

# Characterization of Cell Therapy Products

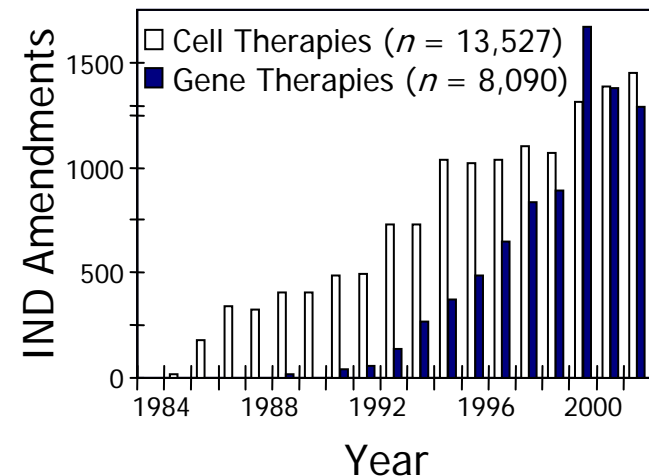
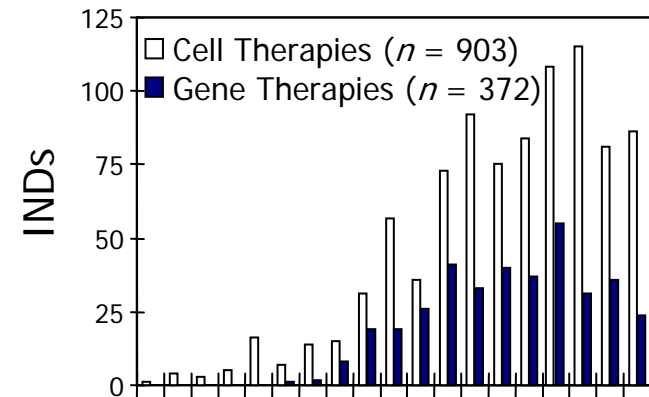
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# Product Characterization Testing

- Safety
  - Sterility, endotoxin, mycoplasma, adventitious agents
- Purity, Identity
  - Cell viability, concentration, morphology, immunophenotype, karyotype, other
- Potency
  - Relevant biological function, real-time surrogates for functional assays
- Stability
- Tumorigenicity
- Reagents, Ancillary Materials

# Product 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
- Analytical Methods
  - 21 CFR 610 methods, *or demonstrate equivalence*
  - At BLA, 21 CFR 610 methods or *validated* alternative methods



# Product Testing - Safety

- Sterility cultures (aerobic, anaerobic, yeast/fungal)
  - Phase I, II - pediatric blood culture bottles. Automated, minimal sample requirements. 14-day culture.
  - Phase III - CFR 610, USP sterility, *or validated equivalent*
- Endotoxin (21 CFR 610.13)
  - Specification <5 EU/Kg/hr for i.v. administration
- Mycoplasma (21 CFR 610.30)
  - Validated PCR-based assay acceptable alternative to PTC
- Adventitious agents
  - Follows practice for blood donor testing, more extensive if using xenogeneic reagents
- Logistics, release policies
  - Results pending, or QCT failure. Clinical release?

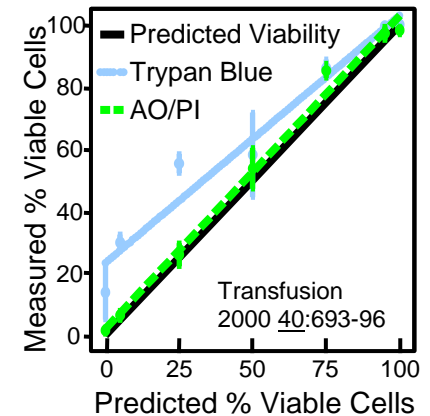
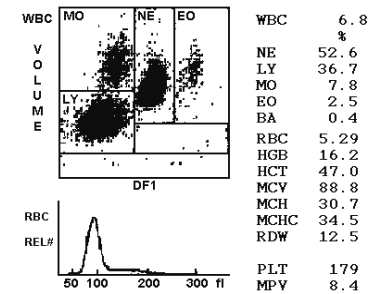
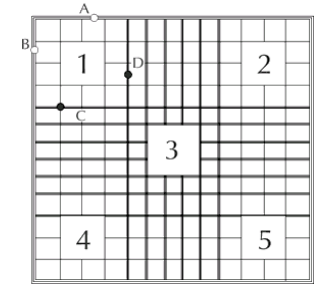
# Safety Testing Strategy

- Cryopreserved products - preferred
  - Thaw and administer product when release testing complete
  - Release testing performed on pre-cryopreservation product
    - Validate cryopreservation and thaw
    - Product thawed and administered *without further testing (!)*
- Products administered fresh
  - Culture 24-48 hr pre-harvest, repeat at harvest
  - Stat Gram stain and endotoxin, at harvest
  - Release based on negative 48-hr culture, Gram stain, endotoxin, final results pending
    - Policy and action plan for positive culture results, other QCT failures, obtained after product administration

# Product Testing – Purity, Identity

## Cell Counts, Viability, Morphology

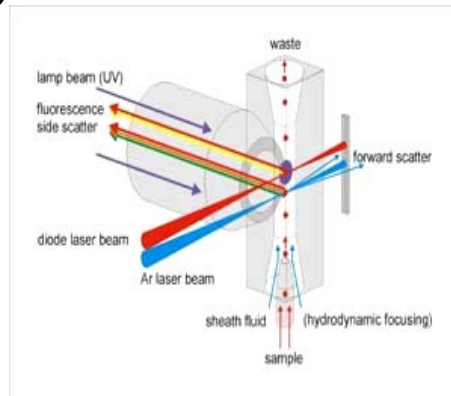
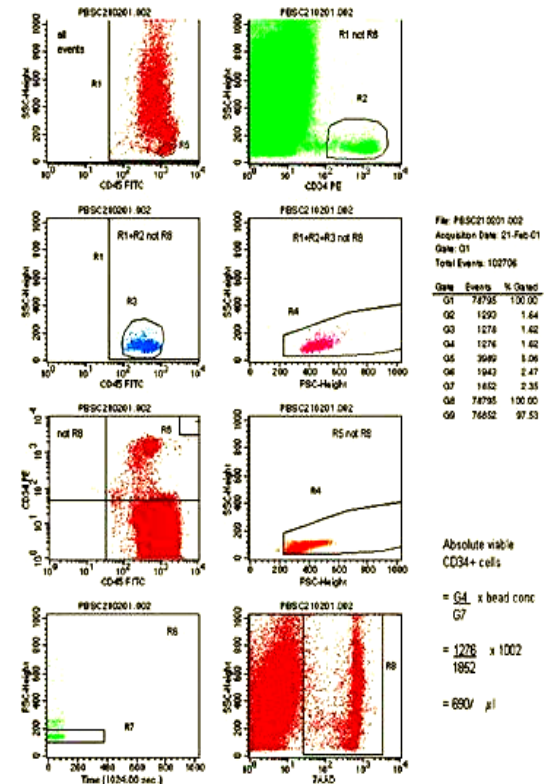
- Manual hemacytometer
  - Impedance technology
  - Other
- Automated electronic cell counters
  - Beckman ViCELL - Trypan blue
  - Guava Technologies PCA - fluorescent dyes
- Qualify viability assay!
  - Trypan blue, acridine orange-propidium iodide, 7-AAD



# Product Testing – Purity, Identity

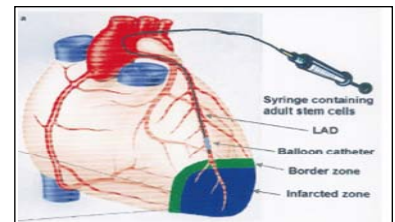
## Immunophenotype Determination – Flow Cytometry

- Essential analytical tool for most cell therapies
  - Purity, identity
  - Applications in potency testing
- Multiple potential sources of error, variability, subjective interpretation
- Requires optimization, rigorous control, *validation*
  - IQ/OQ/PQ - reagents, *cytometer*, software, procedures, analysis

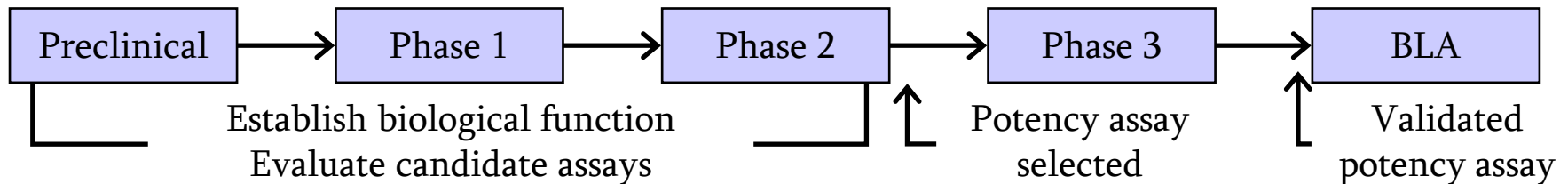
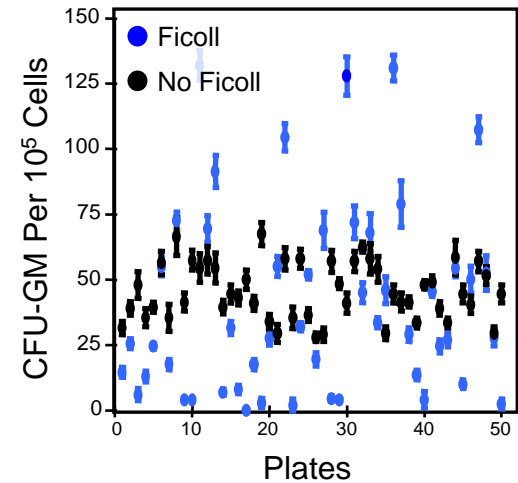


# Product Testing - Potency

- Testing "relevant biological function"
  - Understanding product function refined from preclinical to Phase III
- Potency assays
  - Cytotoxicity, cytokine release, antigen presentation, proliferation, differentiation...
  - Evaluate candidate assays across Phase I, II trials, assess in light of clinical data
  - Functional assay turnaround time problematic, qualify real-time surrogate assays

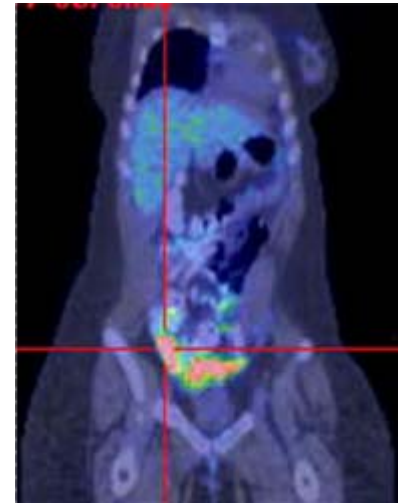


Direct/indirect repair? Humoral factors? *In vitro* correlates for *in vivo* function?

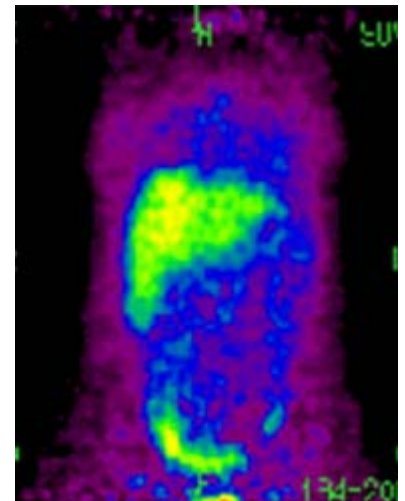


# Cell Distribution *In Vivo*

- 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

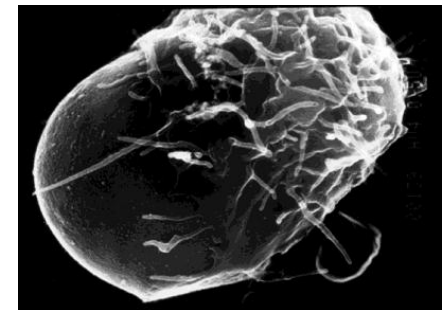


FDG-labelled MAK cells  
t = 3 hr., PET-CT

# Reagents

- GMP manufactured materials needed, but complex, often unique reagents
  - Sera, cytokines, vectors, genes, culture media, supplements, mAbs...
- If GMP reagents unavailable?
  - Qualification
    - Physical characteristics, quality control, qualification tests, storage conditions, expiration
  - Manufacturer Service Level Agreements
- Qualify removal of ancillary materials from final product

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



J Immunol Methods 2001

# Summary

- Cell, gene, and tissue therapy products are not well characterized, and so process control, consistency, and characterization efforts are especially vital.
- Processes, analytical methods, and product definition develop over multiple clinical trials.
- Clinical trial experience often is necessary to elucidate relevant biological function and support potency testing development.