

Characterization of Stem Cell Therapy Products

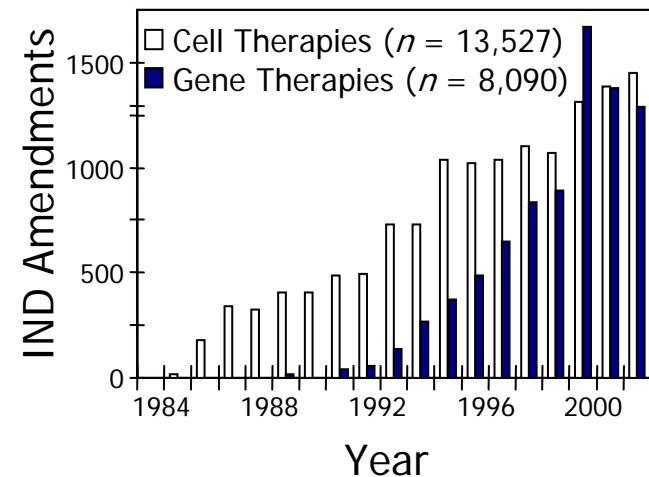
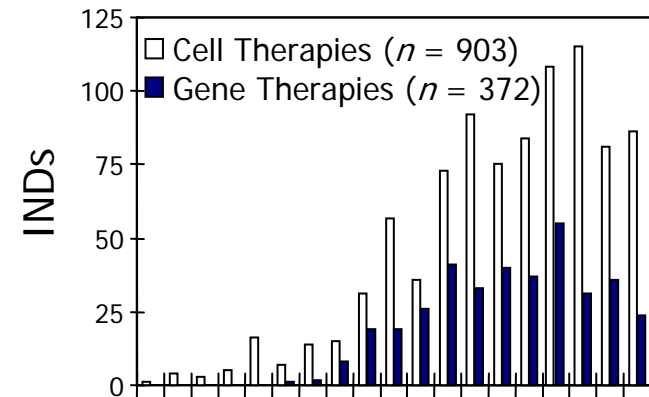
Scott R. Burger, MD
Advanced Cell & Gene Therapy

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?

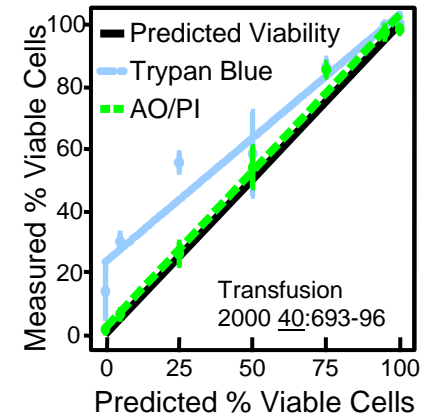
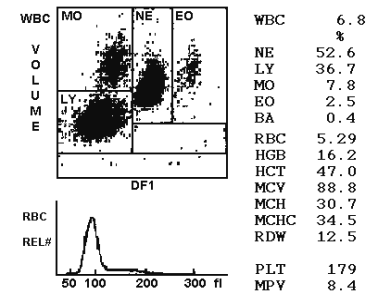
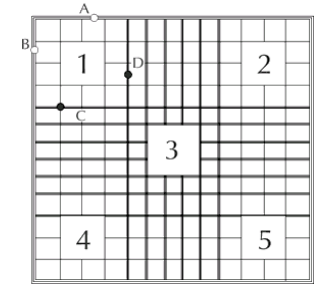
Safety Testing Strategy

- 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

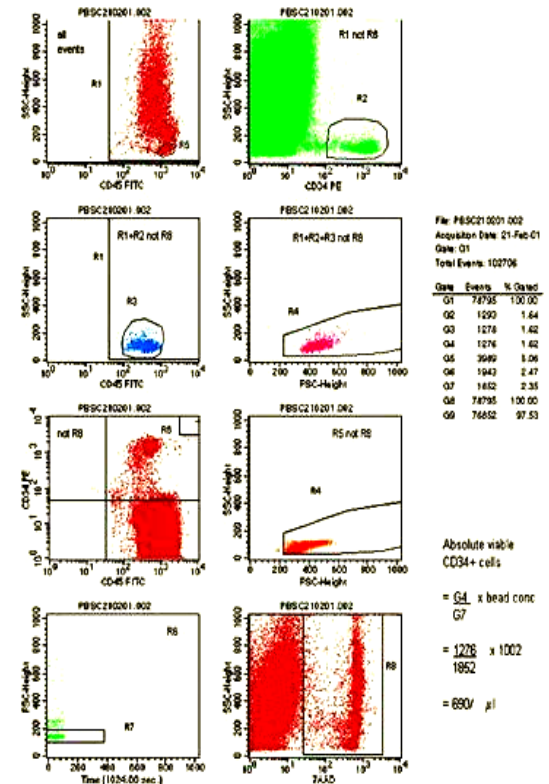
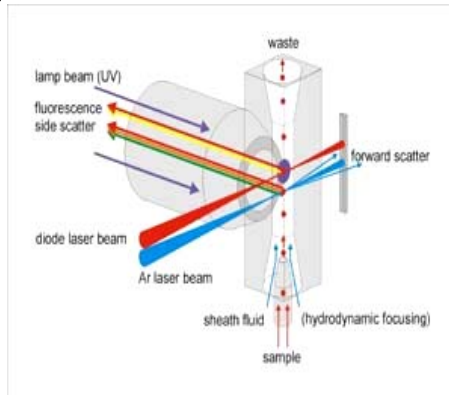
- Manual hemacytometer
 - Impedance technology
 - Other
- Automated electronic cell counters
 - Beckman ViCELL - Trypan blue
 - Guava Technologies PCA - fluorescent dyes
- Qualify viability assay!
 - Trypan blue, acridine orange-propidium iodide, 7-AAD



Product Testing – Purity, Identity

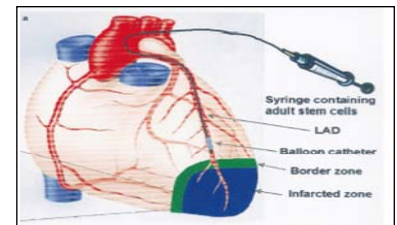
Immunophenotype Determination – Flow Cytometry

- 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

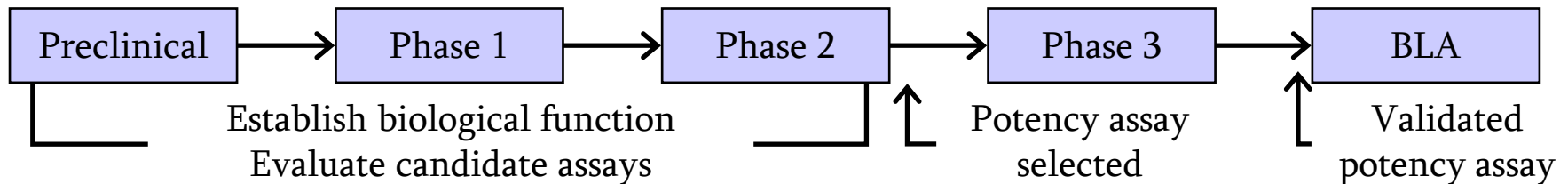
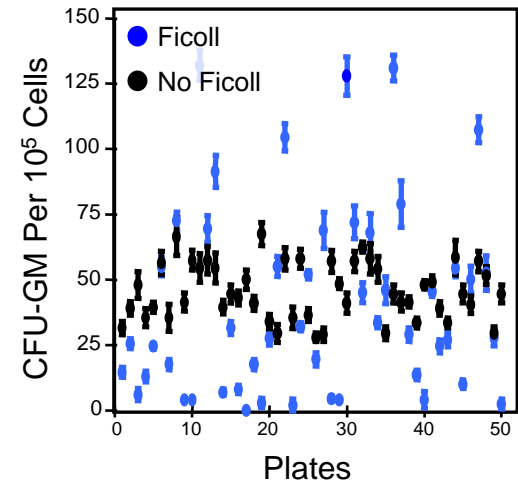


Product Testing - Potency

- 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

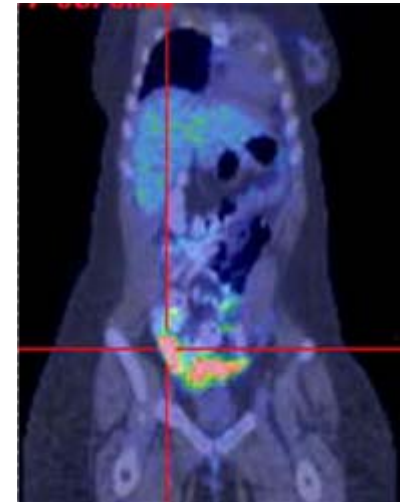


Direct/indirect repair? Humoral factors? *In vitro* correlates for *in vivo* function?

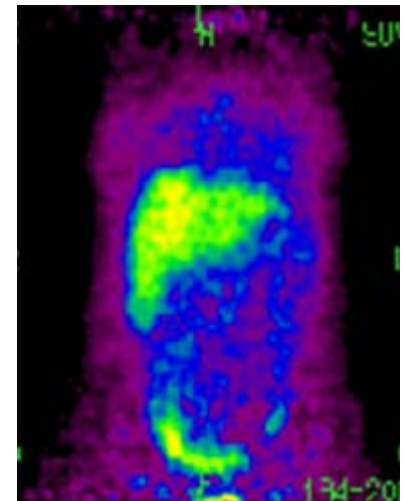


Cell Distribution *In Vivo*

- 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

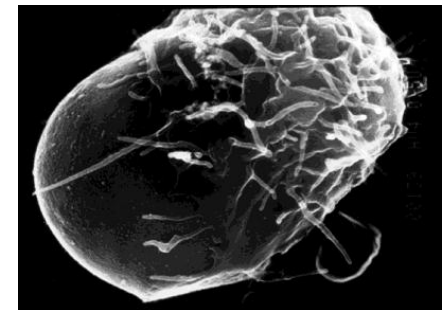


FDG-labelled MAK cells
t = 3 hr., PET-CT

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 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

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.