ISCT Stem Cell Translation: Strategies, Best Practices, and Regulatory Considerations
September 28, 2010

USP Standards for Cell and Tissue Therapies

Fouad Atouf, Ph.D.
Senior Scientific Liaison
Biologics and Biotechnology
Pharmacopeial Standards

- Scope of a Pharmacopeia is to provide standards that ensure safe, effective and high quality medicines
- Pharmacopeia Should provide suitable public standard
  - Global use
  - Global recognition
- Should facilitate registration
  - Quality attributes of specific products
  - Test specifications and acceptance criteria
- Should support regulatory agencies
  - General chapters aligned with regulations
The United States Pharmacopeia Convention (USP)

- Oldest Pharmacopeia: founded 1820
- Independent, science-based, nonprofit public health organization
- Official compendia in the U.S.
  - 1906: First Federal Food and Drugs Act recognizes the U.S. Pharmacopeia as an official compendium
  - 1938: More prominent role for USP’s standards for identity, and strength, quality and purity, in modern Federal Food, Drug, and Cosmetic Act (FDCA)
- USP standards enforceable by the US FDA, and many state authorities and foreign governments (USP does not enforce)
- USP’s standards used to facilitate regulatory filings
- USP/FDA, Private/Public partnership: USP’s mission complements the mission of the FDA's which is to ensure patients have access to safe and effective drug products.
Expert Committees and Expert Panels support USP staff to develop Standards for:

- Drugs
- Biologics
  - Blood and Blood products
  - Cell, Gene, and Tissue Therapies
    - Ancillary Materials
  - Proteins and Polysaccharides
- Vaccines
- Excipients
- Dietary supplements
- Food and Food ingredients
USP Standards in the Biopharmaceutical Lifecycle

**USP Vertical Standards:** Monographs and Reference Materials

**USP Horizontal Standards:**
- General Chapters & Reference Materials for Procedures
- General Chapters & Reference Materials for Ancillary & Process Materials

Event Timeline:
- Early
- IND
- Clinical
- BLA/NDA
- Market
Monographs vs. General Chapters

- **Monographs**
  - Product-specific
  - Requirements for the product to meet

- **General Chapters with number <1000**
  - Enforceable
  - Test methods chapters
  - Methods referenced in product monographs

- **General Chapters with number >1000**
  - Informational
  - Used as a guidance
### Standards for Cell Therapies: USP Approach

<table>
<thead>
<tr>
<th>Chapters-Official</th>
<th>Proposed Chapters Pharmacopeial Forum (PF)</th>
<th>Monographs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell &amp; Gene Therapy Products &lt;1046&gt;</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>&lt;1047&gt; Gene Therapy Products</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>&lt;1027&gt; Flow Cytometry</strong></td>
<td>- <strong>Cell Therapy</strong></td>
</tr>
<tr>
<td></td>
<td><strong>&lt;90&gt; FBS Specifications</strong></td>
<td>- <strong>Gene Therapy</strong></td>
</tr>
<tr>
<td></td>
<td><strong>&lt;xxxx&gt; Process reagents</strong></td>
<td>- <strong>Tissue products</strong></td>
</tr>
<tr>
<td><strong>Ancillary Materials &lt;1043&gt;</strong></td>
<td><strong>&lt;1046&gt; Cell Therapy Products</strong></td>
<td>Chapters and RS:</td>
</tr>
<tr>
<td></td>
<td><strong>&lt;92&gt; Cytokines &amp; Growth Factors</strong></td>
<td>- <strong>Ancillary Materials</strong></td>
</tr>
<tr>
<td></td>
<td><strong>&lt;1024&gt; Bovine Serum</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>&lt;xxxx&gt; Cryopreservation</strong></td>
<td></td>
</tr>
</tbody>
</table>

**USP Chapters to support monographs development**
Draft monograph developed based on information received from volunteer organizations, with validation data and reference standards. Chapters are written by USP Expert Committees/Panels

Monograph or chapter reviewed by USP staff, discussed with Expert Committee, and published in *Pharmacopeial Forum*

Public comments are reviewed and addressed

Final monographs and chapters approved by Expert Committees and made official via publication in *USP-NF*
RS ensures that the process, as measured by release assays, does not change over time

USP RS developed in parallel with documentary standard:
- Evaluated through a multi-laboratory study
- RS is made from a lot that passes release testing.
- RS candidate may be subjected to additional characterization

Replacement require testing of new candidate against existing RS

RS used to show compliance with requirements in the official text
Chapters
Rationale of the revision is to update the chapter with changes in technology and regulatory frameworks in the areas of cell, gene and therapy products.

New design is to split <1046> into 2 chapters:
- <1046> Cell and Tissue-based Products
- <1047> Gene Therapy Products
Chapter <1046> -- Revision’s Outline

- Components of Cell Therapy Manufacturing
- Manufacturing of Cell or Tissue-Based Products
- Analytical Methodologies
- Quality Systems
- Facility Design and Operation Considerations
- Considerations for Validation and Qualification
- Clinical Site Preparation and Administration
- Stability
- Storage and Shipping
- Labeling
- Considerations for Technology Transfer
- Regulations and Standards
Ancillary Materials (AMs)

- **Terminology:** Ancillary Materials will also refer to Ancillary Reagents, Ancillary Products, Process reagents

- Biological and biochemical substances that are used to manufacture cell- , Gene-, and Tissue engineered products or therapeutics derived from cell culture (i.e. vaccines, proteins). These substances are not intended to be in final product.

- Highest quality of AMs is needed by end users and demanded by regulators

- AMs included or to be developed in USP-NF:
  - Protein A
  - Fetal Bovine Serum
  - Trypsin, Collagenase, DNA Nuclease
  - Transferrin
  - Interleukin-4
  - FGF, GM-CSF and other Cytokines and Growth Factors

*AMs with official standard in USP-NF*
Ancillary Materials

- Quality of AMs can affect stability, safety, potency and purity of medicinal products
- Validation of manufacturing processes to ensure removal of ancillary materials from final products
- Residual testing is important (potential immune reaction)
- Risk-based classification of AMs
- Qualification programs for AMs used in cell manufacturing:
  - Identification
  - Selection
  - Suitability of use
  - Characterization
  - Vendor qualification
  - QA/QC data
Qualification Programs for AMs.

- Identification
  - Source material
  - Concentration of use
  - Tests for identification
  - Manufacturing steps

- Selection
  - Early phase of development
  - Microbiological
  - BSE/TSE risk assessment
Suitability of use
- Evaluate the risk associated with transmission of diseases, when using human or animal-derived AMs
- Material traceability
- Risk can be reduced by using a quantitative or semi-quantitative risk-assessment tool (e.g. FMEA)

Characterization
- Level of testing is based on the risk assessment profile
- Specifications to be established for each AM used in manufacturing, to ensure quality of the product
- Acceptance criteria should be based on pre-clinical studies and early clinical studies
Vendor qualification
- Vendor should be qualified early during development
- Audit of vendors should include their GMP and testing program for AM
- Vendors should be familiar with principles of validation

QA/QC data
- Qualification program need to comply with cGMPs and should be monitored by QA/QC unit
- QA/QC data to include: Inspection and release of AM prior to use in manufacturing, vendor auditing, certificate of analysis, stability testing
Ancillary Materials Standards

Guidance-Information Chapter

Specific Product Chapters:
- <1024> Bovine Serum
- <90> FBS Quality Attributes
- <92> Cytokines and Growth Factors Quality Attributes

Reference Standards:
- FBS
- Interleukin-4
USP <90> FBS Quality Attributes

- Initial ranges for FBS quality attributes based on data from suppliers, while trying to harmonize with the European Pharmacopeia

- Published chapter <90>: quality attributes adjusted based on USP multi-lab study (USP Lab, 2 Contract Labs, 4 Industry Labs)

  - Osmolality: 280-360 mOsm/Kg
  - Total Protein: 30-45 mg/mL
  - pH: 7.00-8.00
  - Endotoxins: <10 Units/mL
  - Hemoglobin levels <30 mg/dL
  - Identification: Radial ImmunoDiffusion (RID): species ID, IgG levels
  - Functionality Assays
    - Growth Curve
    - Clonal Assay
Chapter <92> Cytokines & Growth Factors used in cell manufacturing
  – Interleukin-4 (Human Recombinant), first candidate included
  – bFGF, GM-CSF to follow

IL-4 required Tests
  – Identification
    ‣ Amino acid sequence analysis (minimum 8 residues)
    ‣ Western blot analysis
  – Purity: 97% (SDS-PAGE and silver stain)
  – Protein content (UV Absorption)
  – Bioidentity (bioactivity)

Reference Standard Candidate
  – Labeled potency of RS will be based on bioactivity
  – Candidate will be assayed against the WHO IL-4 International Standard
Ancillary Materials – Conclusions

- Standards for AMs are part of a USP overall strategy to establish standards for biological therapeutics

- New Chapters under discussions/development
  - <xxx> Enzymes as Ancillary Materials
  - <xxx> Monoclonal Antibodies as Ancillary Materials

- These chapters specify Reference Standards that can be used to demonstrate compliance to compendial tests

- Reference standards may also be qualified for use as markers and calibration standards for residual testing
Monographs

- Official:
  - Small Intestinal Submucosa (SIS) Wound Matrix
  - Graftskin

- Under Development:
  - Sipuleucel-T
Small Intestinal Submucosa (SIS) Wound Matrix -

Official Monograph

- SIS Wound Matrix is marketed under various brand names to treat a wide range of conditions including management of dermal wounds and burns.

- Applications: Dermatology, Podiatry, Wound Care

- SISWM is composed of 70% protein, 20% carbohydrates, and 7% lipids. Major protein is type I collagen (90%).
Tissue origin: porcine small intestine
  • Mechanically and chemically processed to yield a decellularized matrix
Viral inactivation validated for removal of a battery of viruses (Reovirus, Parvovirus, …)
Terminally sterilized
Meets the requirements under <71> sterility
Endotoxins <85>—Less than 20 USP Units per 70 cm²
SIS Wound Matrix – Quality Attributes and Tests

- Glycosaminoglycan content
  - GAG content, Not less than 2 μg per mg of SIS

- FGF-2 Content
  - ELISA assay, Not less than 10,000 pg per g of SIS

- Metabolic activity
  - MTT assay, metabolic activity compared to fresh porcine jejunum

- Bioactivity
  - Test for the activity of endogenous growth factors, and capacity to promote neuronal differentiation (neurite-like extensions formation) in a cell line (PC12)
Living, bi-layered skin substitute derived from neonatal foreskins

Upper epidermal layer-human keratinocytes

Inner dermal layer-human fibroblasts in bovine collagen lattice

Cell banks generated and screened for microbial and viral contaminants

Monographs tests
  • Histology
  • Gene expression profile
  • Barrier integrity
  • Metabolic activity
Graftskin-Histology

- Qualitative assessment of product’s structural quality
  - Epidermal coverage
  - Epidermal development
  - Keratinocyte aspect
  - Dermal matrix thickness
  - Fibroblast density
  - Matrix aspect

- Authentic Visual References
  - Passing units
  - Failed units
PCR analysis to demonstrate that keratinocytes and fibroblasts are producing cytokines documented to influence wound healing:
- Transforming growth factor beta (+)
- Interleukin 1 alpha (+)
- Interleukin 4 (-)
- PDGF A (+)
- Glyceraldehyde-3-phosphate dehydrogenase (+) (housekeeping gene)
Barrier Integrity: demonstrates presence of stratum corneum and functionality of the epithelium in Graftskin
- Employs cell culture well inserts in a 6 well plate in a percutaneous absorption insert
- Tritiated water placed on upper surface of setup
- 6 hour incubation – assess rate of penetration
- Limit: No more than 1.97% penetration

Metabolic Activity: demonstrates cell viability
- Employs cell culture assay/MTT
- 3 hour incubation
Title
   – Sipuleucel-T

Description/Definition
   – Autologous cellular immunotherapy, the active components are autologous antigen presenting cells (APCs) and a recombinant human protein, PAP-GM-CSF.

Labeling
   – The final product is labeled to indicate the identity of the patient, the expiration date and time, the required storage conditions, and the lot number.

Packaging and storage
   – Supplied in a sealed infusion bag labeled for the specific recipient.
Assay
  – Potency assay by Flow Cytometry

Identity
  – ELISA assay for antigen

Other Requirements
  – Viability by trypan blue exclusion
  – Total nucleated cell count
  – Sterility
  – Bacterial Endotoxins Test <85>
Cell-based Therapies- Future USP Standards

- Standards for ancillary materials will allow access to quality reagents and aids, with defined quality attributes, to use in the manufacturing of cell-based products.

- Standards for specific products will help defining quality attributes for a products or class of products. Defined acceptance criteria will ensure consistency within acceptable levels of biological variation.

- References materials associated with monographs and chapters are used
  - To show compliance with official text
  - For standardization of procedures
  - For system suitability

Comments on USP Chapters and Monographs: FA@usp.org
Thank You