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

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

September 25, 2014
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

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-mbI16-41BBL)
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
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
**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

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Method</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial Endotoxin</td>
<td>Kinetic chromogenic LAL</td>
<td>&lt; 5 EU/mL</td>
</tr>
<tr>
<td>Mycoplasma</td>
<td>PTC method (direct and indirect culture)</td>
<td>No contamination detected</td>
</tr>
<tr>
<td>Sterility Test</td>
<td>21 CFR 610.12 bacteristasis and fungistasis</td>
<td>No contamination detected</td>
</tr>
<tr>
<td>Post-thaw viable cell recovery</td>
<td>Trypan Blue</td>
<td>&gt; 70%</td>
</tr>
<tr>
<td>Residual uninactivated K562 cells</td>
<td>Click-it® Cell Proliferation assay</td>
<td>&lt; 0.1%</td>
</tr>
</tbody>
</table>
**NK Cell Manufacturing Process**

- K562-mbIL15-41BBL Expansion (10-12 Days)
- Donor Apheresis
- PBMC Isolation
- CD3 Depletion (CliniMACS)
- NK Formulation
- NK Cell/K562-IRR Bioreactor Expansion (11 days)
- NK Harvest (10X vol reduction)
- K562-Irradiation
- K562-Irr QC Testing/Release

- 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

- Donor Apheresis
- PBMC Isolation
- Thaw Cryopreserved K562-IRR
- NK Cell/K562-IRR Bioreactor Expansion (11 days)
- CD3 Depletion (CliniMACS)
- NK Formulation
- NK Harvest (10X vol reduction)

- 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

- 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
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
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 it’s 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…

Waisman Biomanufacturing

Bryan Atkinson  Diana Drier  Ross Meyers  Bryan Atkinson
Chris Bartley  Heather Dunn  Carl Ross  Chris Bartley
Jaime Bellon  Rebecca Ertel  Natalie Russell  Jaime Bellon
Alan Bettermann  Michael Hainstock  Josh Sotos  Alan Bettermann
Neehar Bhatia  Jen Jauquet  Tim Sparks  Neehar Bhatia
Janice Boyer  Bill Kreamer  Megan Stone  Janice Boyer
Paula Brisco  Laurie Larson  Kari Thostenson  Paula Brisco
Lisa Burdette  Connor Lyons  John Welp  Lisa Burdette
Brian Dattilo  Eric Mauer  Kathy Yee  Brian Dattilo

UW PACT Team

Peiman Hematti,  Deb Bloom,  Jaehyup Kim  Peiman Hematti
Amish Raval,  John Centanni,  Eric Schmuck  Amish Raval
Tim Hacker,  Jill Koch  Tim Hacker
Marlowe Eldridge,  Ruedi Braun  Marlowe Eldridge

UW Carbone Cancer Center

Ken De Santes, MD  Ken De Santes
Paul Sondel, MD, PhD  Paul Sondel
Kim McDowell, MD, PhD  Kim McDowell