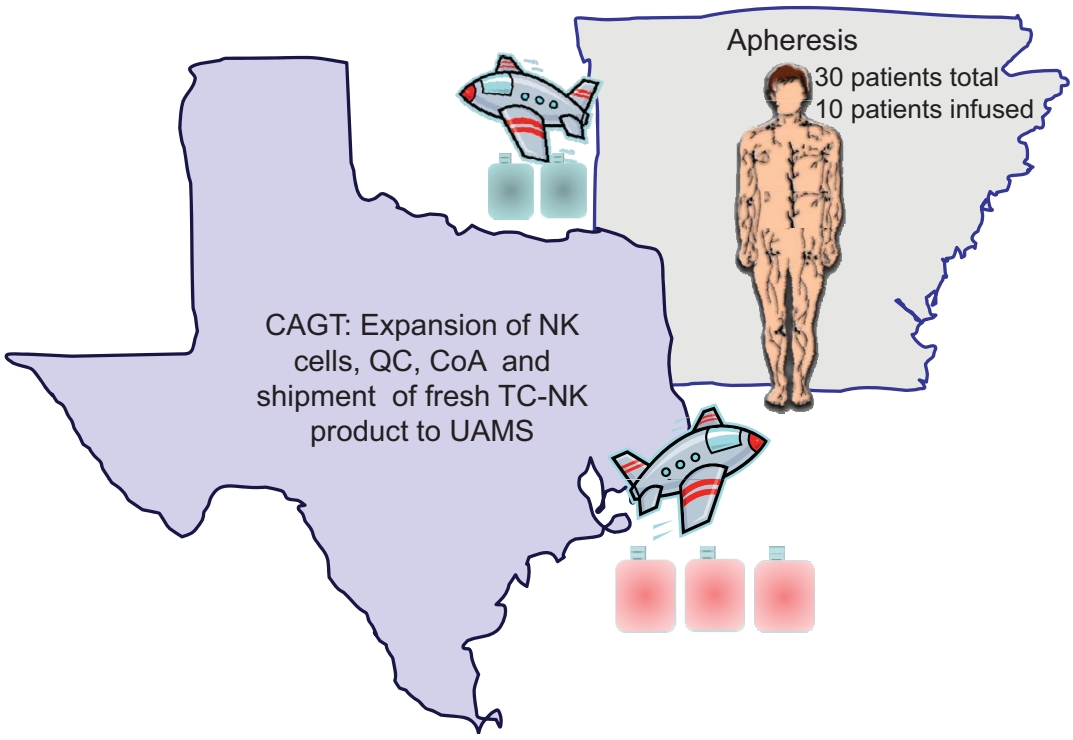
Before CliniMACS **$CD3^-$ fraction**



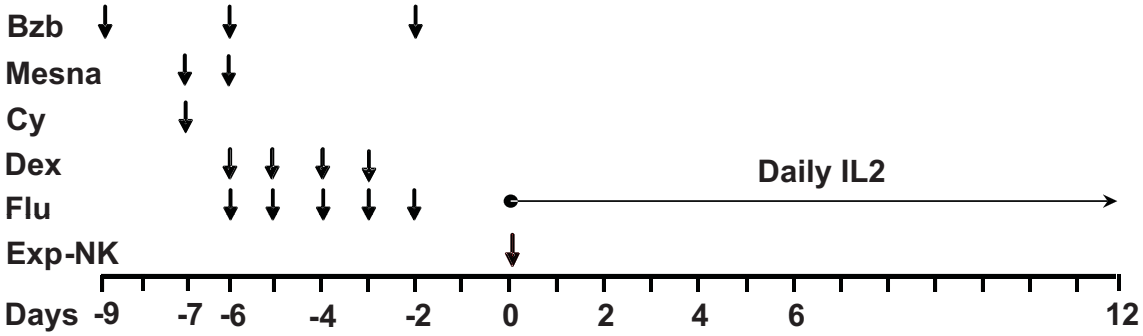
Release criterion: $<5x10^5$ **(<0.2-1%)** $CD3^+CD56^-$ T cells per kg for $2\text{-}10x10^7$ TNC per kg

Clinical Protocol Logistics

Texas **Arkansas**

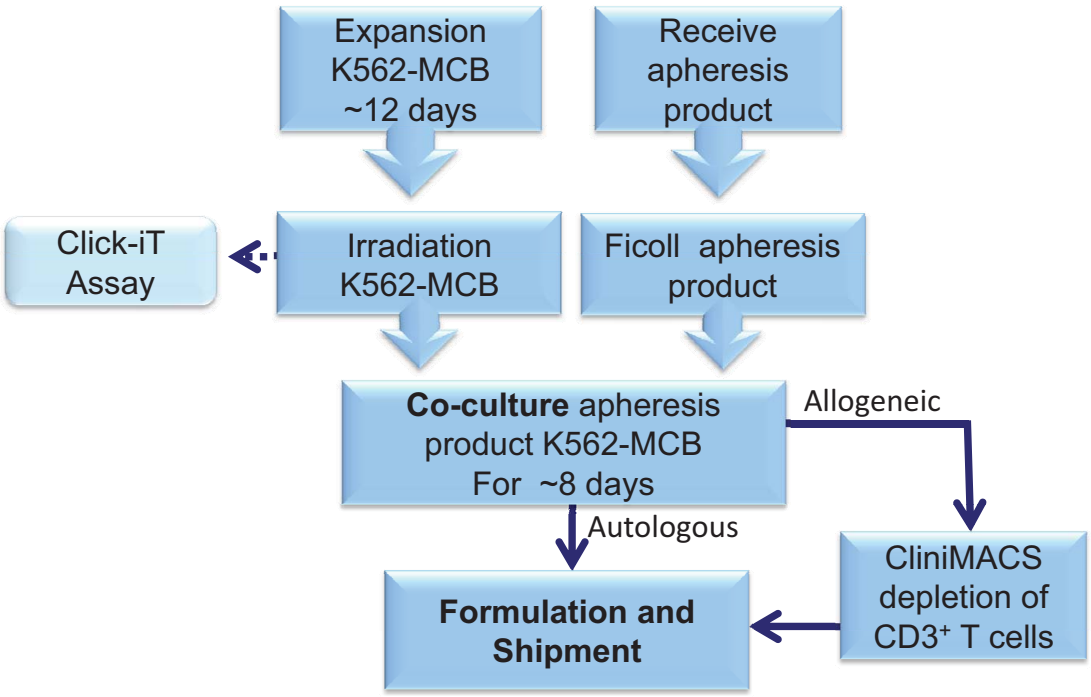


Treatment Schema for Allo-NK In Myeloma Patients



Treatment	Dose	Purpose
Bortezomib	1.0 mg/m^2	Reduce HLA, increase TRAIL on MM
Mesna	30 mg/kg i.v.	Prevention of hemorrhagic cystitis
Cyclophos	60 mg/kg i.v.	Tumor debulking, immunosuppression
Dex	40 mg PO	Tumor debulking, immunosuppression
Fludarabine	25 mg/m^2 i.v.	Immunosuppression
Exp-NK cells	2-10x10^7/kg	Lysis of MM
Interleukin-2	3x10^6 U s.c.	Support NK activity, persistence

NK Cell Production Outline



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

Non CD3 Depleted products

autologous

Subject	NK %	T %	Viability %	Potency
Val1	52	34	72	63.4
Val2	69	19	86	61.9
1	88	4	83	65
4	84	6	92	39

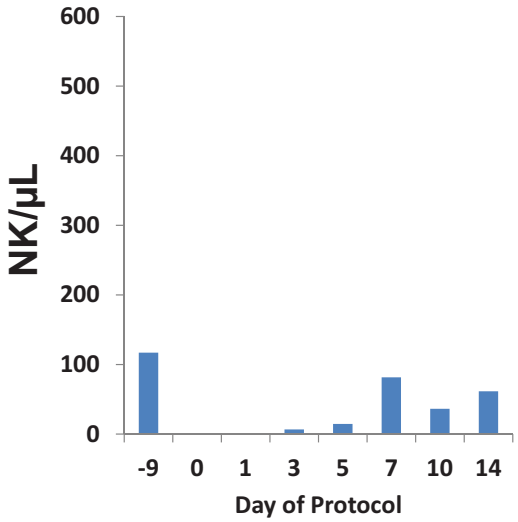
CD3 Depleted products

allogeneic donors

Subject	NK %	T %	Viability %	Potency
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

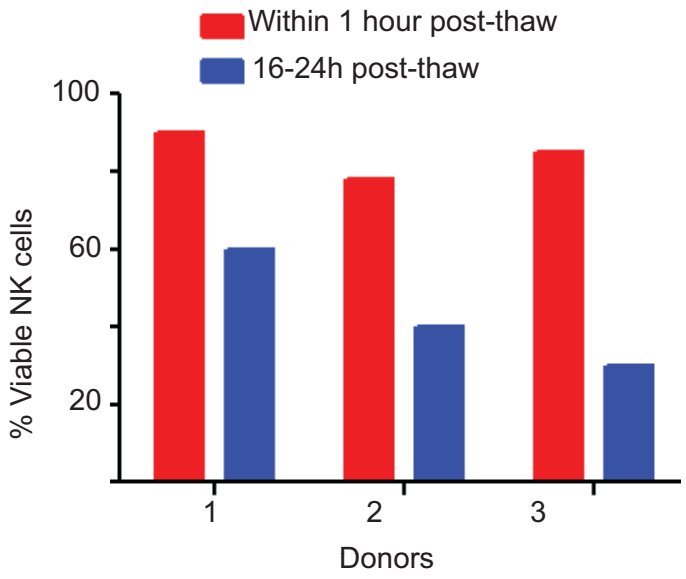
No Increase in Frequency of NK Cells After Infusion



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	$3.2x10^7$/kg	94%	94%
2A	$2.1x10^7$/kg	100	$3.4x10^7$/kg	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

Frozen NK Cells Are Suboptimal



Can we ship fresh NK products?

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

Frozen or Fresh NK Products

Frozen

One product for multiple infusions

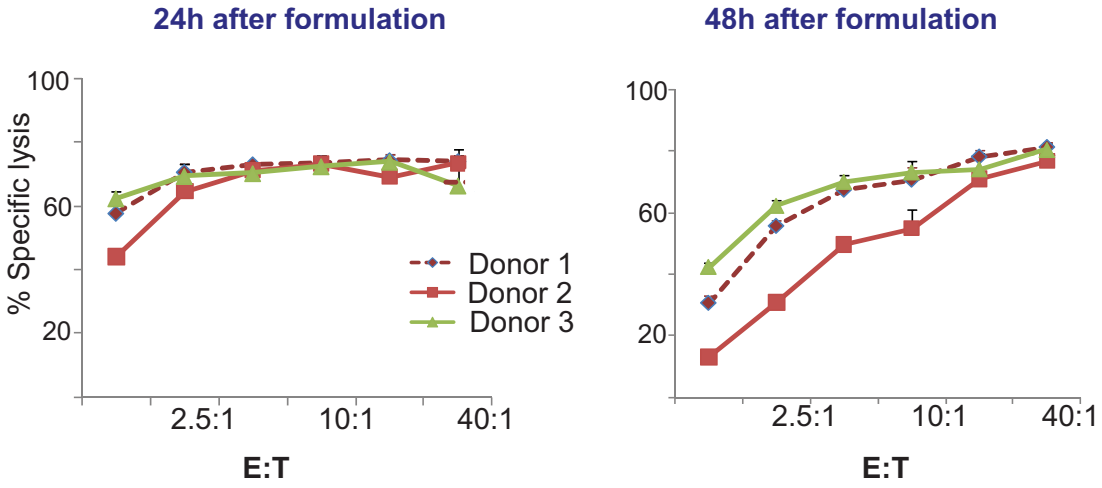
Post- thaw
- Immediately
  - Good viability
  - Poor cytoxicity
- 24hours post thaw
- Poor viability
- Good cytotoxicity
- Expand poorly *in vivo*

Fresh

One product for one infusion

How do Fresh NK cells ship?
- Viability?
- Cytotoxicity?
- Subsequent expansion?

Fresh NK Potency is Retained After Shipping



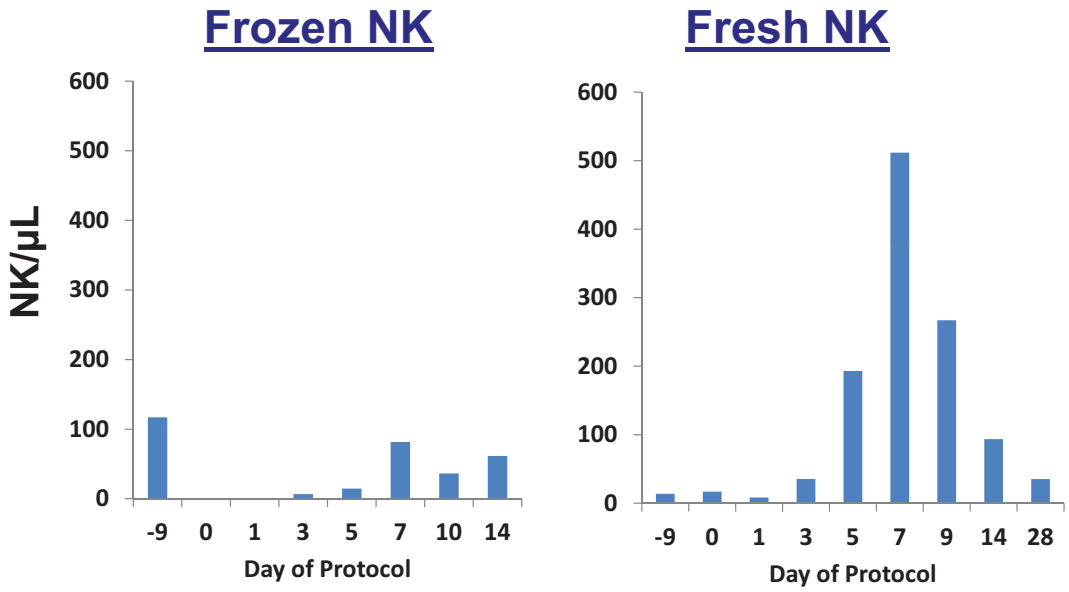
Fresh NK Continue to Expand During Shipping

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

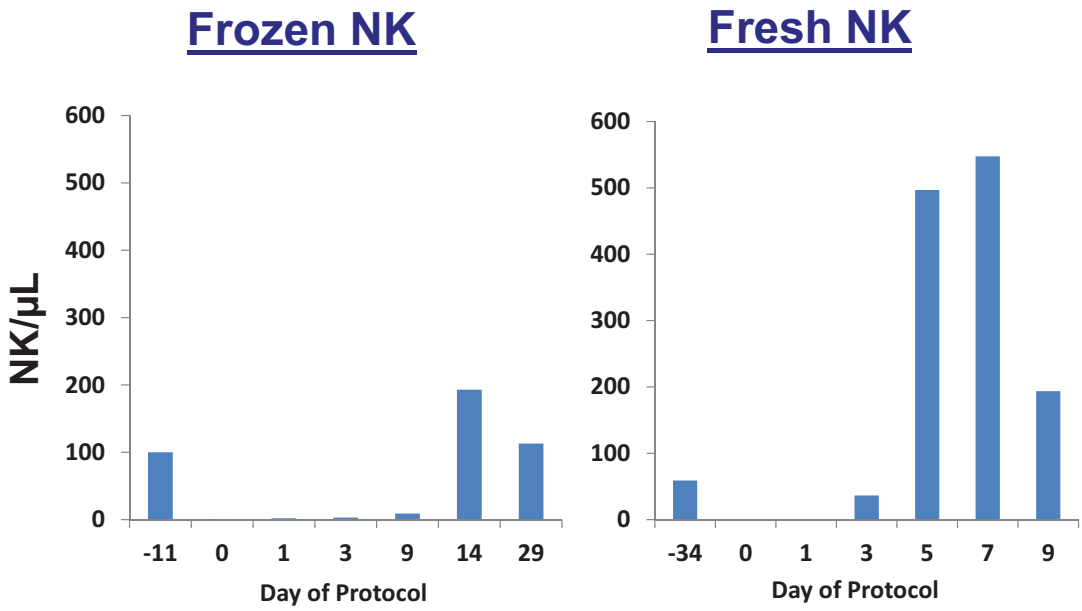
Fresh NK Purity and T cell Content

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*



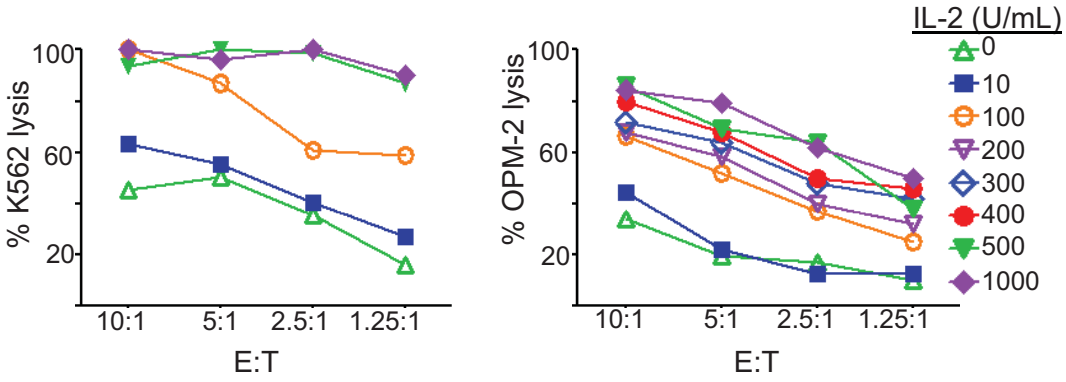
Fresh Allo-NKs Expand *in vivo*



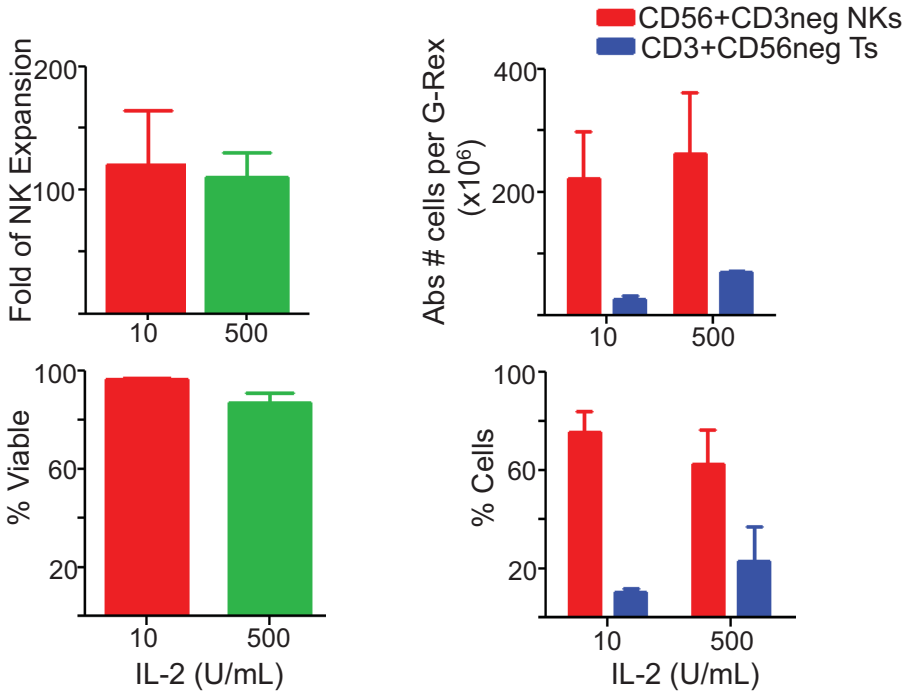
- Good In Vivo Expansion
- No Tumor Responses

Can we grow more potent NK cells?

Increasing IL-2 *In Vitro* Improved NK Potency

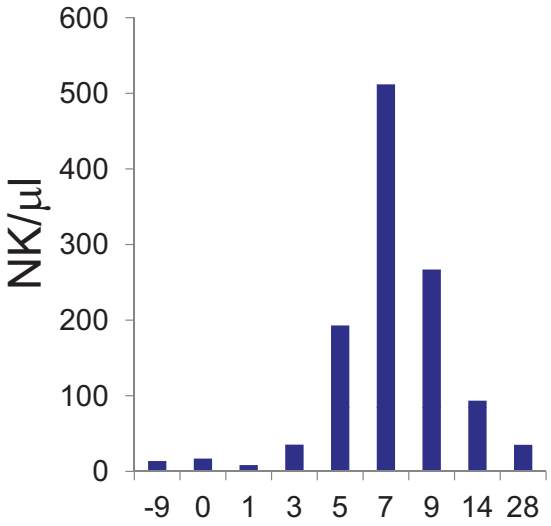


Similar Rate of Expansion
10 U/mL Vs 500 U/mL IL-2

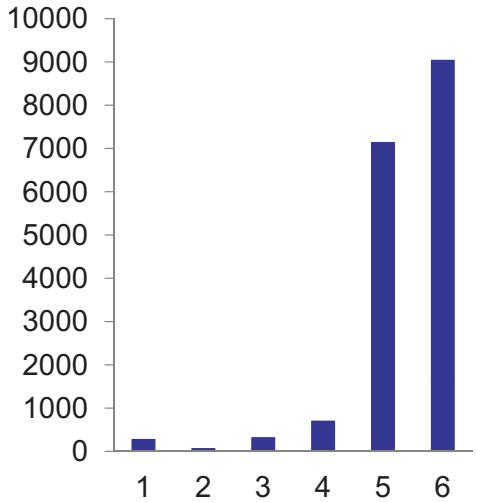


Expansion in vivo Auto-NKs

Fresh NK low IL-2



Fresh NK high IL-2



Days of protocol

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 is improved with higher IL-2 during manufacturing**

Acknowledgements

CAGT

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