

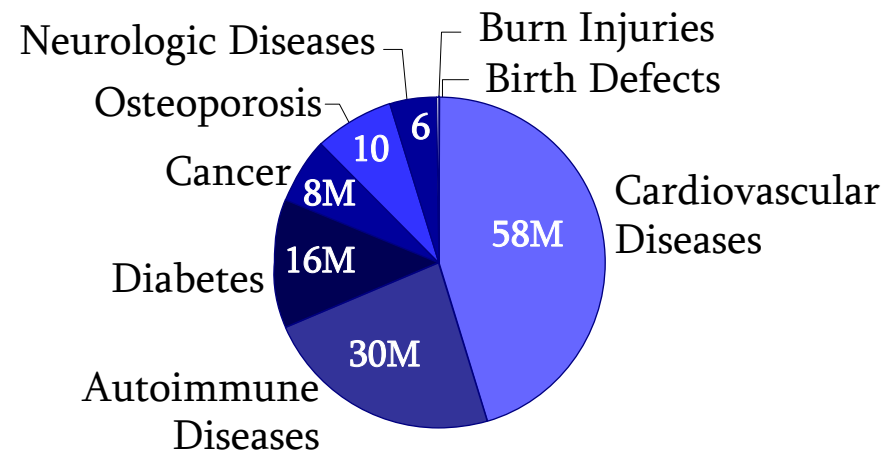
Translational Development: From Bench, to Clinic, to Commercial Manufacture

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Advanced Cell & Gene Therapy

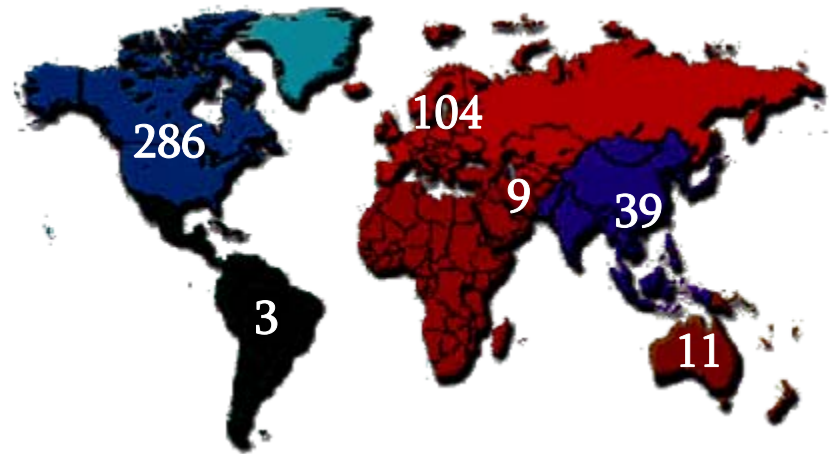
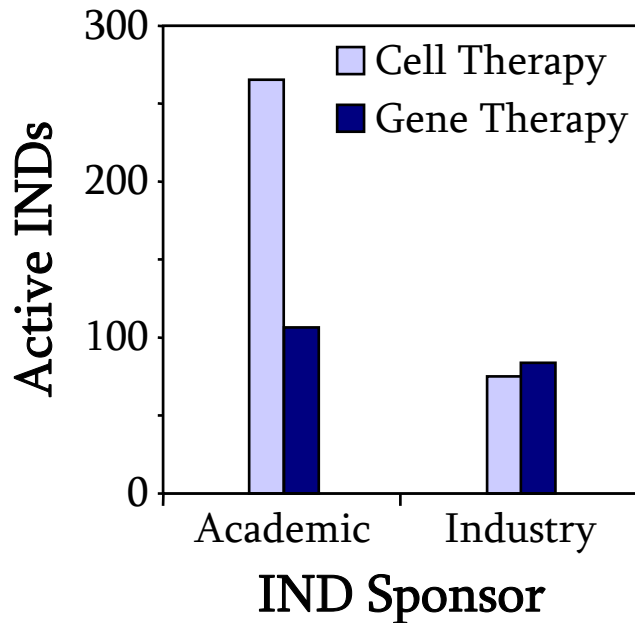
Cell and Gene Therapies - Potential, Promise, and Plenty of Challenges

- 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)



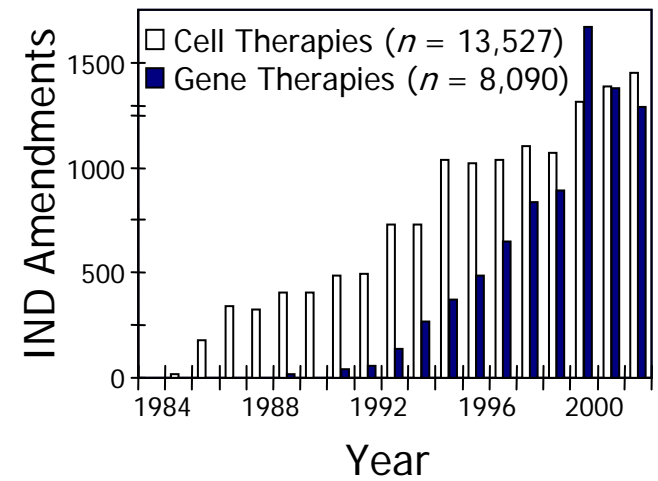
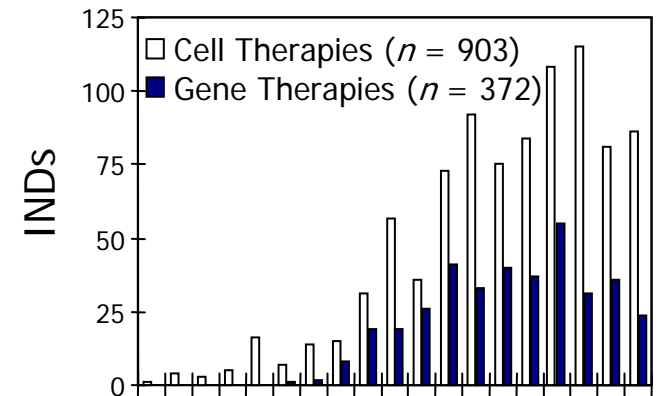
An Emerging Gene Therapy Industry



- Over 1,000 gene therapy clinical trials worldwide
- Industry sponsors 44% of gene therapy INDs
- Over 400 cell or gene therapy companies worldwide

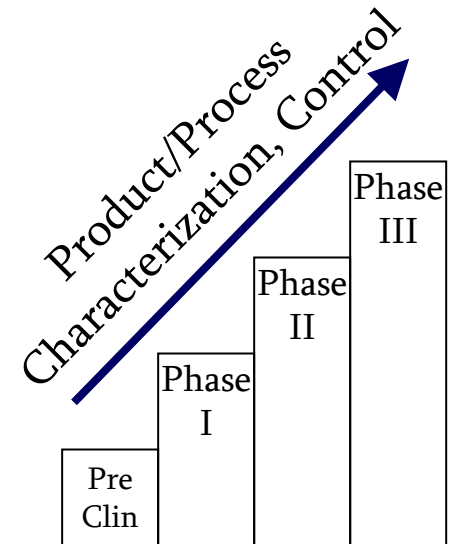
Process and Product Definitions Develop Through Clinical Trials

- Process, testing, product definition evolve based on manufacturing experience, characterization data, clinical outcome data
 - Refine specifications and thresholds over time
 - Determine biological function, and cells mediating function
 - Final potency assay in place by Phase III



Development Throughout Clinical Trials

- Evolution of manufacturing process, characterization profile
 - Processes, specifications refined based on experience
- FDA expects \uparrow control/characterization as clinical development progresses
 - Preclinical - basic process control, characterization
 - Phase III - full characterization, GMPs, GTPs



Common Process Development Issues

- Optimize gene transfer
 - *In vivo?* *Ex vivo?*
 - Gene transfer methods
 - Viral vectors
 - Non-viral - electroporation (closed-system), liposomes, other?
 - Transduction efficiency, cell recovery, viability, function
- Manufacturing process scale
 - Individualized, patient-specific or bulk manufacturing?
 - Functionally-closed systems
- Raw materials
 - Sourcing, availability, qualification

Automated, Functionally-Closed Systems: Making Coffee One Cup at a Time

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



Preloaded, disposable,
individualized raw
material sets



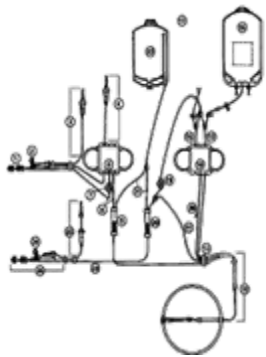
Separate process
environment
for each product



Automated
processing
device

Automated, Functionally-Closed Systems: High Throughput Manufacturing

Individualized manufacturing, run in parallel for high throughput - cell selection, expansion, activation, centrifugation, cryopreservation.



Preloaded, disposable,
individualized raw
material sets



Separate process
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for each product

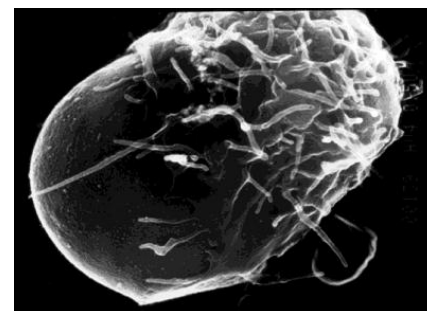


Automated
processing
devices

Raw Materials

- Complex, often unique - vectors, cytokines, vectors, genes, culture media, supplements, mAbs...
- Need for GMP manufactured reagents
 - Vector sources – NGVL, others
 - Avoid animal-derived reagents
 - Defined reagents whenever possible
- Develop reagent specifications, qualify
 - Physical characteristics, quality control, qualification tests, storage conditions, expiration

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

IND Specifications - Manufacturing

- Vector
 - Production, purification
 - Storage
- *Ex vivo* modified cells
 - Collection methods
 - Processing methods
 - *Ex vivo* modification procedures
 - Gene modification, expansion, activation, selection, irradiation
 - Final harvest
- Final Product Formulation
 - Formulation buffer
 - Excipients
 - Vector concentration
 - Cell density
 - Storage

IND Specifications - Vector and Cells

- Vector
 - Description, history, *detailed* derivation of construct
 - Vector diagram
 - Sequence analysis
- Cells
 - Source (tissue and cell type)
 - Collection procedure
 - Donor screening (GTPs!)
 - Allogeneic cells - screen using standard blood banking criteria
 - Autologous cells - screen to prevent increasing viral load or transmitting adventitious agents

Ancillary Materials

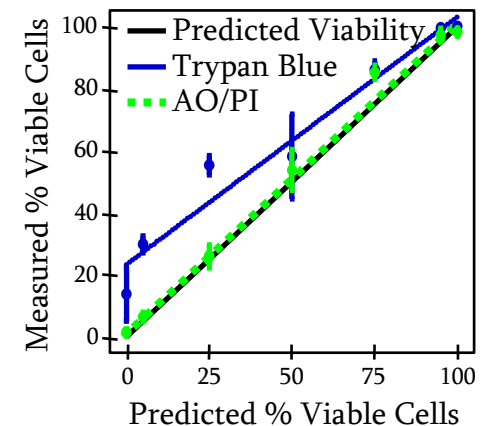
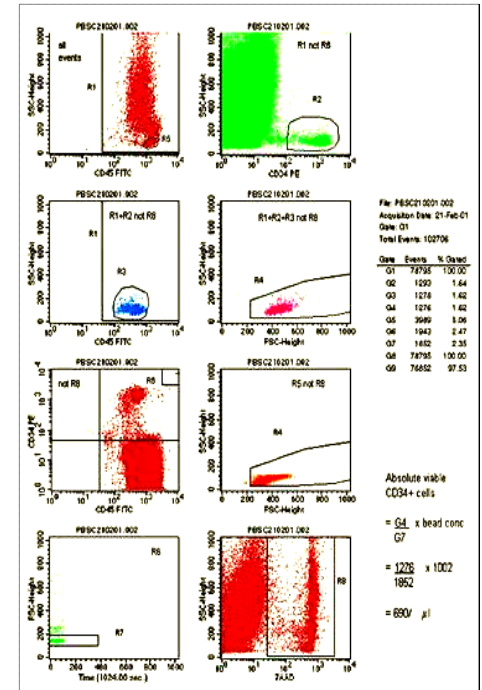
- Materials used in manufacturing, not in final product
 - Cytokines, antibodies, adhesion molecules, beads, media...
- Qualification programs - vendors, reagents
- Qualify removal of ancillary materials
- Include in IND
 - Description, final concentration
 - Vendor, source (human, bovine, etc.)
 - Grade (licensed product, clinical grade, reagent grade)
 - Certificates of Analysis
 - Cross reference permission letters

Analytical Development Issues

- Safety, purity/identity, potency
 - Sterility cultures
 - When? Cryopreserve product or administer fresh?
 - Mycoplasma, endotoxin
 - RCR
 - Recombination events
- Transduction efficiency, function
- Patient monitoring
 - Retroviral gene transfer protocols - detect recombination events
 - Long-term monitoring, LM-PCR

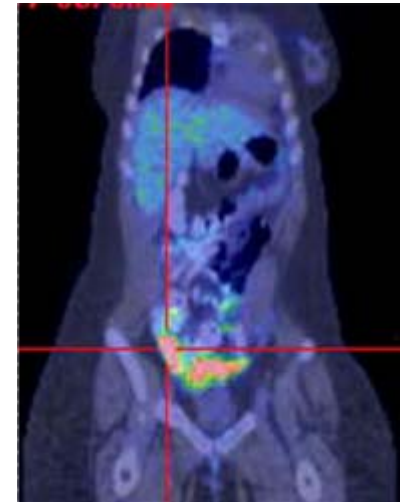
Product Characterization

- Characterization strategy
 - Test multiple parameters, establish *pattern* of product characterization data, *refine over time*
 - May need novel analytical tools
 - Robust, qualified analytical methods
 - Documented reproducibility, predictive value, sensitivity, specificity
 - Consistency, variability
- Safety, Purity/Identity, Potency

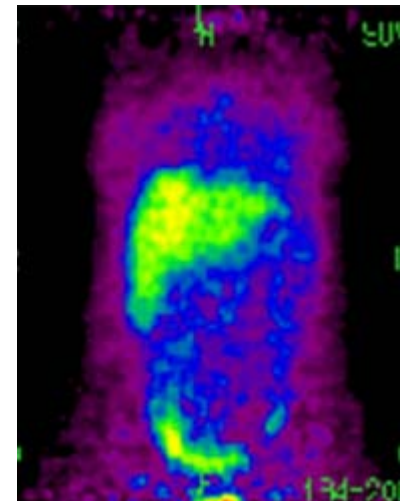


Cell Distribution *In Vivo*

- 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

Testing - Master Cell Bank

- Safety Testing
 - Sterility
 - Mycoplasma
 - Adventitious viruses
 - *In vitro* and *in vivo*
 - Bovine and porcine viruses
 - Human cell lines
 - EBV, HBV, HCV, HIV 1/2, HTLV 1/2, CMV, B19, others
- Characterization
 - Morphology, karyotype
 - Isoenzyme analysis
 - Tumorigenicity
 - Other
 - Cell viability

Testing - Master Virus Bank

- Safety Testing
 - Sterility
 - Mycoplasma
 - Adventitious viruses
 - *In vitro* and *in vivo*
 - Bovine and porcine viruses
 - Human viruses
 - EBV, HBV, HCV, HIV 1/2, HTLV 1/2, CMV, B19, others
 - Murine - MAP
 - RCV
- Characterization
 - Identity
 - Vector sequence
 - Restriction map
 - Activity, expression
 - Transgene-specific protein expression
 - Other
 - Titer
 - What cells to use in titer assay?

Testing - Final Product

- Safety
 - Sterility, Mycoplasma, endotoxin, pyrogenicity
 - Adventitious virus
 - *In vitro* virus
 - RCV
- Characterization
 - Purity
 - Cell substrate DNA, RNA, protein
 - Identity
 - Restriction map, structural characterization
 - Titer
 - Transgene-specific activity
 - Potency (Phase II)
 - Stability

IND CMC Section - Common Problems

- No vector sequence provided
- Virus testing
 - No *in-vitro* adventitious viral testing of final vector product
 - Incorrectly performing *in vitro/in vivo* adventitious testing
- Not performing complete testing for human pathogens on human cell lines
 - Inadequate QA/QC program
 - Inadequate description of segregation and cleaning procedures, to prevent cross-contamination from production of multiple gene therapy vectors

IND CMC Section - Common Problems

- Inadequate lot release testing - *ex-vivo* vector transduced cell products
 - Replication competent retrovirus assays
 - Endotoxin testing
 - Sterility testing, Gram stain

Contract Services

- Academic-based laboratories, n <5
 - Decades of experience in cell/gene therapy development, production, state-of-the-art cell/gene 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/gene therapy
 - Contract services are primary mission
- Cell/gene therapy-specific experience, capabilities, GMP/GTP and QA infrastructure



Summary

Unprecedented numbers of gene therapy products are in development for a remarkable range of clinical applications. Cell-based gene therapy products present unique challenges in development, manufacturing, characterization, and delivery

- Rigorous, evolving characterization and process control vital to address biological heterogeneity, variability, incomplete definition.
- Begin preparing for manufacturing *early*, and expect changes. Processes/analytical methods/product definition must evolve over multiple clinical trials.