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Considerations for cGMP Production of Patient-Specific Cell Therapeutics – A PACT Case Study

Derek J. Hei, Ph.D.
Director, Waisman Biomanufacturing
University of Wisconsin

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National Heart Lung and Blood Institute
People Science Health

PACT Production Assistance for Cellular Therapies
National Heart Lung and Blood Program

Challenges for Patient-Specific Cell Therapeutics

- Autologous or allogeneic cell therapeutics with patient-specific donor (not “off-the-shelf” therapy)
- Manufacturing cost – cost of goods, Quality Control testing, cleanroom time
- Scale-up vs. scale-out
- Manufacturing logistics – timing, QC testing
- Patient/donor variability and impact on manufacturing process
- In some cases repeat dosing is highly desirable – increased cost

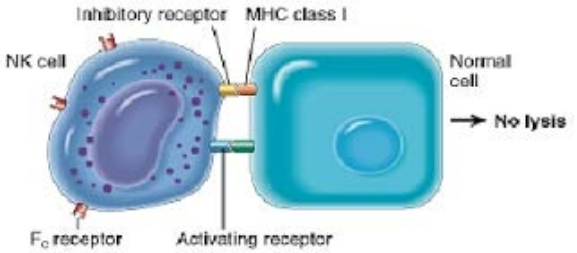
PACT Project Overview

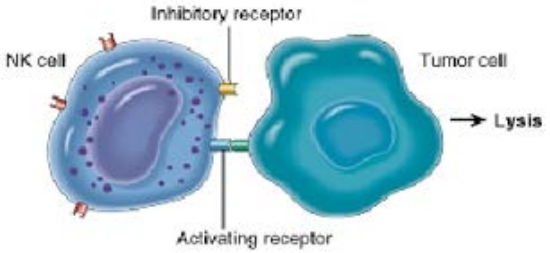
Solving Kids' Cancer™
every kid deserves to grow up

- Proposed clinical trial -
  - PI – Dr. Ken DeSantes, UW Carbone Cancer Center
  - Neuroblastoma – relapsed/refractory in pediatric patients
  - Haploidentical NK cells from KIR-mismatched parent
  - hu14.18-IL2 immunocytokine - humanized anti-GD2 mAb linked to IL-2 (Osenga et al., Clin Cancer Res 2006; 12(6):1750)
  - Goal – multiple doses, preferably from single manufacturing process
- Initial proposed target dose –
  - 1E6 NK cells/kg escalate to 1E8 NK cells/kg
  - Potential for up to 4 doses per patient
  - Requires up to 2.8E10 CD56+ cells for 70 kg patient
  - T cell reduction is critical < 5E4 $CD3^+$ cells/kg
- Ex-vivo expansion of NK cells using K562-mbIL15-41BBL feeder cells (Campana et al., Cancer Res 2009; 69(9):4010-7)

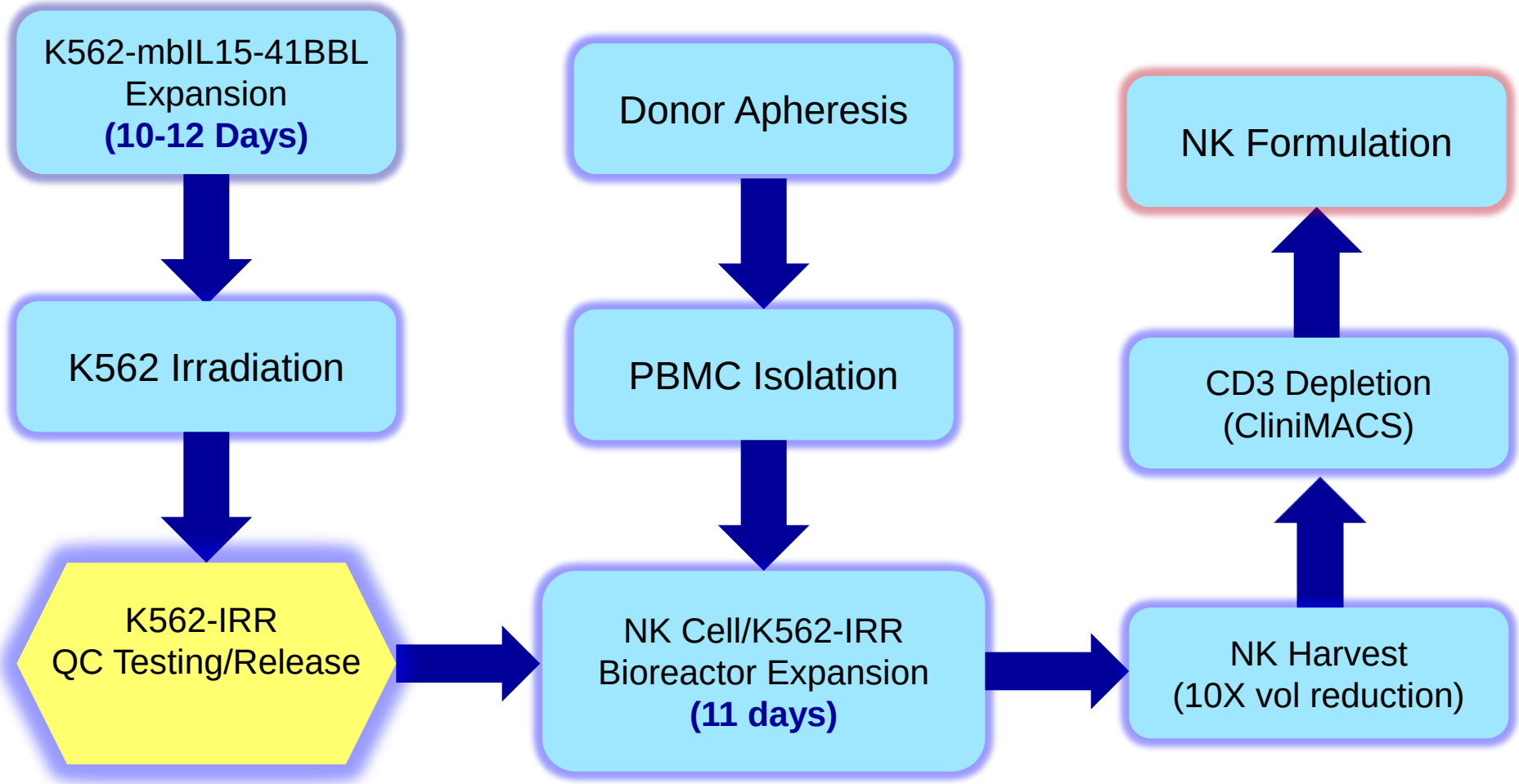
Natural Killer Cells

- Cytotoxic lymphocytes that are active against cells infected with viruses and intracellular pathogens
- NK cell killing determined by inhibitory and stimulatory ligands expressed by malignant and infected cells including lack of MHC expression
- Currently under evaluation as therapy against a wide range of cancers including AML, ALL, NSCLC, multiple myeloma
- $CD56^{+}$ $CD3^{-}$ cells comprising 5-20% of circulating monocytes
- Potential sources –
  - PBMNCs from Apheresis with immunomagnetic selection (CD56+ / CD3-), IL-2/IL-15 activation
  - Ex vivo expansion using stimulatory “feeder” cells (K562-mbIL16-41BBL)





NK Cell Manufacturing Process



- 21-23 day manufacturing process
- Quality Control testing logistics for K562-IRR intermediate
- Goal to produce 3-4 doses/patient

Potential Improvements to NK Cell Manufacturing Process

- K562-mbIL15-41BBL production and testing
  - Ability to produce irradiated, cryopreserved cells that retain function in NK cell expansion?
  - Ability to scale-up K562-IRR production
- NK cell expansion -
  - Ability to produce multiple doses in a bioreactor?
  - Ability to cryopreserve NK cell product and maintain function

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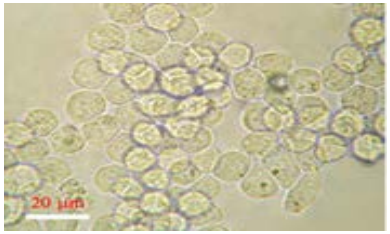
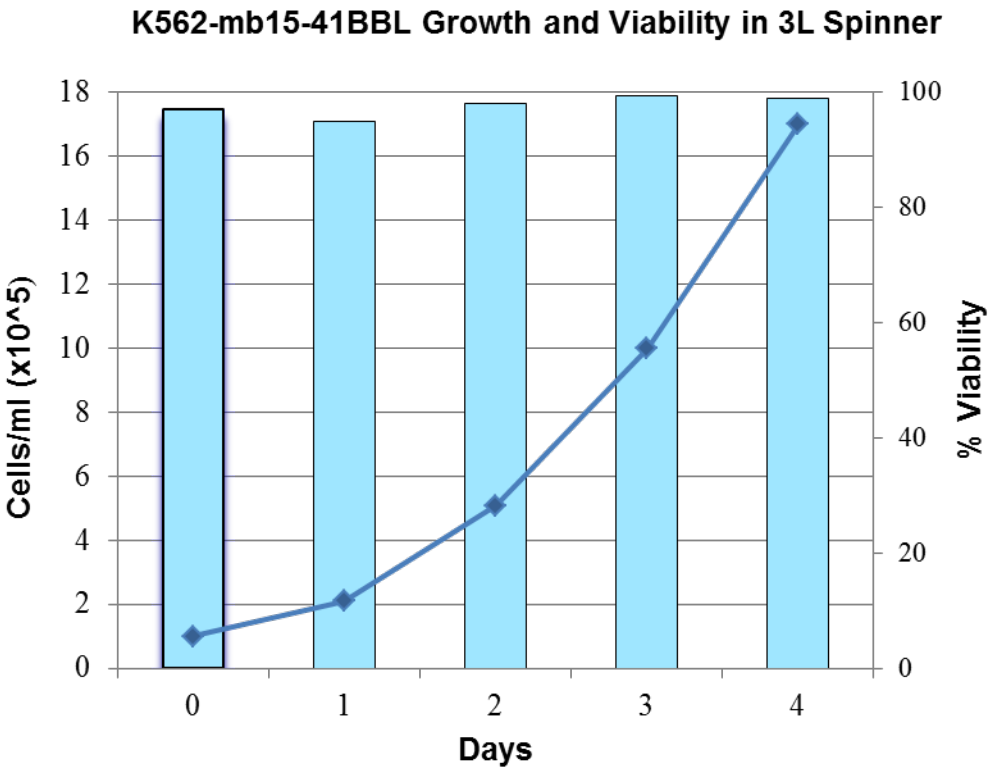
K562 Expansion and Irradiation

- Processing logistics –
  - Require 10:1 K562:CD56+ cells for NK expansion – >2E9 K562s/run
  - Expansion time for K562 cell line is approximately 2 weeks
  - QC testing for release of K562-IRR cells – time and money
- K562-mbIL15-41BBL cell banks
  - Master Cell Bank provided by Baylor PACT facility
  - Produced Working Cell Bank under cGMP
  - Adventitious agent testing



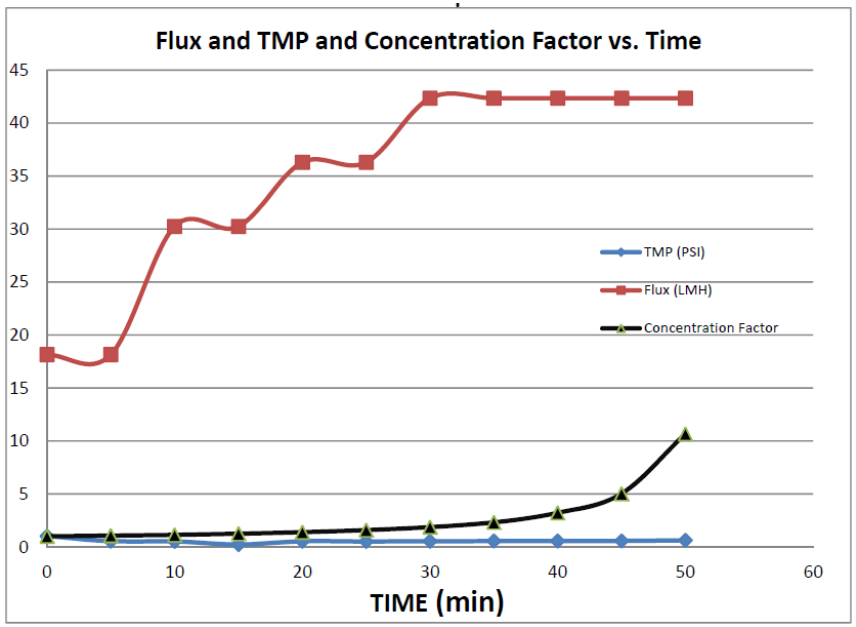
- K562-IRR production process
  - Expand K562 WCB in suspension culture – spinner flasks, bioreactor
  - Harvest and irradiate (100 Gy)
  - Cryopreservation – demonstrated acceptable recovery and function of cryopreserved K562-IRR for NK cell expansion
  - Final dose – 1E7 cells/mL, 200 mL RPMI-1640 + 20% FBS + 10% DMSO
  - Is the process scalable?

K562-mbIL15-41BBL Suspension Culture



- Maximum density for late-logarithmic growth = 1.5E6/mL
- Expansion from spinner flasks to 50L Single-Use Bioreactor (SUB)
- Projected yield = 15-20 bags at 2E9 K562 cells/bag
- Cleanroom time savings = 25-35 weeks/year

K562-mbIL15-41BBL Harvest and Recovery



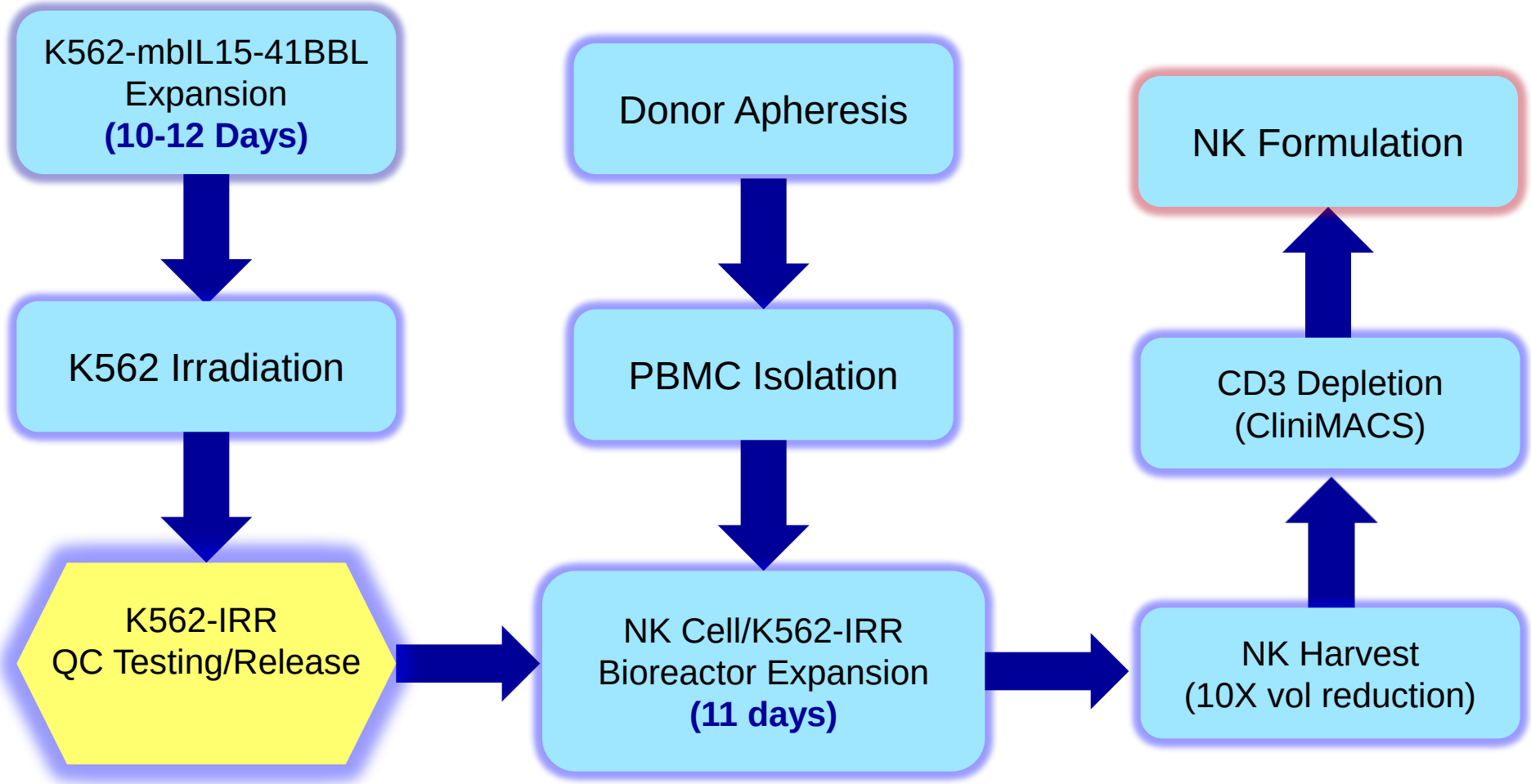
- Hollow fiber TFF system (0.65 μm)
- Sterile, completely closed single-use system
- Three pump system – fed batch with permeate flow control
- 10X volume reduction, < 1 hour, > 95% recovery

K562 Expansion and Irradiation

- Advantages of irradiated/cryopreserved K562 cells
  - Cells can be thawed for immediate expansion of NK cells
  - Decreased cleanroom time and overall process cost – 25-35 weeks/yr
  - Decreased QC testing requirements due to increased batch size
  - Improved consistency in K562 cells

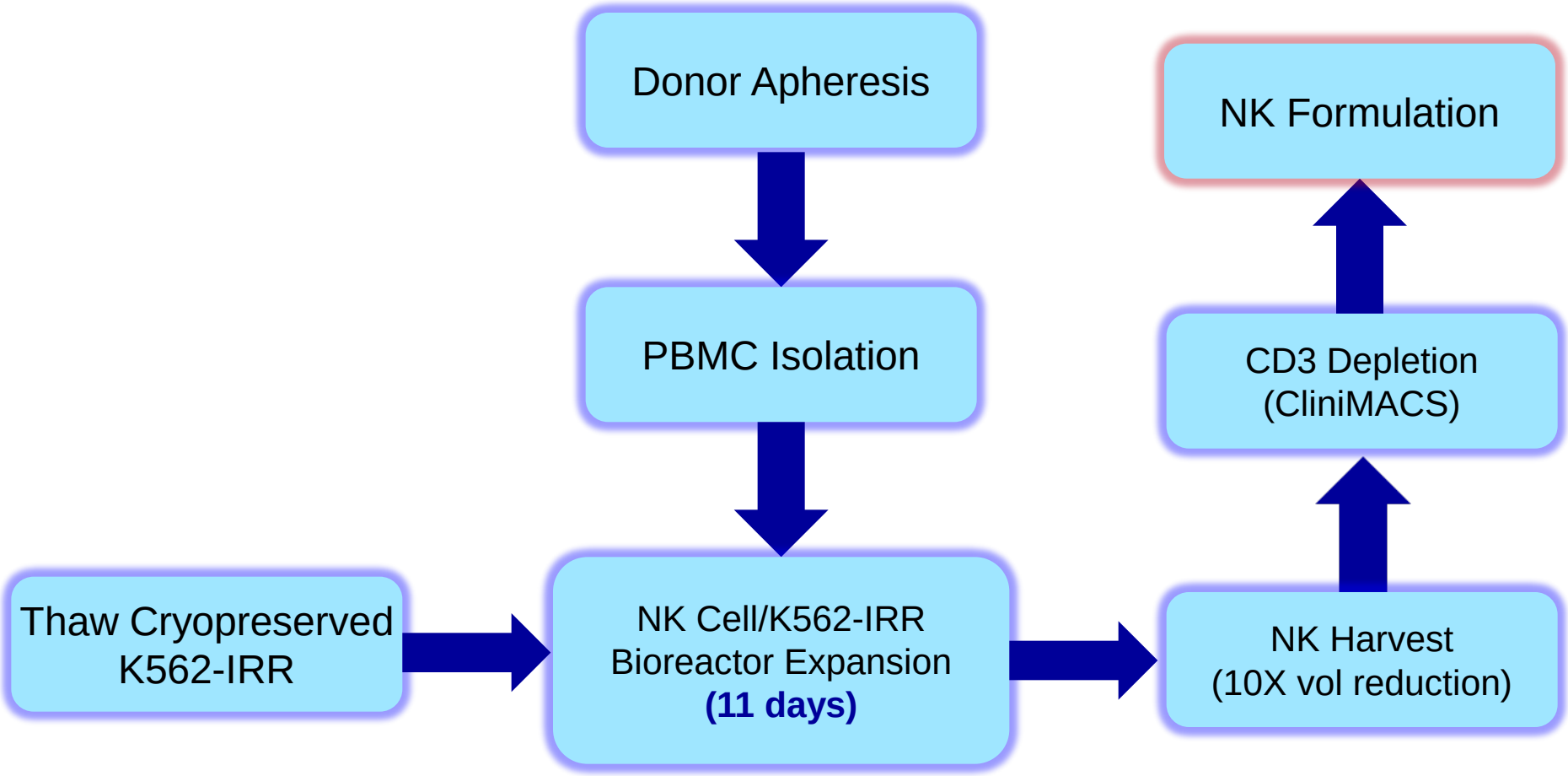
Attribute	Method	Specification
Bacterial Endotoxin	Kinetic chromogenic LAL	< 5 EU/mL
Mycoplasma	PTC method (direct and indirect culture)	No contamination detected
Sterility Test	21 CFR 610.12 bacteristasis and fungistasis	No contamination detected
Post-thaw viable cell recovery	Trypan Blue	> 70%
Residual uninactivated K562 cells	Click-it® Cell Proliferation assay Perform post-irradiation	< 0.1%

NK Cell Manufacturing Process



- 21-23 day manufacturing process
- Quality Control testing logistics for K562-IRR intermediate
- Goal to produce 3-4 doses/patient

Overview of NK Cell Manufacturing Process



- 11 day manufacturing process
- Cryopreserved K562-IRR intermediate with full QC testing
- Goal to produce 3-4 doses/patient

Potential Improvements to NK Cell Manufacturing Process

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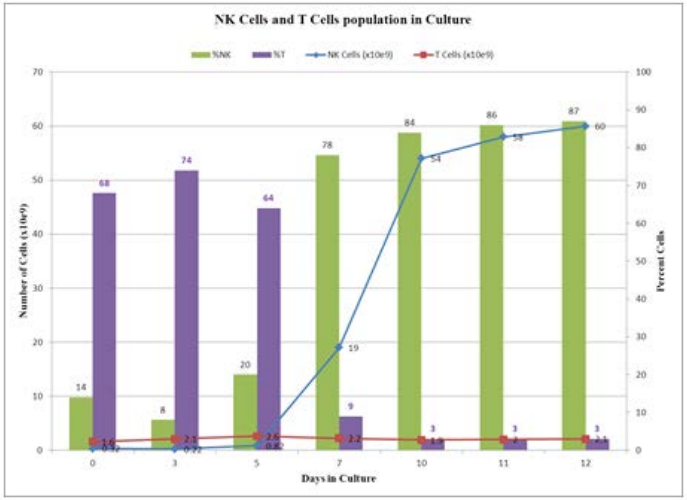
  - Ability to produce irradiated, cryopreserved cells that retain function in NK cell expansion?
  - Ability to scale-up K562-IRR production
- NK cell expansion -
  - Ability to produce multiple doses in a bioreactor?
  - Ability to cryopreserve NK cell product and maintain function

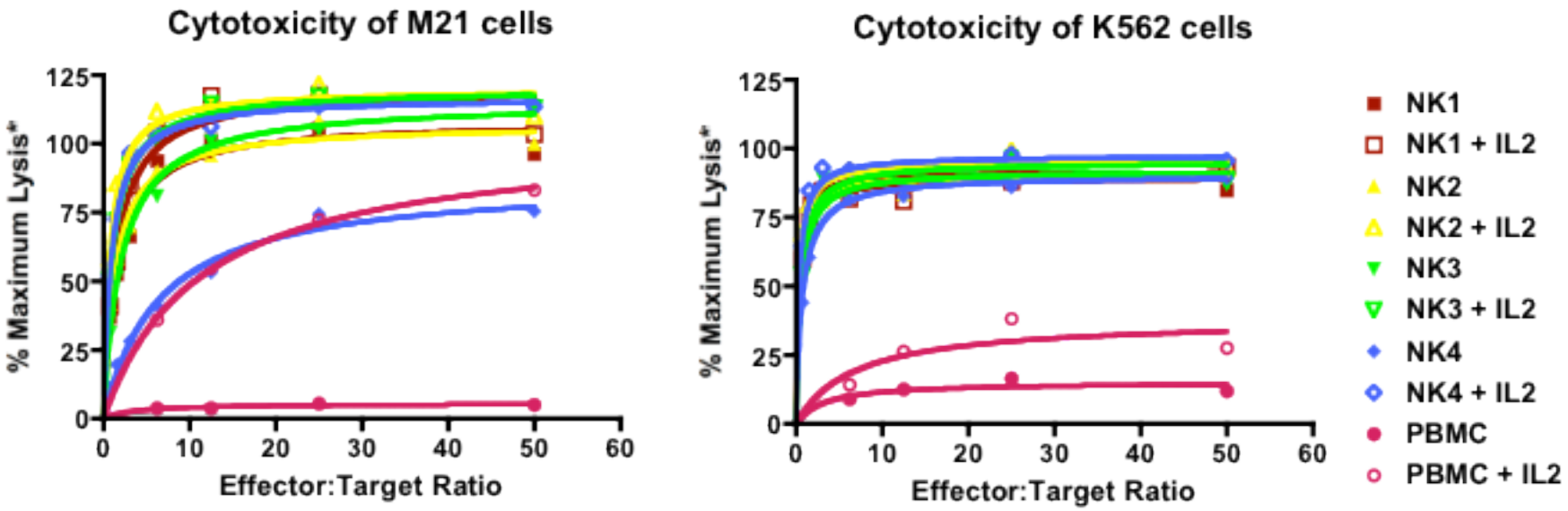
NK Cell Expansion Process

- Apheresis unit – 4-8E8 PBMCs, 5-20% NK cells
- NK expansion in Wave 20 Bioreactor
  - 10:1 K562:NK cells (irradiated, cryopreserved)
  - XVivo 10/hAB serum, 100 U/mL hIL-2
  - 60-150X expansion over 11 days
- NK harvest – Cobe, TFF
- CD3 depletion - CliniMACS
- Release first dose as fresh NK cells



- Cryopreserve additional 3 doses of NK cells
  - Goal: 1E6 NK/kg escalate to 1E8/kg, up to 4 doses per patient
  - 2E7 cells/mL, 100-350 mL dose
  - Can NK cells be cryopreserved and maintain viability and activity?

Cytotoxicity is Maintained in Expanded NK Cells



*Maximum Lysis based on ^{51}Cr release using Cetrimide Detergent

Kimberly A. McDowell MD, PhD
Department of Pediatrics
Division of Hematology, Oncology and Bone Marrow Transplant

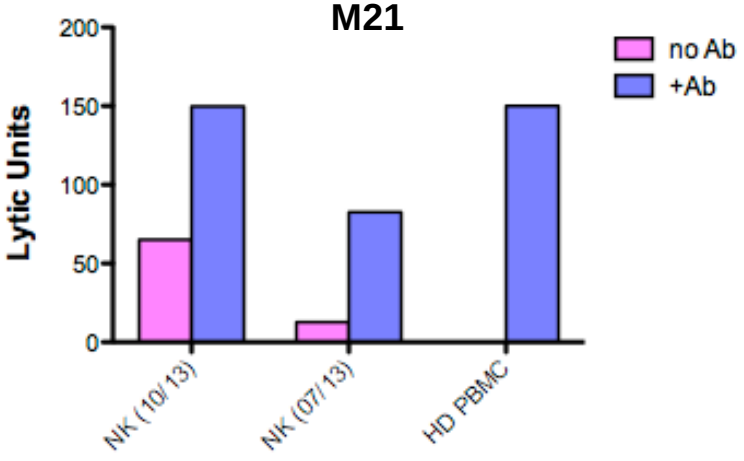
Cryopreservation of Expanded NK Cells

- NK cells cryopreserved following expansion in Wave bioreactor at $2.0x10^7$cells/mL
- 1 fresh dose, 3 cryopreserved doses
- Two cryopreservation media evaluated in initial PD studies
  - Initial viable cell recovery >80%, delayed onset cell death
  - Plasmalyte + 5% HSA + 5% DMSO 18-36% recovery
  - BioLife Solutions Cryostor CS5 Medium 45-55% recovery
- Process Qualification trials (N=3)
  - 40% human AB serum, 50% Plasmalyte, 10% DMSO (Dean Lee, MDACC)
  - Viable NK recovery > 90% post thaw and wash
  - Cell washing process qualified

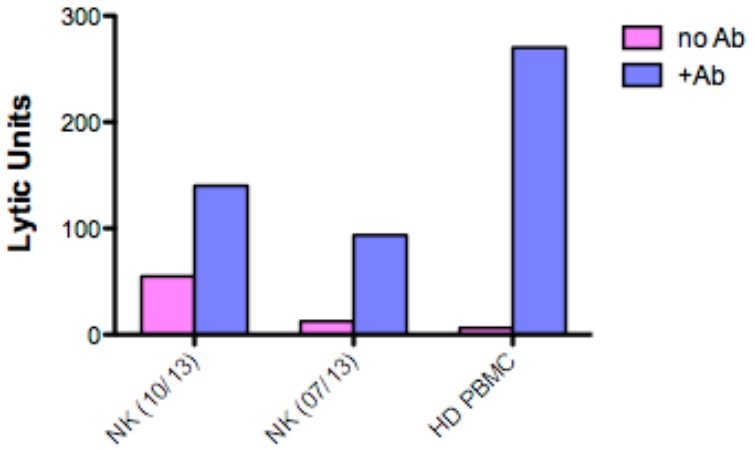
Cytotoxicity Testing of Cryopreserved NK Cells

Cytotoxicity of NK cell resistant cell lines

Melanoma cell line M21

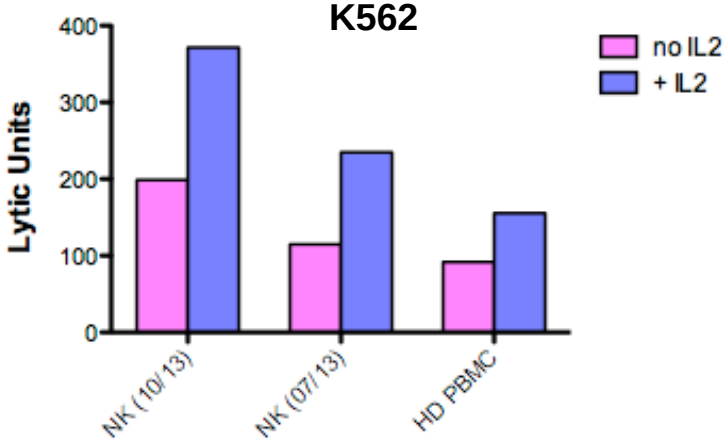


Neuroblastoma cell line



Cytotoxicity of NK cell sensitive cell line

CML cell line K562



Ab = hu14.18K322A (100 ng/ml)
IL2 = Interleukin 2 (100 units/ml)
4 hour ^{51}Cr assay

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Process Qualification Trials

- Process Qualification trials – demonstrate process reproducibility and impact of donor variability
- NK cell expansion in Wave bioreactor
  - 1.3 – 6.0 E10 NK cells
  - 30-200 fold expansion
- CliniMACS CD3 depletion
  - < 0.1% residual T cells
  - > 87% $CD56^{+}$ $CD3^{-}$ cells
- Thaw/wash qualification studies
  - Validate process – sterility, endotoxin, viable cell recovery
  - Stability study performed out to 6 months on frozen NK cells
- Donor variability – still a challenge
  - In-process testing to predict final process outcome
  - Process adjustments based on in-process tests?

Conclusions

- For autologous/patient-specific cell therapeutics its critical to address manufacturing logistics, scale-out, and manufacturing cost issues early in development
- Donor/patient variability will continue to be a challenge – in-process testing and process adjustments are key in addressing this issue
- QC testing logistics on final fresh and thawed/washed product should be addressed along with shipping and post-thaw processing issues

Special Thanks To…

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A National Heart Lung and Blood Institute Resource

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