

Manufacturing Cell Therapy Products

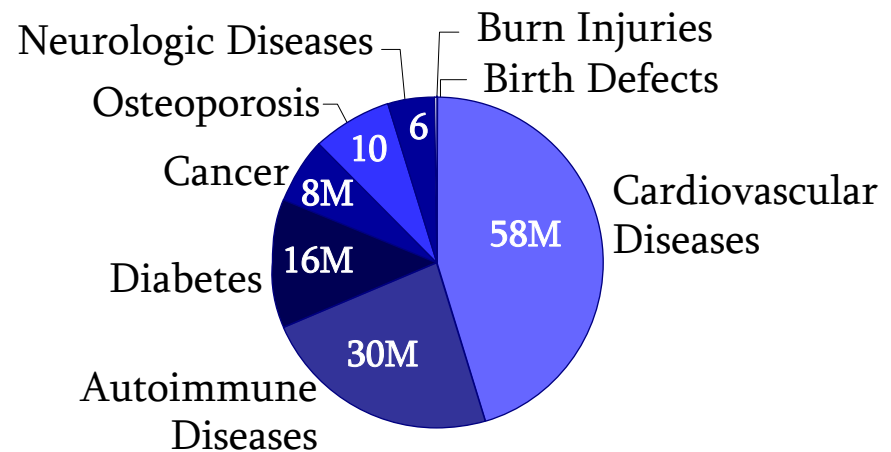
Challenges and Opportunities

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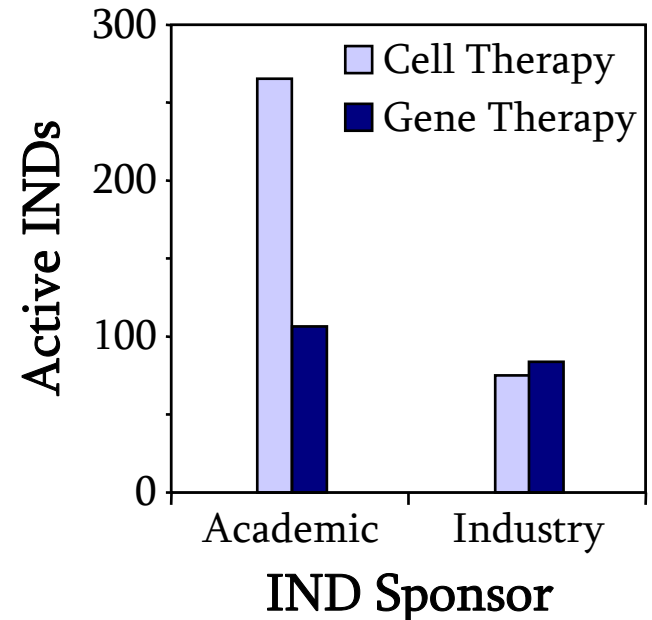
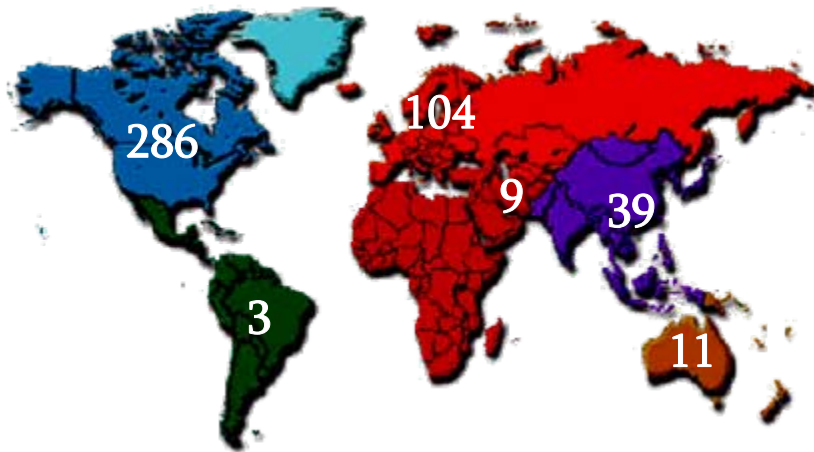
Cell Therapies - Potential, Promise, and Plenty of Challenges

- Numerous potential applications, often for unmet needs
 - Potentially definitive therapies
- Novel uses of cells/genes
 - Difficult to evaluate efficacy, predict clinical risks
- Complex cell/gene engineering
 - Risk of manufacturing problems, product/process characterization challenges

Potential Patients (USA)



An Emerging Cell Therapy Industry



- Over 400 cell or gene therapy companies worldwide
- Over 500 cell or gene therapy products in clinical development
- Over 1,000 gene therapy clinical trials worldwide
- Industry sponsors 25% of cell therapy INDs

Cell Therapy Applications

Immune, hematopoietic replacement

Cancer, autoimmune, immunodeficiency disorders

Blood, bone marrow cord blood, HPC, HSC, MSC, cord tissue cells

Immune effector cell therapy

Cancer, autoimmune, infectious diseases

Dendritic cells, NK cells, lymphocytes, macrophages

Tissue repair, regeneration

Cardiovascular, neurologic, orthopedic disorders, wound healing

Bone marrow MSC, other stem cells, neural cells, cardiomyocytes, macrophages, chondrocytes, keratinocytes, dermal fibroblasts

Metabolic replacement, support

Diabetes, renal, liver failure, other metabolic disorder

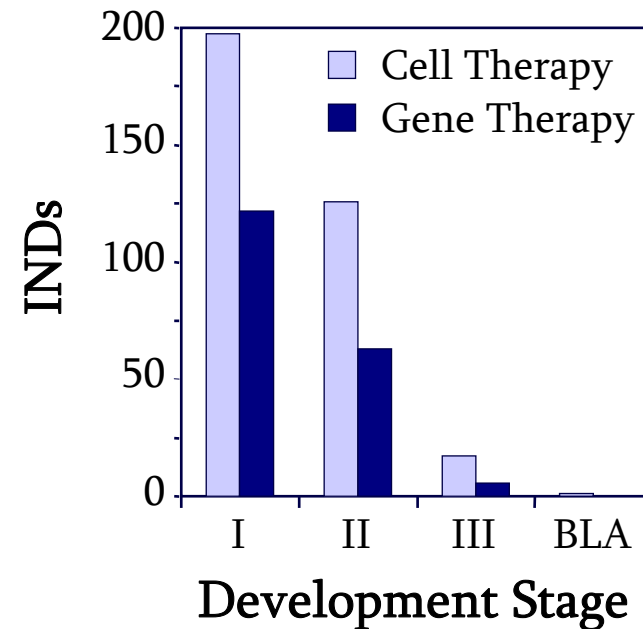
Islet cells, stem cells, bioartificial liver, bioartificial kidney, hepatocytes

Biotechnology and Cell Therapy Models - Critical Differences

	<i>Biotechnology</i>	<i>Cell Therapy</i>
<i>Product</i>	Cultured cells generate product	Living cells <i>are</i> product
<i>Raw Material</i>	Seed cell lines	Unique, primary tissue
<i>Biological Variability, Heterogeneity</i>	Limited	Substantial
<i>Process, Testing</i>	Established early	Evolve through trials
<i>Product Definition</i>	Well-defined, definable products	Product defined through trials Full definition likely unattainable
<i>Process Scale</i>	Bulk processes predominate	Patient-specific products common

Why Do Cell Therapy Products Fail in Clinical Development?

- Major drop-off post-Phase II
 - Damage is often self-inflicted
- Common root causes of failure
 - Inability to manufacture, deliver product (inadequate/undeveloped processes)
 - Inability to adequately characterize product (inadequate/undeveloped characterization)
 - Inability to evaluate efficacy - animal model limitations, problematic clinical endpoints
 - Lack of critical enabling infrastructure
 - Financial considerations
- Even successes can fail - Carticel



Major Obstacles to Cell Therapy Product Manufacturing

- Effective process scale for patient-specific products
- Evolving manufacturing processes, product and process characterization
- Lack of informatics support
- Limited enabling infrastructure

Cell Therapy Manufacturing Processes

- Cryopreservation
- Cell selection
- *Ex vivo* culture
 - Expansion, maturation, selection, other
- Cell activation
- Genetic modification
- Tissue processing
- DNA/RNA purification, amplification

Cell Therapy Product Development/Manufacturing Strategies

- Manufacturing process must protect product, patient
 - Focus on product characterization, process control
 - Controlled, consistent processes → controlled, consistent products
- High throughput, parallel processing to achieve scale
 - Functionally-closed processing systems, automation
- Rigorous process development, characterization

Automated, Functionally-Closed Systems for Making a Nice Cup of Coffee

Individualized, cup-specific brewing of a variety of coffees, teas, cocoa, *even mochaccino with extra foam*. But how?



Preloaded, disposable, individualized raw material sets



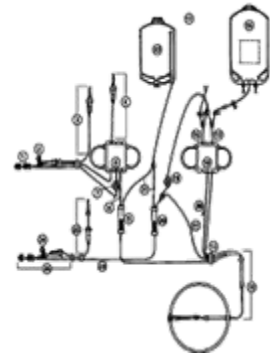
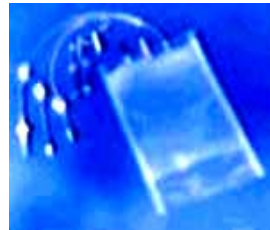
Separate process environment for each product



Automated processing device

Functionally-Closed Systems - Key Tools

- Separate, enclosed process environment for each product
 - Based on robust, established blood processing/i.v. infusion technology
 - Presterilized, disposable
- Access and manipulate system contents without violating system integrity
 - SCDs to sterile-weld tubing, sealed septum/spike ports
- Perform multiple manufacturing functions in integral functionally-closed system
 - Centrifugation, cell washing, cryopreservation, sampling, immunomagnetic cell selection, *ex vivo* expansion...
 - Multiple bags/connections/access points



Manufacturing in Open vs. Closed Systems

	<u>Open</u>	<u>Closed</u>
<i>Contamination Risk</i>	↑	↓
<i>Cell Yield</i>	↓	↑
<i>Manufacturing Space</i>	Cross-contamination risk limits options	Maximal effective use
<i>Consistency, Control</i>	Limited	↑ ↑ ↑
<i>Throughput Potential</i>	↓ ↓ ↓ Patient-specific	↑ ↑ ↑ Parallel processing Readily automated Presterilized, disposable



Limited Informatics Capabilities

- Process control, documentation systems often primitive
 - Pen and paper worksheets, pocket calculators
- Need for robust informatics capabilities
 - Process complexity necessitates better control, documentation
 - Regulatory requirements (GMPs, GTPs, other), particularly tracking, traceability, software validation requirements

Contract Services

- Academic-based laboratories, n <5
 - Decades of experience in cell therapy development, production, state-of-the-art cell therapy technology
 - Not primarily contract service labs, use external contracts to help support facility
- Commercial laboratories, n <5
 - Experience emphasizes cell line and vector production, banking, rather than clinical cell therapy
 - Contract services are primary mission
- Cell therapy-specific experience, capabilities, GMP/GTP and QA infrastructure



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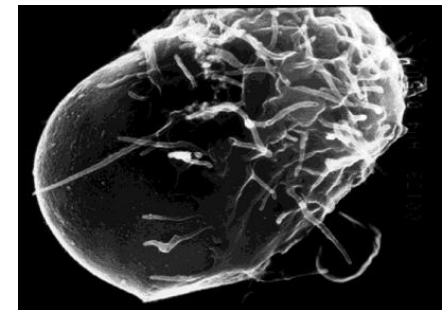
Common Problems in Cell Therapy Manufacturing - Examples

- Raw materials sourcing
- Mononuclear cell enrichment
- *Ex vivo* expansion - adherent cells
- Solid tissue processing for cell isolation
- Product preservation and delivery

Raw Materials

- Viable, functional biological raw material
 - Cell/tissue source for development studies?
- Reagents and supplies
 - Complex, often unique - cytokines, vectors, genes, culture media, supplements, mAbs...
 - Need for GMP manufactured reagents
 - Develop reagent specifications, qualify
 - Physical characteristics, quality control, qualification tests, storage conditions, expiration
 - Manufacturer Service Level Agreements
 - USP Chapter 1043
 - Ancillary Materials for Cell, Gene, and Tissue-Engineered Products

Human serum (AB-, autologous)
Fetal calf serum, horse serum
Monoclonal antibodies
Recombinant vectors
G-CSF, GM-CSF, EPO, TPO
IL-1 α , IL-1 β , IL-2, IL-3
IL-4, IL-6, IL-7, IL-8,
TNF- α , PG-E₂
SCF, FL, Flt3, VEGF
BMP-4, EGF, IGF
PDGF-BB, MIP-1 α , MCP-1
TGF- β 1, aFGF, bFGF
N-desulfated O-sulfated heparin



J Immunol Methods 2001

Cell Separation - MNC Enrichment

- Ficoll density gradient centrifugation
 - Produces MNC at high purity, common research method
 - Ficoll is not a clinical reagent
 - GMP product helps, but is this a clinical reagent?
 - Manual process, tubes (open system), variable MNC recovery
 - Sepax device may help, but any other device options?
 - Other clinical-grade density-gradient solutions? Engineering alternative?

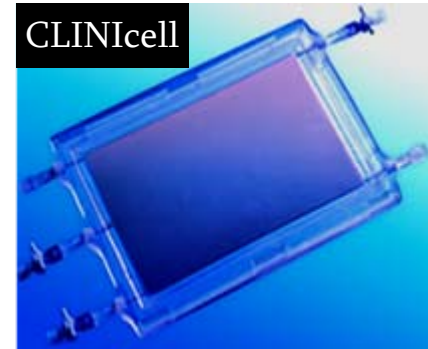


Ex Vivo Expansion - Adherent Cells

Increasing applications for adherent cell types

MSC, MAPC, islets, myocytes, chondrocytes...

T-150, T-225 flasks, Nunc Cell Factory	Open-system, problematic
Aastrom RepliCell bioreactor	Large but open-system, limited adapability, problematic
MABio CLINiCell	Closed-system, very promising
Miltenyi culture bag	Closed-system
TissueGenesis BOS bioreactor	Closed-compatible, automated yet flexible, very promising
Puramatrix, porous silicon, others	Substrate in non-adherent, closed-system - maybe



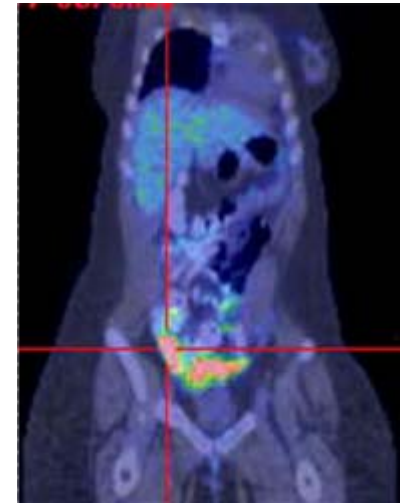
Formulation - Stability - Shelf-Life

- Fresh (non-cryopreserved) products
 - Product stability drives release, distribution, administration
 - Release on 24/48 hr sterility culture, endotoxin, Gram stain
 - Need effective rapid sterility testing
- Cryopreserved products
 - Manufacturing and administration flexibility, but...
 - Thaw and administer product with cryoprotectant
 - Thaw, wash, administer - manufacturing at clinical site
 - Post-wash, stat Gram stain and endotoxin
 - *Need functionally-closed device for thaw/wash/resuspension*
- Novel preservation alternatives
 - Investigate possibilities for DMSO-free, non-frozen preservation

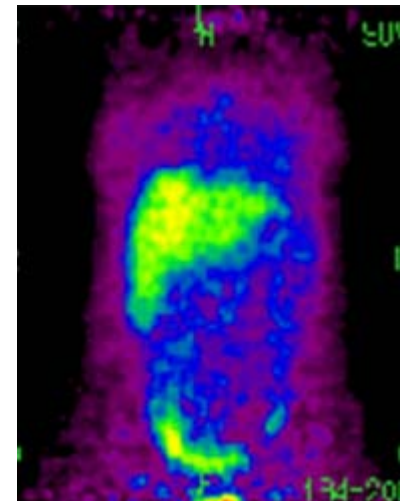


Cell Distribution *In Vivo*

- Imaging technology for *in vivo* cell tracking
 - Cell distribution, kinetics
 - Fluorescence, magnetic particle-based imaging
 - Isotopic imaging - PET-CT, SPECT
- Development and regulatory applications
 - Clues to biological function
 - Animal model qualification
 - Human cells? Animal cells?
 - Bridge manufacturing changes
 - Fresh *vs.* frozen/thawed? Serum *vs.* serum-free?
 - Patient monitoring



Pre-Rx PET



FDG-labelled MAK cells
t = 3 hr., PET-CT

Enabling Cell and Gene Therapy Commercialization

- Process development
- Raw materials
- Raw material/product characterization
- Enabling technology, devices
- Contract services infrastructure
- Centralized or distributed manufacturing?
- Cryopreserved or non-cryopreserved products?
- Raw material, product transport
- Regulatory environment

Summary

- Unprecedented numbers of cell therapy products are in development for a remarkable range of clinical applications, some nearing commercialization.
- These living biological products present unique challenges in development, manufacturing, characterization, and delivery.
- Infrastructure to support commercial-scale cell therapy is needed
 - Challenges to cell therapy manufacturing represent opportunities for companies supplying enabling technology