ISCT Annual Meeting
Rotterdam, The Netherlands
19 May 2011

Technical Applications Track 2
Safety Testing for Cell Therapy Products: Requirements, Relevance, and New Technologies

Speakers
Scott Burger (USA)
Regulatory Requirements for Safety Testing

Marianna Sabatino (USA)
Determining if Mesenchymal Stromal Cells Have Passed (Passaged) Their Prime

Mark Bonyhadi (USA)
Technologies for Rapid Testing
Technical Applications Track 2

Safety Testing for Cell Therapy Products: Requirements, Relevance, and New Technologies

Technologies for Rapid Testing

Mark Bonyhadi (USA)
Director Clinical Business Development
Cellular Medicine, Life Technologies
Cell Therapy = Manufacturing Challenges

• Cell therapies present a variety of manufacturing challenges that can impact the delivery of a safe, consistent and potent product
  
  - **Variability and complexity inherent in the components used to generate the final product**
    
    > Autologous vs. Allogeneic
    > Potential Adventitious Agent Contamination
    > Aseptic processing
    > No “terminal sterilization” possible
  
  - **Distribution challenges due to stability issues and cell product shelf life**
  
  - **Need to release final product before lot release test results are available.**
“An extensive characterisation......should be established in terms of identity, purity, potency, viability and suitability for the intended use....”
Technologies for Rapid Testing

• Current Industry Standards
• Are they adequate?
• What’s available and under development that will:
  
  - Increase speed and sensitivity of testing
  
  - Increase test reliability and facilitate “harmonization” of results

  > As an emerging product area, cell and gene therapies are prime area for prospective harmonization and convergence of regulatory approaches

From: Celia M. Witten, Ph.D., M.D., Director, Office of Cellular, Tissue, and Gene Therapies, FDA
# Current Industry Standards

<table>
<thead>
<tr>
<th>EMEA</th>
<th>FDA</th>
<th>Analyses</th>
<th>Analytic Tools/Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identity</td>
<td>Identity</td>
<td>Cell surface markers, genetic polymorphisms, gene-expression, biochemical activity (phenotype &amp; genotype)</td>
<td>Flow cytometry, SNP, HLA-typing, PCR, Immunohistochemistry, Morphology, Gene Sequence/Expression, Epigenetics, etc.</td>
</tr>
<tr>
<td>Cell Purity</td>
<td>Purity</td>
<td>Freedom from residual contaminants: endotoxins, residual proteins/peptides, growth factors, antibodies, serum, unintended cellular phenotypes, viable vs. non-viable cell content</td>
<td>ELISA, flow cytometry, luminex, vital stains, LAL, CastPCR, PLA, qPCR, etc.</td>
</tr>
<tr>
<td>Impurities</td>
<td>Microbiological Testing</td>
<td>Sterility (bacterial/fungal), mycoplasma, adventitious agents</td>
<td>endosafe®, PyroGene™, BacT/ALERT®, Milliflex®, MycoAlert®, Myco Scan, MycoSEQ™, MycoTOOL™, MicroSEQ™, in vitro cell-line-based virus tests, ViralSEQ™, TaqMan® Open Array®, etc.</td>
</tr>
<tr>
<td>Potency</td>
<td>Potency</td>
<td>Measure of the appropriate biological activity of the product (quantitative/qualitative)</td>
<td>Cell type and therapeutic application specific: may include in vitro or in vivo analyses, may be simple or multivariant analyses (i.e. culture, flow cytometry, gene-expression, etc.)</td>
</tr>
<tr>
<td>Tumouri-genicity</td>
<td>Other: Safety, Cell #, Dose)</td>
<td>Viability, Cell Number/Dose, Karyotype, Tumourogenicity</td>
<td>Cell counters, vital stains, karyotype, genetic analysis, animal models, etc.</td>
</tr>
</tbody>
</table>

Some of the methods above are approved for product release, some are used as “in-process” controls, while others will need to be validated prior to regulatory approval for product characterization/release.
**Are current standards adequate?**

- **What is meant by “safety” and “characterization?”**
  - Identity, purity, potency, viability, tumorigenicity, adventitious agents, etc…
  - Suitability for the intended use?

- **Need for rapid test methods**
  - Some existing tests can take up to 4 weeks, or longer (e.g. in vivo tumorigenicity)
  - As new cell types (e.g. ESC), complex cell mixtures/scaffolds and applications (e.g. gene-modification) evolve, current test methods may not be adequate for proper evaluation

- **Some test methods have inherent variability**
  - Operator-to-operator variability
  - Lack of automation
  - Site-to-site variability
  - Sensitivity of tests
  - Different “reagents” (e.g. different vendors, lot #’s, etc.)
Are current standards adequate?

- Why there is a need for rapid test methods with reduced variability and increased sensitivity

<table>
<thead>
<tr>
<th>Safety</th>
<th>Cell Characterization</th>
<th>Turnaround Time</th>
<th>Variability</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sterility Cultures</td>
<td>14 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gram Stain</td>
<td>Same day</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>Mycoplasma</td>
<td>Same day or 28 days</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>Endotoxin</td>
<td>Same day</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>Viral In Vitro</td>
<td>28 days</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>Viral In Vivo</td>
<td>Weeks</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Human Viral Panel (HIV, HCV, etc.)</td>
<td>Next day</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td></td>
<td>In Vivo tumorigenicity</td>
<td>Weeks</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Purity, Identity</td>
<td>Karyotype</td>
<td>1-2 days</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phenotype (Flow Cytometry)</td>
<td>Same day</td>
<td>✓</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Isoenzyme</td>
<td>Same days</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Potency</td>
<td>Variable</td>
<td>Variable</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
What’s available and under development that will:

- Increase speed and sensitivity of testing
- Increase test reliability and facilitate “harmonization” of results
  - Flow Cytometry Systems

WHERE WE ARE HEADED

- Rapid Rare Cell Event Detection
- Rapid Mycoplasma Detection Systems
- Rapid Microbial Identification
- Rapid Sequencing
- Rapid Molecular Arrays
Flow Cytometry Systems: Differentiation (Identity)

• Cells were immunophenotyped using 192 antibodies to cell surface markers
  - Antibodies were lyophilized into 96 well plates

• Cells were screened by flow cytometry and immunohistochemistry

• Signatures were validated by cell sorting experiments

• Sorted cells were validated by expression of cell-type specific markers and differentiation potential

• BD Lyoplate® Human Cell Surface Marker Screening Panel

• Additional panels in development

  Contents
  - Lyophilized antibody to over 200 cell surface markers arrayed in three 96-well plates (5 tests)
  - Isotype controls
  - Secondary antibodies
  - Flow or bioimaging
Multiple markers of immune function can be measured by flow cytometry (Potency)

**Cytokine flow cytometry (CFC):**
- Anti-IL-2 PE-Cy7
- Anti-IFN-α PE
- Anti-IFN-γ PE

**CD107 degranulation assay:**
- CD107 APC
- Anti-IFN-γ FITC

**pDC function:**
- CD123 PerCP-Cy5.5
- Anti-IFN-α PE
- 28.8%

**Combined BrdU and CFSE:**
- Anti-BrdU APC
- CFSE
- 85%
- 3%

**Cell Signaling:**
- pSTAT-1 A647
- % of Maximum
Flow Cytometry Systems: Purity

Improving “rare event” detection

Attune® - Acoustic Focusing Cytometer

- Faster sample acquisition times (10x)
- Can use dilute samples/no-wash techniques
- Absolute cell counts with external counting reference (e.g. beads)
- Allows for rapid “rare-event” detection/enumeration
Rapid Rare Cell Event Detection: castPCR™ reagents

High specificity TaqMan® qPCR method with potential to assess product purity & lineage commitment

- **Detection of Rare undifferentiated hESCs (methylation-specific)**
  - Data suggests sensitivity down to <0.01% contaminating cells
- **Detection of Specific Lineage Methylation Signatures**
- **Detection of contaminating tumor cells (mutations)**
  - Detect mutation in heterogenous samples, capable of detecting one mutant copy in background of $10^7$ WT copies

Assay Sensitivity and Selectivity

Dilution analysis detecting single mutant copy with 7 log dynamic range: detecting 1 mutant allele in 10,000,000 wild-type molecules
# Mycoplasma Detection Systems

<table>
<thead>
<tr>
<th>Company</th>
<th>Life Technologies (AB)</th>
<th>Company 2</th>
<th>Company 3</th>
<th>Company 4</th>
<th>Company 5</th>
<th>Company 6</th>
<th>Service Labs</th>
<th>Service Labs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Product</strong></td>
<td><strong>MicroSEQ® Mycoplasma</strong></td>
<td><strong>Product 2</strong></td>
<td><strong>Product 3</strong></td>
<td><strong>Product 4</strong></td>
<td><strong>Product 5</strong></td>
<td><strong>Product 6</strong></td>
<td><strong>PCR</strong></td>
<td><strong>28-day test</strong></td>
</tr>
<tr>
<td><strong>Technology</strong></td>
<td>Real-Time PCR</td>
<td>Microarray</td>
<td>End-point PCR</td>
<td>RNA labeling</td>
<td>Luminescence</td>
<td>Q-PCR</td>
<td>PCR</td>
<td>Points-to-Consider Test</td>
</tr>
<tr>
<td><strong>Species Coverage</strong></td>
<td>&gt;90</td>
<td>40 + &quot;universal&quot; probe to detect all Mycoplasma</td>
<td>Unknown</td>
<td>22</td>
<td>based on &quot;universal&quot; Mycoplasma enzyme</td>
<td>25</td>
<td>Variable</td>
<td>All</td>
</tr>
<tr>
<td><strong>Test Sample Volume</strong></td>
<td>100ul-50ml</td>
<td>&lt;1ml</td>
<td>&lt;1ml</td>
<td>1-2ml</td>
<td>100ul</td>
<td>100ul-1ml</td>
<td>low</td>
<td>10ml</td>
</tr>
<tr>
<td><strong>Sensitivity</strong></td>
<td>~1 genome copy/test reaction</td>
<td>&lt;10 cfu/ml</td>
<td>~1 genome copy/test reaction</td>
<td>100,000 cells/ml</td>
<td>&lt;50 cfu/ml</td>
<td>4.5 GC/ul</td>
<td>unknown</td>
<td>1 cfu</td>
</tr>
<tr>
<td><strong>Viability Assessment</strong></td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>unknown</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td>No detection of other species</td>
<td>Detection of other species</td>
<td>Detection of other species</td>
<td>Detection of other species</td>
<td>Bacteria can be detected</td>
<td>Unknown</td>
<td>Detects some bacteria</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Time to Results</strong></td>
<td>Same day 5 hrs</td>
<td>Same day</td>
<td>Same day</td>
<td>Same day</td>
<td>Same day 20 minutes</td>
<td>Same day</td>
<td>28+ days</td>
<td>28 days from start of test</td>
</tr>
<tr>
<td><strong>Quantitation</strong></td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>unknown</td>
<td>Yes</td>
</tr>
</tbody>
</table>

* An integrated sample preparation and real-time, quantitative PCR assay for the detection of *Mycoplasma* in cell culture samples

* Rapid sample preparation and same-day results allow for in-process testing
Rapid Microbial Identification: Product Safety

• **MicroSEQ® Rapid Microbial ID System**
  - Next generation, high throughput comparative DNA sequencing system for identification of bacteria and fungi
  - Used in top pharmaceutical companies worldwide
  - Used for environmental/in-process monitoring and microbial identification
  - Accurate genotypic bacterial identification based on the 16S rRNA gene
  - Accurate genotypic fungal identification based on the D2 region of the 26S rRNA gene
  - Easy workflow, high throughput, accurate results in less than five hours
  - Fully validated and implemented in four months
  - Enables 21 CFR Part 11 compliance

“Genotypic methods have been shown to be more accurate and precise than traditional biochemical and phenotypic techniques. These methods are especially valuable for investigations of failures (sterility test; media fill contamination).”
US FDA Guidance for Industry, September 2004
Rapid Sequencing (personal sequencing systems)

<table>
<thead>
<tr>
<th>Genome:</th>
<th>Transcriptome:</th>
<th>Epigenome:</th>
</tr>
</thead>
<tbody>
<tr>
<td>De Novo Sequencing</td>
<td>Gene Expression Profiling</td>
<td>Chromatin Immunoprecipitation Sequencing (ChIP-SEQ)</td>
</tr>
<tr>
<td>Targeted Resequencing</td>
<td>Small RNA Analysis</td>
<td>Methylation Analysis</td>
</tr>
<tr>
<td>Whole Genome Resequencing</td>
<td>Whole Transcriptome Analysis</td>
<td>Structural Variation Analysis</td>
</tr>
<tr>
<td>Cytogenetic Analysis</td>
<td>Gene Regulation Analysis</td>
<td></td>
</tr>
<tr>
<td>SNP Discovery</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Applications

- PACBIO RS Single Molecule Sequencing
- MiSeq™ Personal Sequencing System
Rapid Molecular Arrays

• TaqMan® OpenArray® System
  - Gene Expression
  - Genotyping
  - miRNA Profiling
  - Digital PCR

• Automation for rapid throughput

• 3072 replicates per plate/one person can generate over 30,000 data points a day without robotics.

• OpenArray® System can be readily adapted to validation studies because of its highly flexible format.

• OpenArray® qPCR can be used early in cell therapy development process to identify a panel of essential markers for:
  - In-process monitoring (including scale-up)
  - Safety (senescence, adventitious agents, purity, genotyping, etc.)
  - Product quality & potency
Rapid Molecular Arrays

- TaqMan® Low-Density Array (TLDA)
  - Real-Time PCR method
  - Spatial multiplexing
    - > 1-8 samples
    - > Single sample = multiple wells
    - > 21 assays/load port
  - Once key panel of “markers” have been identified, array may be able to be used for:
    - > In-process monitoring
    - > Product characterization (e.g. unique to cell type)
    - > Site-to-site comparability
    - > Product release
Summary

• There are many options for rapid (<24 hrs) safety testing and product characterization
• Flow cytometry may be most-effective tool for single cell characterization within a bulk population
• Molecular-based approaches may have the best-qualities for:
  • Rapid testing
  • Automation
  • Reproducibility from operator-to-operator & from site-to-site
  • Harmonization
  • Cost-effectiveness
• Many molecular-based approaches can and should be validated for in-process analyses and product release safety testing and characterization
  • Requires extensive effort by tool/reagent/equipment provider & by end-user to validate to regulatory agency specs
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QUESTIONS & DISCUSSION

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