Designing Technologies to Meet the Manufacturing Needs of New Regenerative Therapies

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National Academy of Science Engineering Medicine 2017 RM workshop
Disclosure

- Scientific co-founder: Juno Therapeutics
- Consultant: Juno Therapeutics
- Research support: Juno Therapeutics
**CTCEF Platforms**

- **Cancer**
  - iPSCs
  - Lymphocytes
  - CAR T cells
  - CAR γ-retroviral vectors
  - CRISPR-Cas9

- **Hemoglobinopathies**
  - G-CSF PB HPSCs
  - CD34+
  - Globin Lentiviral vectors

- **Parkinson**
  - ESCs
  - DA neurons

- **NYSTEM Consortium #1**
  - Rafii, Sadelain, Scandura, Boyle, Butler, Barker, Boulad, Wang

- **NYSTEM Consortium #2**
  - Studer, Tomishima, Irion

**Collaborators**: MSKCC/Weill/Chicago/Industry

**Sadelain, Brentjens Eyquem, Mansilla-Soto, Wang**
Examples of Unit Operations for Cell Manufacturing

- **Cell Preparation**
  - COBE 2991
  - Cell Saver
  - LOVO
  - Elutra
  - Sepax

- **Selection**
  - DynaMag CTS
  - Dynabeads MPC

- **Activation**
  - ClinMACS Plus
  - G-Rex
  - ClinMACS Prodigy

- **Expansion**
  - Xuri/Wave
  - Rocking/Motion Bioreactors/ Cocoon™

- **Transduction**
  - Centrifuge

- **Formulation (wash + concentration)**
  - Controlled rate freezers

- **Cryopreservation**
  - Controlled rate freezers

Adapted from Kaiser et al. Cancer Gene Ther. 2015 Mar; 22(2): 72–78
CAR T cell Manufacturing Flow (v1.0)

1. Day 0 Apheresis
   Thaw/Wash (Cell Washer)

2. Incubation
   Dynabeads CD3/28

3. Transduction
   Retroviral Vector

4. CD3+ enriched
   Activated T cells

5. Day ≥5
   Inoculation
   WAVE/Xuri
   Bioreactor

6. CAR/TCR transduced
   T cells

7. DAY ≥ 7
   Debeading
   ClinExVivo MPC

8. CAR+/TCR+
   expanded T cells

9. Cryopreservation
   (CRF)

10. Biosafety/
    QC release tests
    CofA

Validation

In vivo antitumor activity
in SCID/NSG mice, CTL

Hollyman et al, J. Immunother, 2009
Themeli, Riviere, Sadelain, Cell Stem Cell, 2015
# Adult ALL NCT 01044069 (PI: J. Park, MD)

Study Outcome Complete Remission (CR) Rates (ASCO 2016)

<table>
<thead>
<tr>
<th></th>
<th>Morphologic Disease N=30 (%)</th>
<th>Minimal Disease N=20 (%)</th>
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<tbody>
<tr>
<td>CR Rate</td>
<td>23 (77%) [58 – 90]</td>
<td>18 (90%) [68 – 99]</td>
</tr>
<tr>
<td>MRD negative CR Rate*</td>
<td>19/21 (90%) [70 – 99]</td>
<td>14/18 (78%) [52 – 94]</td>
</tr>
<tr>
<td>Time to CR, Mean (SD)</td>
<td>20 days (9)</td>
<td>25 days (9)</td>
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*MRD assessment was not available in 2 patients.

<table>
<thead>
<tr>
<th>Disease Status</th>
<th>CAR T Cell Dose</th>
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<tbody>
<tr>
<td>Morphologic disease (≥5% blasts in BM or EM disease)</td>
<td>1 x 10^6 CAR T cells/kg</td>
</tr>
<tr>
<td>Minimal disease (&lt;5% blasts in BM)</td>
<td>3 x 10^6 CAR T cells/kg</td>
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Hurdles for the establishment of robust manufacturing platforms

Lesson 1

Starting material heterogeneity
Apheresis collection device, disease indication/stage, previous chemo.

Process initiation, cell selection, expansion
PBMCs to selected cell types CD3, CD4, CD8 (CDX: active ingredients)
Removal undesirable cell types (e.g. CD14)
How to maintain the desired cell types in these “living drugs”
Limited number and high cost of GMP grade antibodies

IP encumbered reagents

EQT/tool providers commitment and timelines (e.g. Cytomate)
EQT/tool characteristics (e.g. cell washers processing time vs working volumes)
Single manufacturers back-up needed
Compatibility between unit operations
Alternative cell separation techniques e.g. label free cell sorting fluidics
Alternative CAR T cell Manufacturing Platform (v2.0)

Day 0
Apheresis
Cell Wash
(Cell Saver5, Lovo, Sepax)

Day 0
Positive Selection CD4/CD8

Incubation
Dynabeads or Transact beads

Day 2 or 3
Transduction
CAR Vector

CD3+ enriched
activated
T cells

CAR transduced
T cells

Patient Infusion

Comparability
Validation/Process
CAR T cell characterization,
In vivo antitumor activity
in NSG mice, CTL

Changes under investigation:
- Positive selection on Clinimacs
- Activation with TransAct beads
- No debeading EQT post activation

Wang et al, J Immunother., 2015
Alternative CAR T Cell Manufacturing Platform: CliniMACS Prodigy (v3.0)

- Cell washing and density gradient separation
- Cell Separation
- Cell cultivation
- Complex protocols
- Final product formulation
- Closed system

Comparability
Validation/Process
CAR T cell characterization,
In vivo antitumor activity in NSG mice, CTL

Prodigy evaluation for CAR T cell manuf.: NCI funding
Hurdles for the establishment of robust manufacturing platforms
Lesson 1 (cont.)

Starting material heterogeneity
Apheresis collection device, disease indication/stage, previous chemo

Process initiation, cell selection, expansion
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Early Academy-Industry partnership limitations

Lesson 2

Difficulty of providing patient samples to industry partners (Aph, CAR T cells, blood samples post-infusion) for PD, analytical assay improvement/establishment: timing of partnership agreements vs timing of patient consent.

Data collection and analysis collection of manufacturing data at academic centers (electronic BPCRs difficult to achieve due to process evolution), know-how/cost/time.

Identify early on data/parameters that are valuable for manufacturing process transfer to industry.

Difficulty to reverse transfer manufacturing to improve early Phase trials at academic centers (e.g. IP, know-how).
Manufacturing at the POCs

Limited $$ for PD
Manufacturing automation & analytics development
Infrastructure (e.g. GMP facilities, testing labs)
Multicenter trials

Manufacturing at the POC vs centralized

• Viable for orphan diseases (BMT model?)
• Phase II and beyond: not likely within mission of hospitals: limited resources, infrastructure
• Multicenter trials logistics: Multiple POCs?
• Standardization: minimal IP testing
• Requirement for centralized analytical lab unless analytics are integrated and fully automated
• What level of automation can we afford at POC for multiple processes?
Michael G. Harris Cell Therapy & Cell Engineering Facility @ MSKCC

- Cellular products (e.g. T cells, HSCs, Cord Blood, ESC, iPSC)
- RNA vectors, plasmid DNA
- Phase I/II clinical trials
- Multicenter trials with other academic centers
Towards Automated Platforms for Cell Manufacturing
Increase process control, product consistency, throughput...

- In Process Analytics
  - Process – e.g. pH, O₂, CO₂, metabolites, omics, secretome, cytokine profiles, cell phenotype
  - In-Process Cells and Final Product – e.g. identity, safety, viable cell count, purity

- Microfluidics-based separation for target cell enrichment

- Real-time, built-in, non-invasive sensors
- Feedback loops
- Automated in-line QC sampling (MS, NMR or bio-analyzers)
- Predictive in vitro product potency assays (organ on a chip model)

Center for Cell Engineering
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CTCEF
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Medical Teams, Nurses, RSA

Stem Cell Center
L. Studer MD
M. Tomishima PhD & Team
S. Irion MD

Funding Sources
NCI PO1 CA008748, NCI PO1 CA008748-T cell Therapies; Mr. and Mrs. Goodwin Commonwealth Foundation for Research, MSKCC ETC, ACGT, Major Family, NYSCF, Stand Up To Cancer/AACR, NCCN Young Investigator Award, Leukemia and Lymphoma Society CDA, ASCO CDA, DOD, STARR Foundation

Weill Cornell
S. Rafii MD
J. Scandura MD
J. Butler PhD
R. Boyle PhD

Cytotherapy Lab
Blood Donor Room

Biovec Pharma
M. Caruso
K. Ghani

Our Patients!

Memorial Sloan Kettering Cancer Center
Available Resources

**US national roadmap on cell therapy manufacturing**: collaboration between companies, academic institutions, and government agencies to accelerate the path to commercialization ([http://cellmanufacturingusa.org/road-map](http://cellmanufacturingusa.org/road-map))


**ARM, Standards Coordinating Body**: public-private partnerships to facilitate standards development ([http://www.regenmedscb.org/](http://www.regenmedscb.org/))


**MC3M** ([http://www.cellmanufacturing.gatech.edu](http://www.cellmanufacturing.gatech.edu))

**NIIMBL** ([http://www.niimbl.us](http://www.niimbl.us))


**Catapult** ([https://ct.catapult.org.uk](https://ct.catapult.org.uk))

**CCRM** ([http://ccrm.ca](http://ccrm.ca))

**C3i, Center for Commercialization of Cancer Immunotherapy** ([http://centrec3i.com](http://centrec3i.com))

Adapted from I. Rivière and K. Roy. *Mol. Ther.* 2017