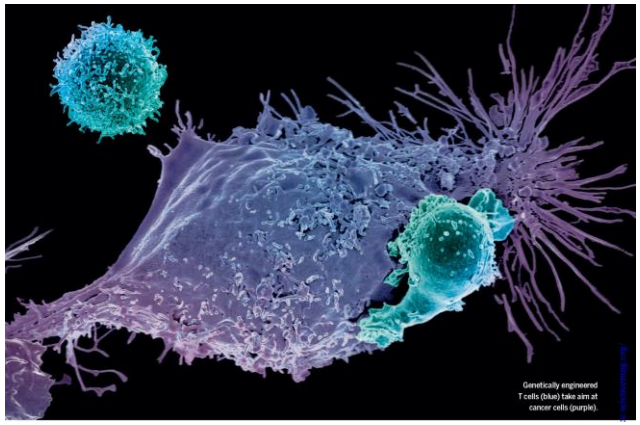


Designing Technologies to Meet the Manufacturing Needs of New Regenerative Therapies

Isabelle Rivière, PhD

Director,
Michael G. Harris
Cell Therapy and Cell Engineering Facility
Memorial Sloan Kettering Cancer Center

National Academy of Science Engineering Medicine 2017
RM workshop

Disclosure

- Scientific co-founder: Juno Therapeutics
- Consultant: Juno Therapeutics
- Research support: Juno Therapeutics

Cancer

Hemoglobinopathies
Cancer

Parkinson

iPSCs

Lymphocytes

G-CSF PB
HPSCs

CB and BM
expansion
E4ORF-HUVECs

ESCs

2018

2007

2012

2019

2018

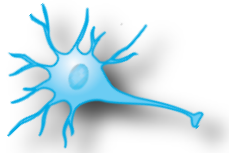
iCAR-T cells

CAR T cells

CD34+

CD34+

DA neurons

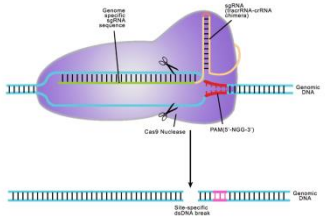


CAR
γ-retroviral vectors

Globin Lentiviral
vectors

Globin Lentiviral
vectors

CRISPR-Cas9



CTCEF Platforms

Sadelain,
Mansilla-Soto,
Themeli,
van der Stegen

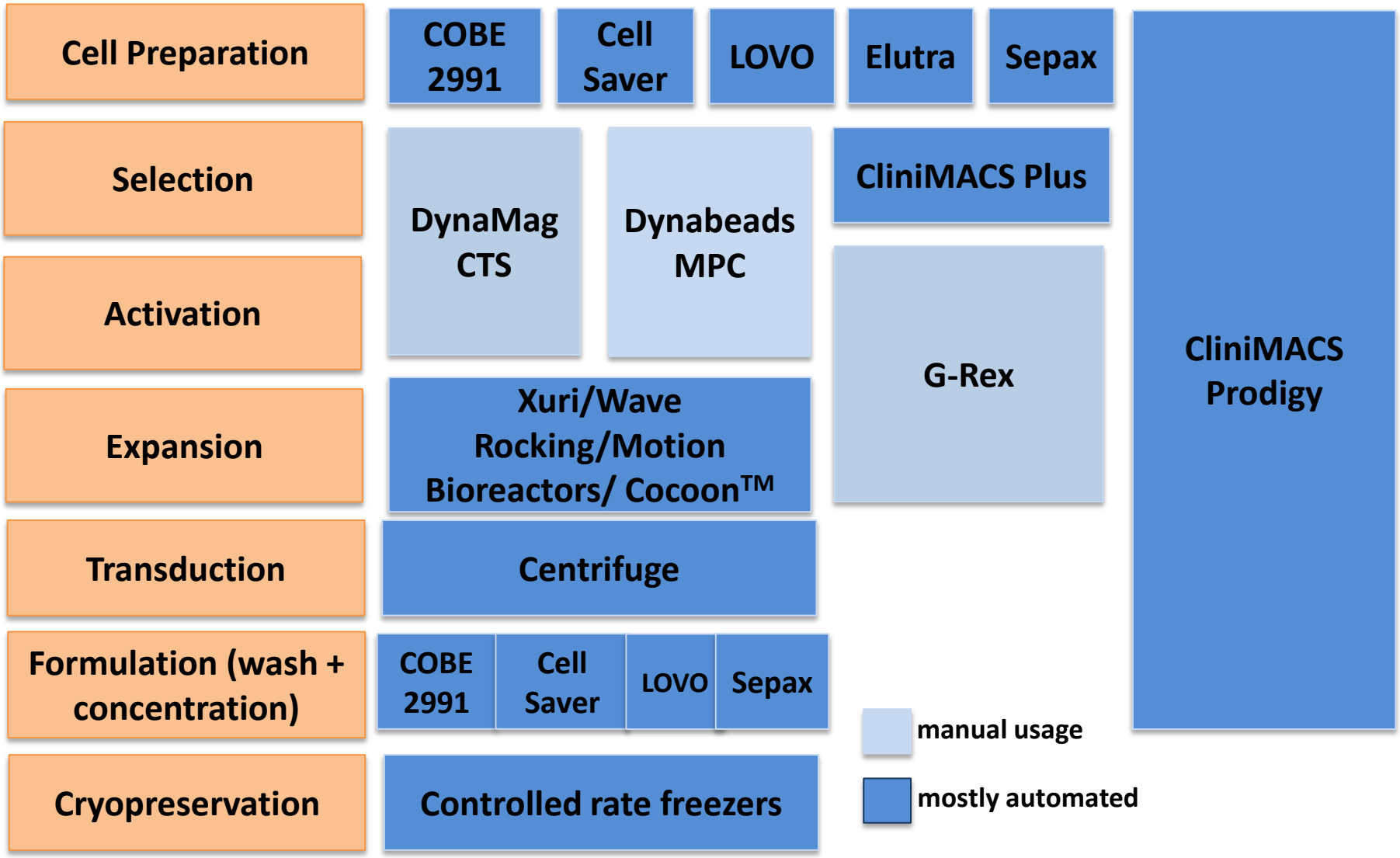
Sadelain, Brentjens
Eyquem,
Mansilla-Soto, Wang

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

NYSTEM Consortium #2
Studer
Tomishima
Irion

Collaborators MSKCC/Weill/Chicago/Industry

Examples of Unit Operations for Cell Manufacturing



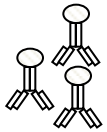
Adapted from Kaiser et al. Cancer Gene Ther. 2015 Mar; 22(2): 72–78

CAR T cell Manufacturing Flow (v1.0)

Day 0
Apheresis
Thaw/Wash
(Cell Washer)

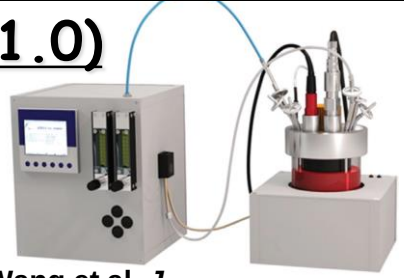
1

Incubation
Dynabeads CD3/28



2

Apheresis
Patient T cells

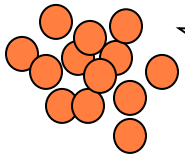


Wang et al, *J Immunother.*, 2015

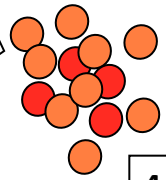
Przybylowski et al, *Gene Ther*, 2006

Transduction
Retroviral Vector

3



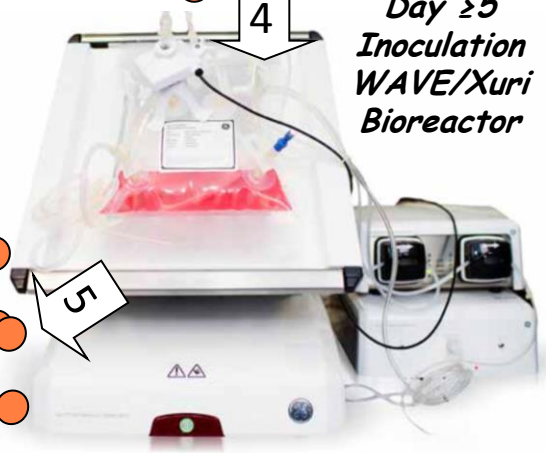
CD3+ enriched
Activated T cells



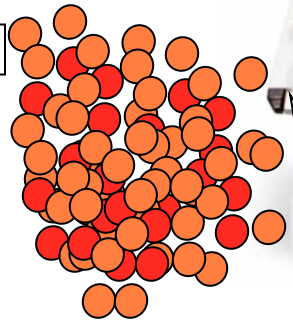
CAR/TCR transduced T cells

4

Day ≥5
Inoculation
WAVE/Xuri Bioreactor

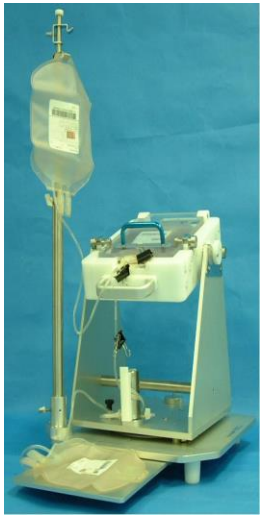


5



CAR+/TCR+ expanded T cells

6

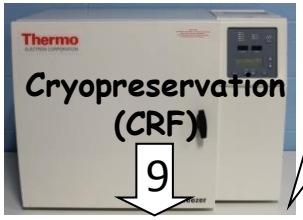


DAY ≥ 7
Debeading
ClinExVivo MPC

7

Wash
(Cell Washer)

8



9

Biosafety/
QC release tests
CofA

10

Patient Infusion

Validation
In vivo antitumor activity
in SCID/NSG mice, CTL

Memorial Sloan Kettering Cancer Center

Hollyman et al, *J. Immunother*, 2009
Themeli, Rivière, Sadelain, *Cell Stem Cell, 2015*

Adult ALL NCT 01044069 (PI: J. Park, MD)

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

	Morphologic Disease N=30 (%)	Minimal Disease N=20 (%)
CR Rate	23 (77%) [58 – 90]	18 (90%) [68 – 99]
MRD negative CR Rate*	19/21 (90%) [70 – 99]	14/18 (78%) [52 – 94]
Time to CR, Mean (SD)	20 days (9)	25 days (9)

*MRD assessment was not available in 2 patients.

Disease Status	CAR T Cell Dose
Morphologic disease (≥5% blasts in BM or EM disease)	1 x 10^6 CAR T cells/kg
Minimal disease (<5% blasts in BM)	3 x 10^6 CAR T cells/kg

Memorial Sloan Kettering Cancer Center

MSKCC FDA Breakthrough & FDA Orphan drug designation 2014

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)

Wang et al, *J Immunother.*, 2015

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

Day 0
Positive Selection CD4/CD8

Incubation
Dynabeads or
Transact beads

+

Apheresis
Patient T cells

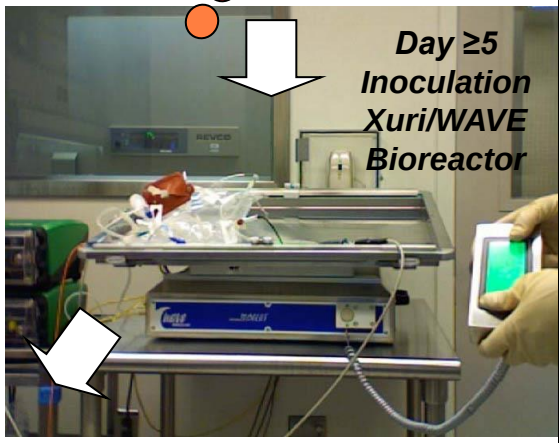
Day 2 or 3
Transduction
CAR Vector

CD3+ enriched
activated
T cells

iCellis Bioreactor

CAR
transduced
T cells

Day ≥5
Inoculation
Xuri/WAVE
Bioreactor



CAR+
expanded T cells

Cell Wash &
Formulation
(Cell Saver5, Lovo, Sepax)

Cryopreservation

Biosafety/
QC release tests

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**

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
PBMCs to selected cell types CD3, CD4, CD8 (CDX: active ingredients)
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EQT/tool characteristics (e.g cell washers processing time vs working volumes)
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Compatibility between unit operations
Alternative cell separation techniques e.g. label free cell sorting fluidics

Early Academy-Industry partnership limitations Lesson 2

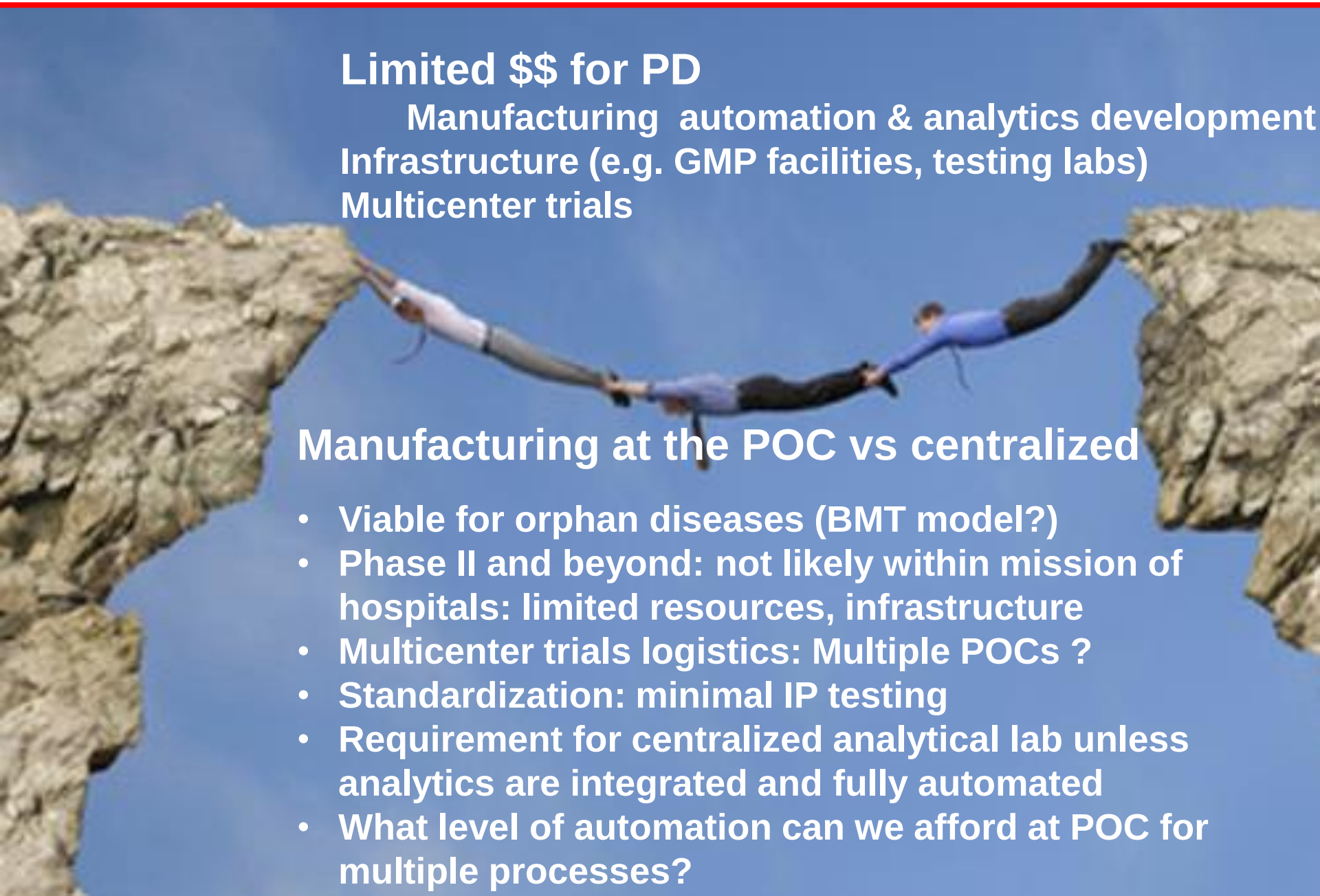
Difficulty of providing patient samples to industry partners (Aph, CAR T cells, blood samples post-infusion) for PD, analytical assay improvement/establishment:
timing of partnership agreements vs timing of patient consent

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

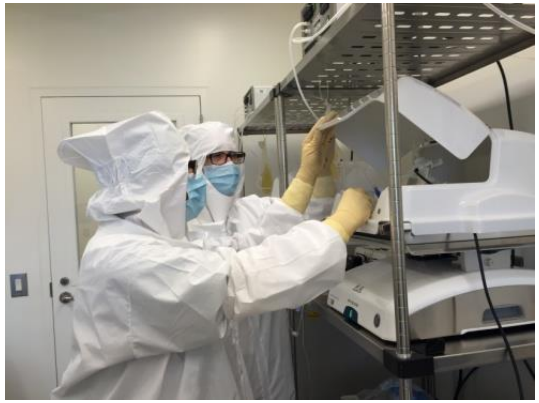
Manufacturing automation & analytics development
Infrastructure (e.g. GMP facilities, testing labs)
Multicenter trials

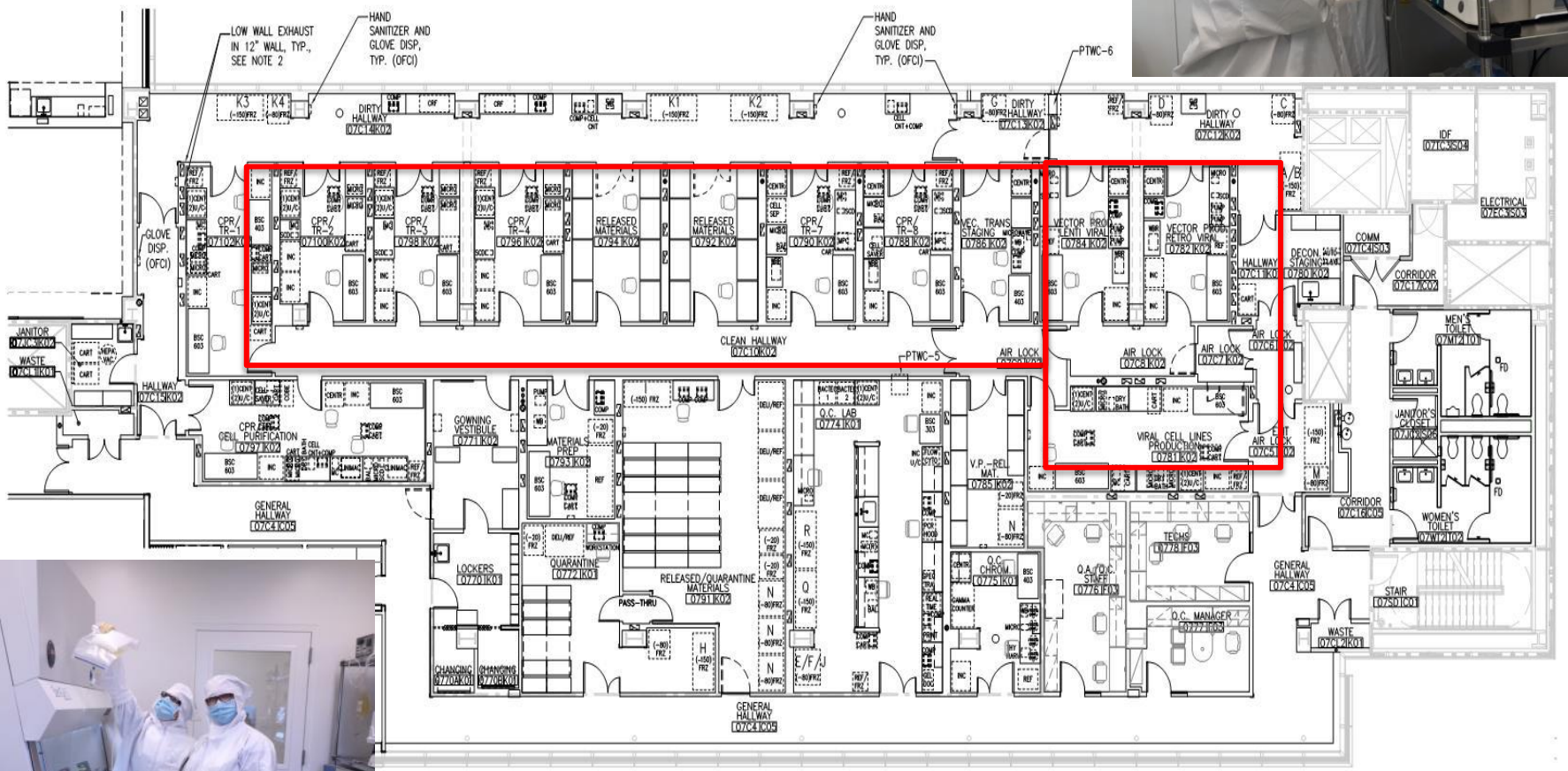
Manufacturing at the POC vs centralized

- 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

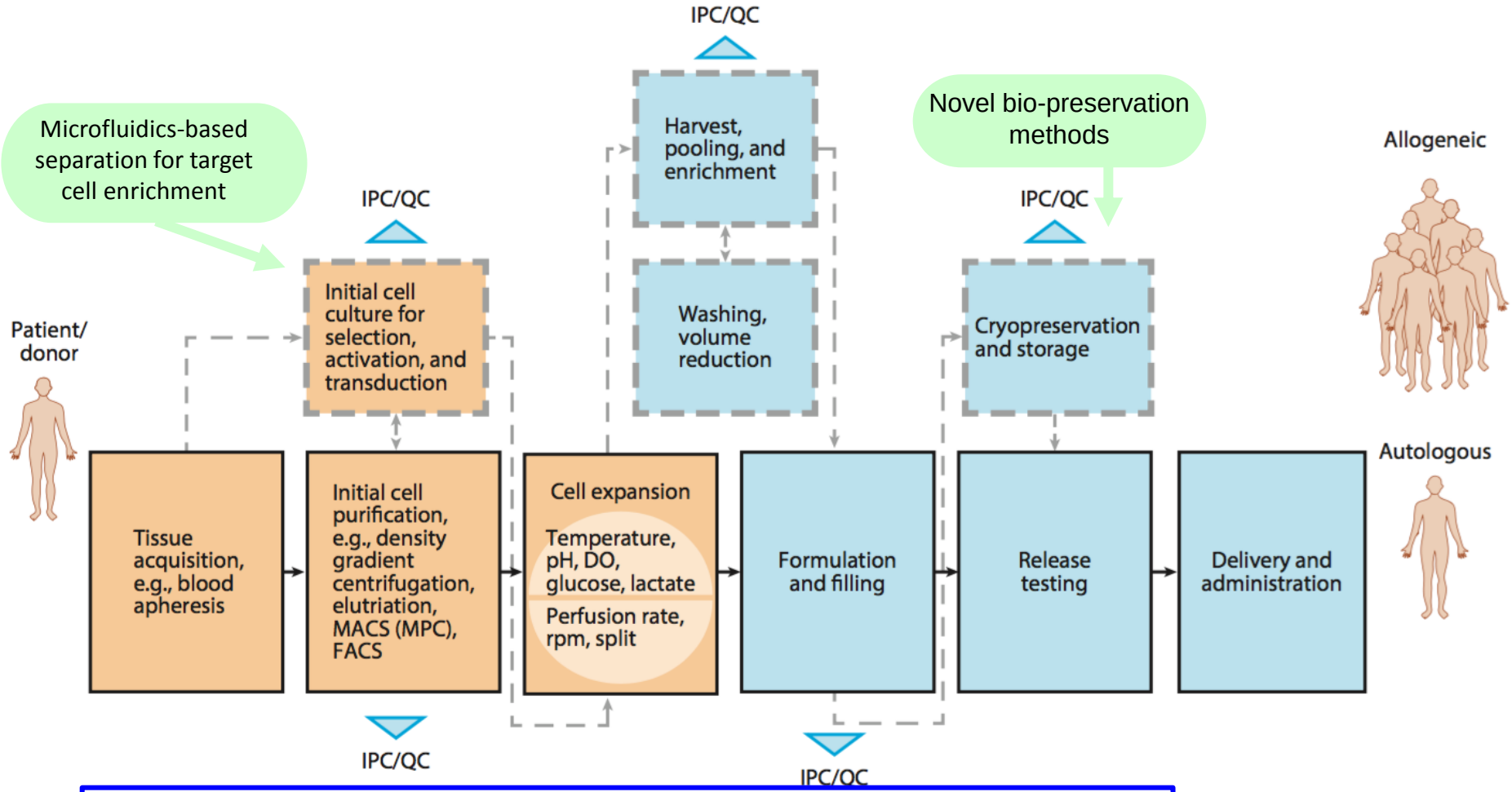
- Cellular products (e.g. T cells, HSCs, Cord Blood, ESC, iPSC)
- RNA vectors, plasmid DNA
- Phase I/II clinical trials
- Multicenter trials with other academic centers





Towards Automated Platforms for Cell Manufacturing

Increase process control, product consistency, throughput…



In Process Analytics

- Process –e.g. pH, O_2, CO_2, metabolites, omics, secretome, cytokine profiles, cell phenotype
- In-Process Cells and Final Product – e.g. identity, safety, viable cell count, purity

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)

Adapted from Roh KH, Nerem RM, Roy K. *Annu. Rev. Chem. Biomol. Eng.* 2016

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Y. Wang Ph D

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Stem Cell Center

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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

NIMBL

NYSTEM

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)

NIST, FDA workshops (https://www.nist.gov/news-events/events/ 2017/04/nist-fda-cell-counting-workshop-sharing-practices-cell-counting)

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

ARMI (https:// www.defense.gov/News/News-Releases/ News-Release-View/Article/1035759/dod- announces-award-of-new-advanced-tissue- biofabrication-manufacturing-innovat)

MC3M (http:// www.cellmanufacturing.gatech.edu)

NIIMBL (http://www.niimbl.us)

US National Academies: forum on Regenerative Medicine (http://nationalacademies.org/hmd/Activities/ Research/RegenerativeMedicine.aspx)

Catapult (https://ct.catapult.org.uk)

CCRM (http://ccrm.ca)

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

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