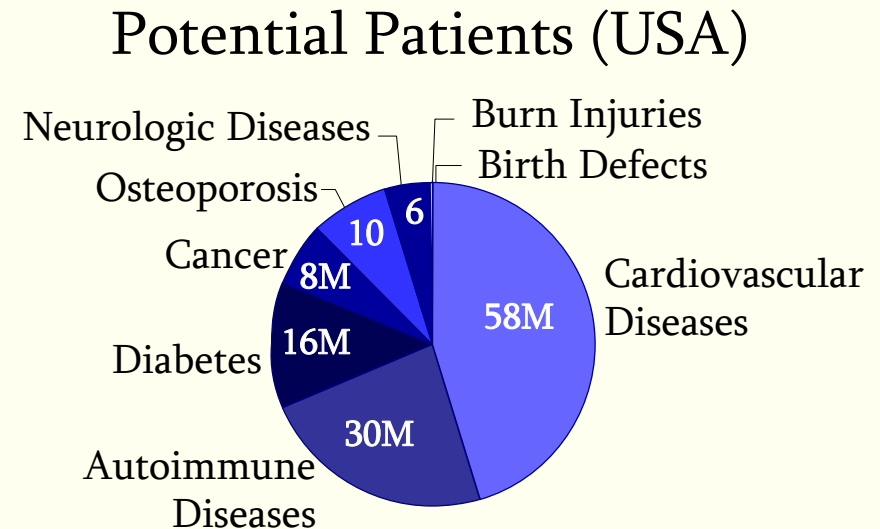


Strategies for Manufacturing Cell Therapy Products

Scott R. Burger, MD
Advanced Cell & Gene Therapy

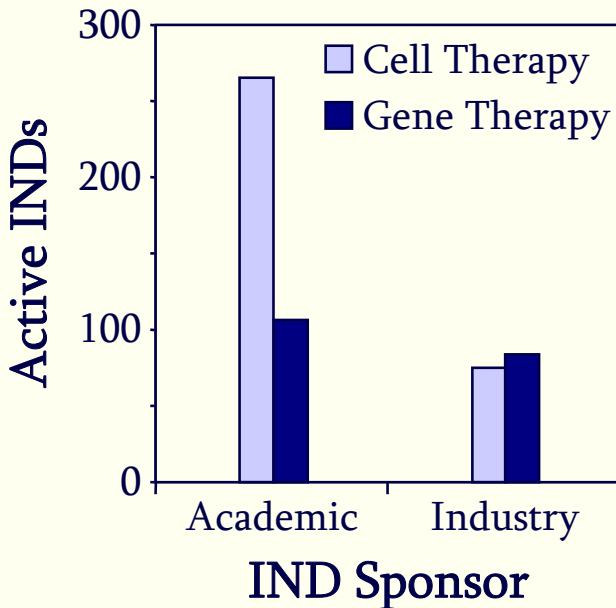
Cell and Gene Therapies - Promise, Potential, and Plenty of Challenges

- Numerous potential applications, often for unmet needs
 - Potentially definitive therapies
- Novel uses of cells/genes
 - How does the product function?
 - Difficult to predict clinical risks
- Complex cell, gene engineering
 - ↑ Risk of manufacturing problems
 - Challenges in product and process characterization

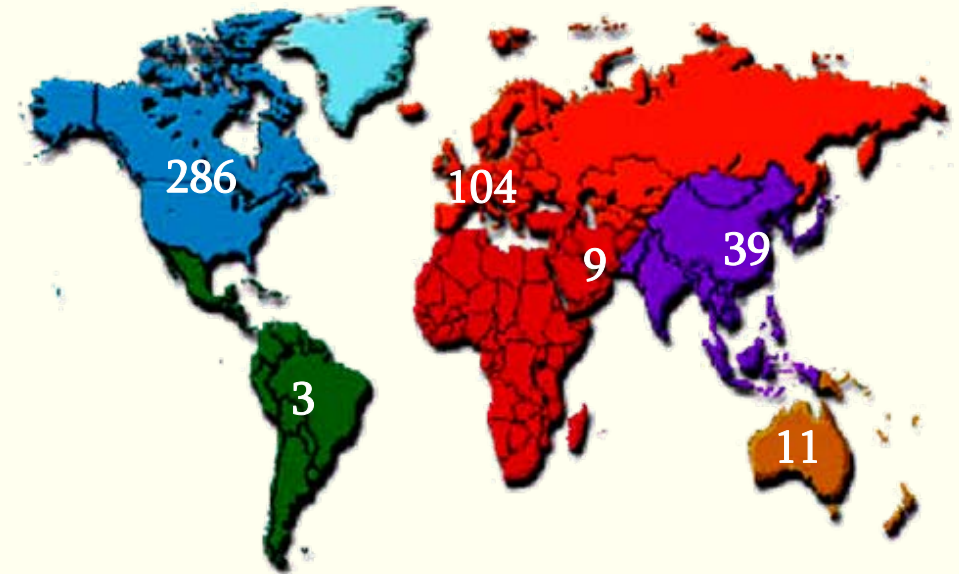


An Emerging Cell and Gene Therapy Industry

Industry sponsors 25%
of cell therapy INDs

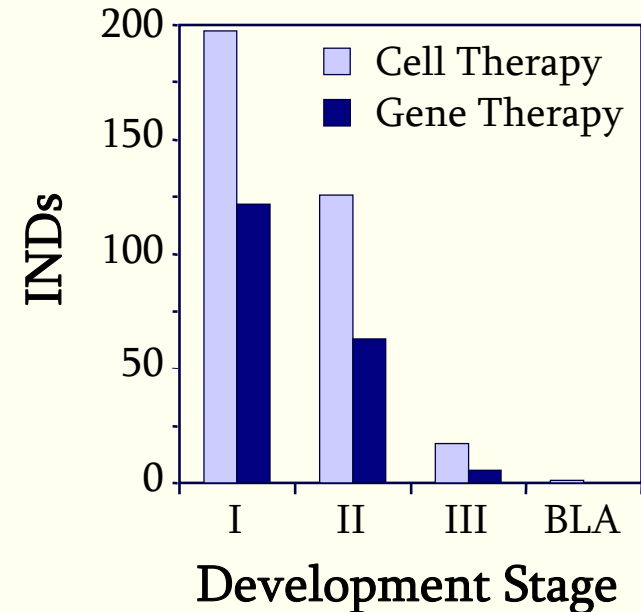


>400 cell and gene therapy
firms worldwide



What Accounts for Clinical Development Failures in Cell and Gene Therapy?

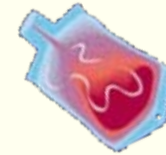
- Common root causes of failure
 - Inability to manufacture product
 - Inability to adequately characterize product



Cell Therapy Products - Intrinsic Challenges

- Product definition
 - Living, functional cells, not the products of cells
 - Biological variability/heterogeneity
 - Product function?
- Process scale
 - Immune compatibility → patient-specific products
 - Commercialize at 1 lot per patient?
- Limited infrastructure, technology, reagents
 - Novel therapies and therapeutic strategies
- Evolving, iterative development
 - Processes, specifications refined through trials

>99% CDX⁺ cells



?% CDY⁺ cells

?% CDY⁻ cells

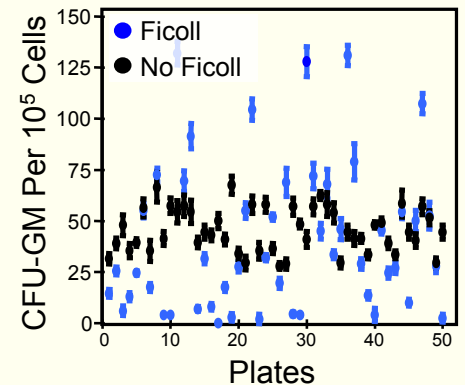
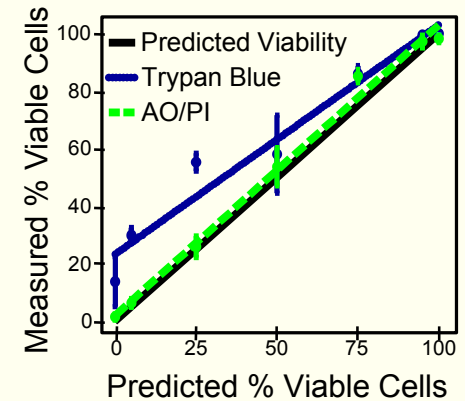
	<u>INDs</u>	<u>Amendments</u>
Cell Tx	903	13,527
Gene Tx	372	8,090

Strategies for Cell and Gene Therapy Product Manufacturing

- Manufacturing *process* must protect product, patient
 - Focus on product characterization, process control
 - Controlled, consistent processes → controlled, consistent products
 - Process qualification (PQ) studies to bridge process changes
- High throughput, parallel processing to achieve scale
 - Functionally-closed processing systems, automation
- Ongoing process development, and rigorous product characterization

Product Characterization

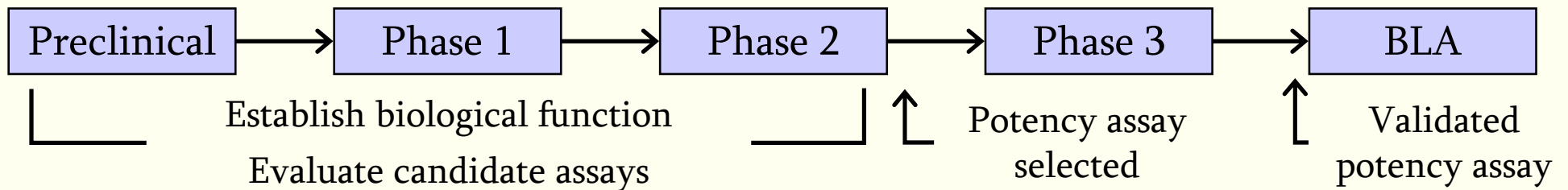
- Safety
 - Sterility, endotoxin, mycoplasma, adventitious agents
- Purity, Identity
 - Cell viability, concentration, morphology, immunophenotype...
- Potency
- Characterization strategy
 - Test multiple parameters, establish *pattern* of product characterization data, refine over time
 - May need unfamiliar or novel analytical tools
 - Robust, qualified analytical methods
 - Reproducibility, predictive value, sensitivity, specificity



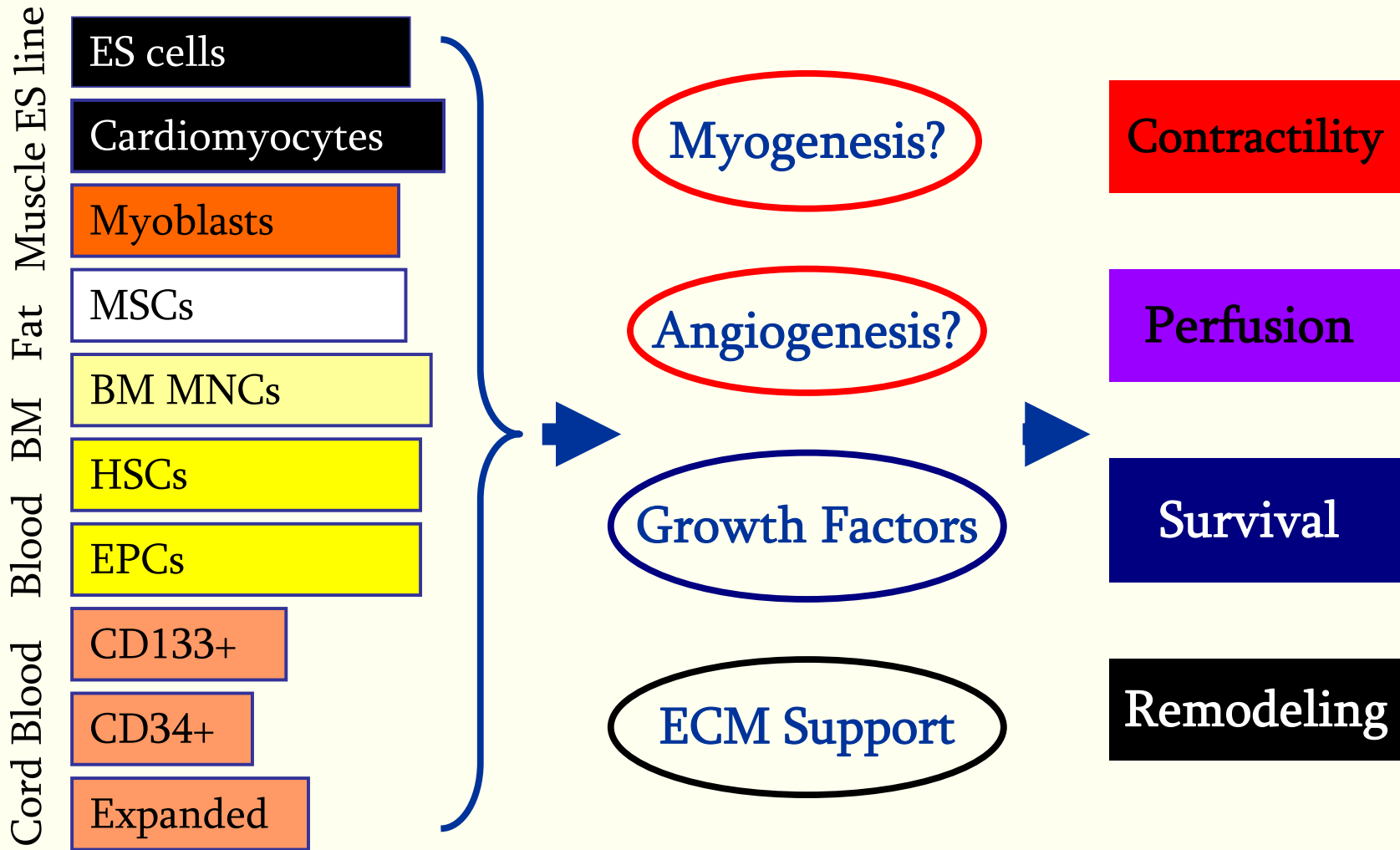
Transfusion 2000 [40:693-96](#)
Transfusion 1999 [39:451-56](#)

Potency Testing

- Testing “relevant biological function”
 - Understanding of product function developed/refined from preclinical-Phase III
 - Antigen presentation, cytotoxicity, cytokine release, clonogenicity, other functional assays...
- Potency assays
 - Evaluate candidate potency assays across Phase I, II trials. Assess candidate assays in light of clinical outcome data.
 - Compensate for long functional assay turnaround time by qualifying/validating real-time surrogate assay.

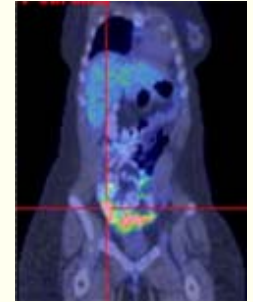


Cell-Based Therapy for Myocardial Repair

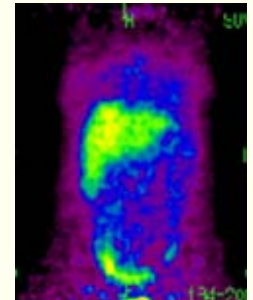


Cell Distribution *In Vivo*

- 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
 - Model qualification
 - Human cells in animal model?
 - Animal cells in animal model?
 - Bridge manufacturing changes
 - Fresh *vs.* frozen/thawed? Serum *vs.* serum-free?
 - Patient monitoring



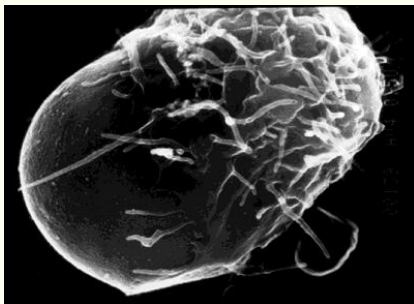
Pre-Rx PET



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

Raw Materials for Cell and Gene Therapies

- Viable, functional biological raw material
 - Cell/tissue source for development studies?
- Reagents and supplies
 - Cytokines, vectors, genes, culture media, supplements, mAbs
 - GMP manufactured reagents
 - Complex biologicals, often unique to product
 - Sometimes reagents aren't even reagents

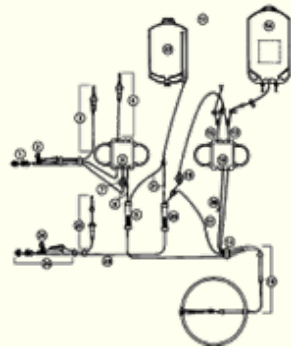
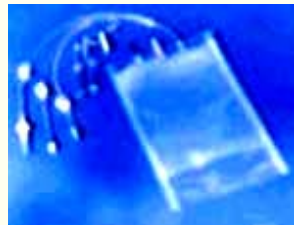


J Immunol Methods 2001;249:111-9

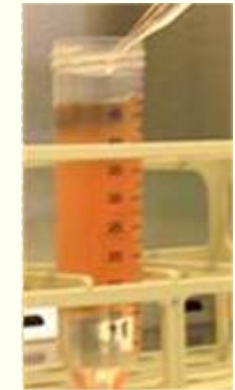
Human serum (AB-, autologous)
Fetal calf serum, horse serum
Monoclonal antibodies
Recombinant vectors
G-CSF, GM-CSF, EPO, TPO
IL-1 α , IL-1 β , IL-2, IL-3
IL-4, IL-6, IL-7, IL-8,
TNF- α , PG-E₂
SCF, FL, Flt3, VEGF
BMP-4, EGF, IGF
PDGF-BB, MIP-1 α , MCP-1
TGF- β 1, aFGF, bFGF
N-desulfated O-sulfated heparin

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

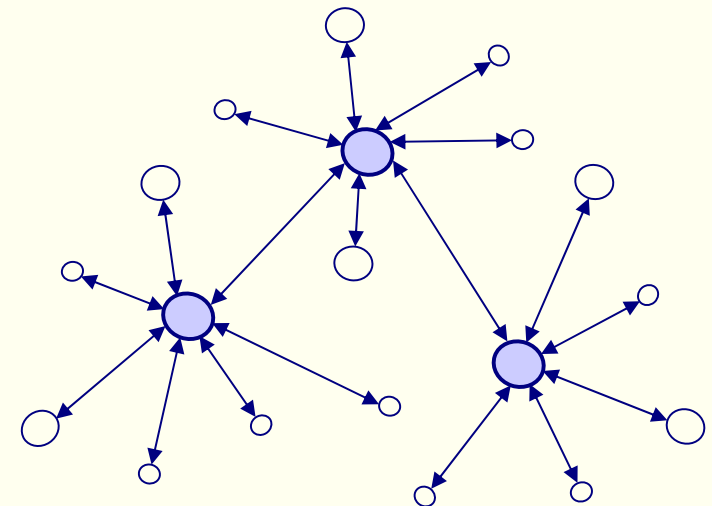
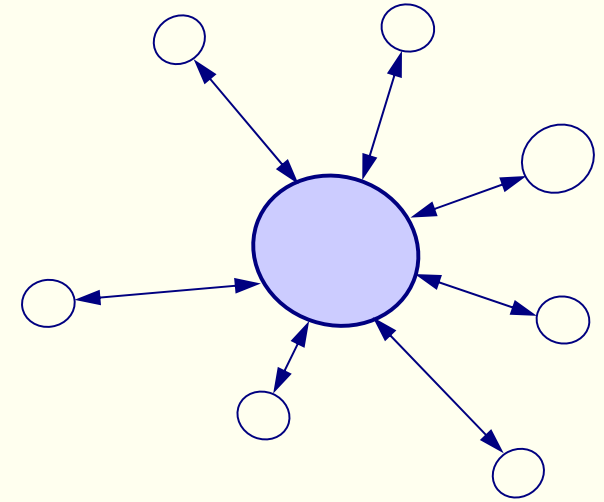


	Open	Closed
Contamination Risk	↑	↓
Cell Yield	↓	↑
Manufacturing Space	Limited options X-contamination	Maximizes effective use
Consistency, Control	Limited	↑ ↑ ↑
Throughput Potential	↓↓↓ Patient-specific	↑ ↑ ↑ Parallel processing Readily automated Presterilized, disposable



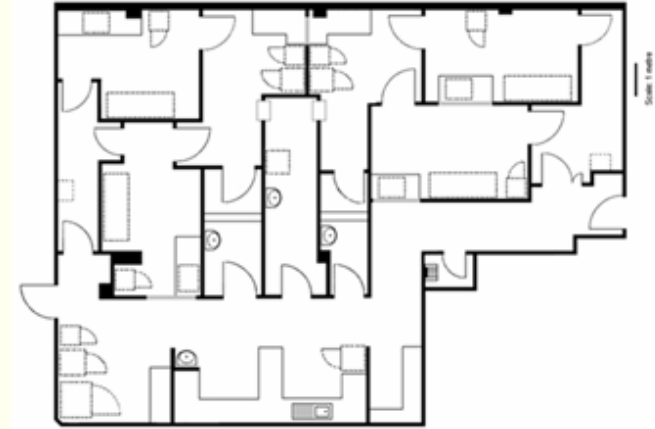
Centralized or Distributed Manufacturing?

- Centralized
 - One facility to control
 - Requires robust, controlled, validated transport, and environmental monitoring during shipping
 - Raw material and product stability
- Distributed
 - Must control multiple GMP/GTP cell therapy facilities
 - Shorter-range transport, but shipping still must be controlled, qualified, monitored



Contract Services

- Academic-based laboratories, n <5
 - Decades of experience in cell therapy development, production, state-of-the-art cell therapy technology
 - Cell therapy GMP usually well established, GTP in progress
 - Not primarily contract service labs, use external contracts to help support facility
- Commercial laboratories, n <5 worldwide
 - Experience emphasizes cell line and vector production, banking, rather than clinical cell therapy
 - Generally GLP, some offer GMP capabilities
 - Contract services are primary mission
- Vendor audit, qualification - essential



Fresh or Frozen Products?

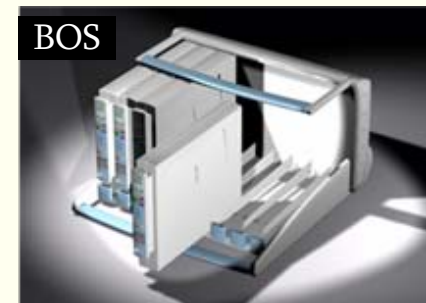
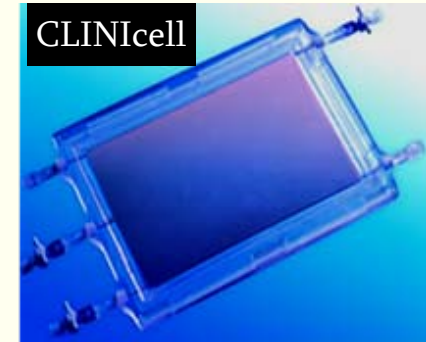
- Cryopreserved products
 - Maximum manufacturing and administration flexibility
 - Thaw and administer product with cryoprotectant
 - Simple, but problems for some products, administration routes
 - Thaw, wash, administer - manufacturing at clinical site
 - Post-wash, stat Gram stain and endotoxin
 - Functionally-closed device for thaw/wash/resuspension
- Fresh (non-cryopreserved) products
 - Product stability drives release, distribution, administration
 - Release based on 24/48 hr sterility culture, endotoxin, Gram stain
 - Action plan for response to positive culture post-administration
 - Stability may be <48 hours - transport critical

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



Summary

- Unprecedented numbers of cell therapy products are in development for a remarkable range of clinical applications. These living biological products present unique challenges in development, manufacturing, characterization, and delivery.
 - Evolving characterization and rigorous process control are especially vital to counter the intrinsic heterogeneity, variability, and incomplete definition of cell therapy products.
 - Processes, analytical methods, and product definition must evolve over multiple clinical trials.
 - Manufacturing in functionally-closed systems can overcome process scale limitations of patient-specific cell therapy products by enabling automated, parallel processing at high throughput.