Perspectives on Potency Assays for Complex Biological Products

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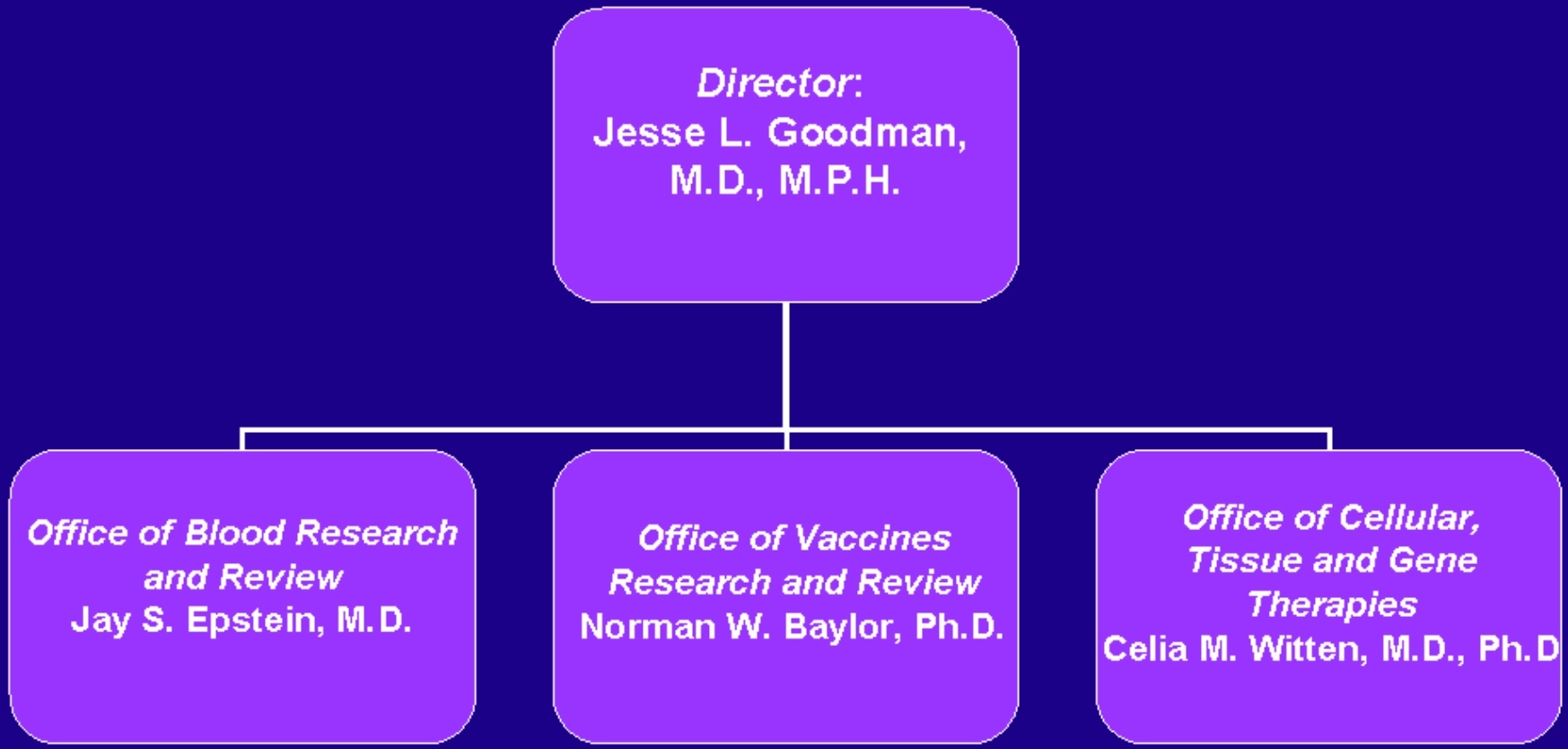
Center For Biologics Evaluation And Research: Product Review Offices



Successful Product Development

- Demonstrate product to be safe, pure, potent, effective and stable
- Full product characterization
- Demonstration of manufacturing and product consistency (e.g. adherence to cGMPs)

Characterization for Product Release 21 CFR 610

- Sterility
- Safety
- Purity
- Identity
- Potency

Potency Regulations

21 CFR 600.3 (s):

The word potency is interpreted to mean the specific ability or capacity of the product…to effect a given result.

21 CFR 610.10:

Tests for potency shall consist of either in vitro or in vivo tests, or both, which have been specifically designed for each product so as to indicate its potency…

Potency Assay Attributes for Licensed Biological Products

- Indicate biological activity (s) specific/relevant to the product
- Results available for lot release
- Provide quantitative readout
- Meet predefined acceptance and/or rejection criteria (demonstrate lot to lot consistency)
- Include appropriate reference material/controls
- Validated for licensure
- Measure activity of all components DEEMED necessary for activity
- Indicate product stability

Progressive Assay Implementation

- During preclinical and early clinical development:
  - Characterize biological activity of the product
    - Wide variances and no (or widely-set) specifications
  - One assay may not capture all critical attributes
    - Determination of product consistency
- Phase II:
  - Potency assay further developed with relationship to biological activity
  - As product development proceeds -- assays evolve:
    - Assays added, deleted, refined
    - Specification modified as needed
- By Phase III
  - Assay well-characterized
  - Specifications should be defined and justified
  - Generally fully qualified, validation on going
- For BLA: validated potency assay
  - used for in process testing, release of DS/DP and drug product stability

Approaches for Potency Measurements

- **Direct measure of biological activity**
  - Biological assay methods: unique and specific product characteristics
    - ***In vivo:***
      - Animal models or clinical data
        - e.g. structural repair, gene function, immune response and/or protection, cell survival, neutralization of venoms, toxins, and viral infections
    - ***In vitro:***
      - Cell & tissue culture
        - e.g. signaling pathways, proliferation, immunogenicity, enzymatic activity, neutralization of venoms, toxins, and viral infections

Approaches for Potency Measurements

- **Indirect measure of biological activity**
  - Analytical assay methods: non-bioassay method directly correlated to a unique and specific activity of the product
    - Immunochemical Procedures
      - e.g. ELISA, ELISPOT, Q-flow cytometry, quantitative western blots
    - Molecular and Biochemical Procedure
      - e.g. Q-PCR, RT-PCR, microarray/genomics, proteomics

Approaches for Potency Measurements

- **Multiple Assay Approach** (Assay Matrix)
  - May not be possible or feasible to develop a single assay that encompasses all elements of an acceptable potency assay:
    - Limited knowledge of product and mechanism of action
    - Product has multiple components with multiple biological activities
    - Time constraints due to limited product stability (e.g. cellular therapy)
    - Biological assay is not quantitative
  - Combination of assays where the combined results, constitute an acceptable potency assay
    - e.g. a quantitative physical assay along with a qualitative bioassay
  - Assay refinement

Approaches for Potency Measurements: Surrogate Measures of Potency

- Use of indirect assays as indicators of biological activity
- Substantiated by direct correlation with results obtained from relevant biological assays
  - Sufficient, statistically sound data
  - e.g by comparison to:
    - preclinical/proof of concept data
    - *In vivo* animal or clinical data
    - *in vitro* cellular or biochemical data

Products Regulated by the OBRR

- **Blood Products:**
  - Whole blood, RBC, leukocytes, plasma, platelets
  - Blood collection/processing establishments
  - Blood testing kits
- **Blood Related Products:**
  - Antibody Products
    - Immune Globulin Intravenous (e.g. Human IGIV)
    - Hyperimmune globulins (e.g., Rho(D)IG, Hepatitis B IG, rabies IG, tetanus IG, botulism IG)
    - Antitoxins & Antivenoms (equine & ovine)
  - Coagulation Factors
  - Hemostatic Agents
  - Anti-coagulants

Example Potency Assays: OBRR Products

- Blood Products
  - *In vivo* survival
  - Biochemical analyses
- Protein Replacement assays:
  - Factor IX (Clotting assay)
  - Thrombin (Clotting assay)
  - von Willebrand Factor (Ristocetin Cofactor Activity assay)
- Antibody products:
  - Tetanus IG, botulism IG (Toxin neutralization assays)
  - Measles IG (Plaque reduction neutralization test)

OBRR General Expectations

- Manufacturers should validate their potency assays according to ICH and FDA guidance documents
- The unitage assigned to their products should be traceable to an international standard when available
- They should have a plan in place to maintain the unitage when they change reference standards
- Testing laboratories should demonstrate good control of their assay methods, and track the consistency of their assays over time

Products Regulated by OVRR

- Preventive vaccines
- Therapeutic vaccines for infectious disease indications (i.e. HIV)
- Toxins & allergenic products

Preventive Vaccines

- **Live, attenuated viral vaccines:**
  - ***e.g. MMR, oral polio, varicella, yellow fever***
- **Inactivated viruses:**
  - ***e.g. Hepatitis A, influenza, inactivated polio, rabies***
- **Crude or purified antigens derived from living or killed cells:**
  - ***e.g. Diphtheria and tetanus toxoids, pertussis, anthrax***
- **Subunit vaccines:**
  - ***e.g. Hepatitis B, influenza, HPV***
- **Conjugate vaccines:**
  - ***e.g. Haemophilus and pneumococcal ps-protein conjugate***
- **Recombinant virus and plasmid DNA vaccines**
  - ***e.g. HIV, Influenza, Ebola***

Expectations for Vaccine Potency Tests

A potency assay should be predictive of immune protection.

- Correlation of a laboratory assay or animal immune response to the expected human immunological response in a dose-dependent manner
- Antigen quantitation in the final formulation
- Direct quantitation of replicating immunogen

Example Potency Assays: OVRR Products

- In vivo
  - Response in immunized animals (e.g. acellular pertussis vaccine)
  - Mouse, Guinea Pig protection assays (e.g. anthrax vaccines)
  - Toxin neutralization assay (e.g. diphtheria and tetanus vaccines)
- In vitro
  - Viable counts (e.g. live viral and bacterial vaccines)
  - Antigen characterization
    - Structural integrity, presence of epitopes
      - e.g. pneumococcal conjugate vaccines, pneumococcal polysaccharide vaccines, HPV vaccine

Challenges to Vaccine Potency Assays

- Fall 2005 NIAID workshop for novel vaccines
  - **http://www3.niaid.nih.gov/research/topics/HIV/vaccines/reports/meeting_Oct11_2005.htm**
  - Epitope determination:
    - MHC Class restrictions (culture, animal models), intracellular processing
  - Correlates of protection (e.g. HIV, malaria)
- Malaria Vaccine Initiative: Correlates of protection
  - **http://www.malariavaccine.org/**
- 2005 CaSSS CMC FORUM
  - Multivalent vaccines
    - Specificity, interference, dilution bias, reference materials
    - **http://www.casss.org/cmc/PDFs/2006MAY_BioProcess_Part2.pdf**

Products Regulated by OCTGT

- **Gene Therapy Products**
  - **e.g. Recombinant and Oncolytic vectors**
    - **plasmids, retro-, adeno-, AAV, HSV, pox, paramyxo-, alpha, rhabdo, reo-viruses**
- **Cellular Therapy Products**
  - **e.g. stem, differentiated, tumor cells**
    - **e.g. embryonic, hematopoetic, cord blood, mesenchymal, neural, pancreatic islets, chondrocytes, myocytes, stromal, dendritic cells, lymphocytes**
- **Therapeutic Vaccines**
  - **e.g. cancer, Alzheimer's Disease, addiction**
    - **cellular products, gene therapy products, cell lysates, cells and cell lysates pulsed with peptides, proteins or vectors, peptides, proteins, adjuvants**
- Tissues, Tissue Engineered Products, Xeno-transplantation Products
- Other Novel Products

Challenges-Assay Characteristics

- Variability
- Validation
- Limited availability of reference standards and controls
  - Patient specific therapies
  - Novel vectors
- Time constraints

Challenges-Product Characteristics

- **Complex mechanism of action**
  - e.g. Multiple steps involved in vector transduction
- **Multiple active components with multiple activities**
  - e.g. multiple cell types, vector types, multiple gene products
  - Potential for interference or synergy
- **Product variability due to variability in starting cells or tissue**
  - e.g. patient specific tumor vaccine
- **Limited material to test**
  - e.g. patient specific tumor vaccine
- **Product stability**
  - Many products administered within hours of harvest
  - Storage/holding may effect viability, potency, etc.

Gene Therapy Potency Assay Development

- **Challenge:**
  - Can a single direct or indirect, biological or analytical assay encompass all elements of an acceptable potency assay?
  - Complex multi-step mechanism of action
    - Dose is based on titer (viral vectors) or mass (plasmids)
    - Efficient transduction: binding/entry into cell, uncoat, expression gene product)
    - Functional gene product (e.g. translational modifications, transport, secretion)
- **Development strategy: Use matrix approach**
  - Develop a combination of assays where the combined results constitute an acceptable potency assay

Potential Example Potency Assays: Gene Therapy Products

- **Cytokine-producing viral vector**
  - Viral titer (genomes/particles and infectious)
  - ELISA: measure cytokine quantitatively relative to titer
    - Functional activity by Phase 3 investigation
      - e.g. Cell proliferation assay
- **Oncolytic virus/vector:**
  - Viral titer (genomes/particles and infectious)
  - Measure tumor specific cytopathic effect or differential viral replication

Cellular Therapy Potency Assay Development

- **Challenge:**
  - Product stability
  - Variable, poorly characterized product
- **Development Strategy:**
  - Cross-Over Between Product Characterization Parameters
    - Assays intended to measure one parameter may be relevant to another parameter

Cellular Therapy Characterization

- Cellular impurities profile: identify and enumerate cell types
- Identity: HLA, other unique markers
  - Examples:
    - Flow cytometric assessment of cell phenotype for purity may link to identity and/or potency
    - Morphological evaluation: cell type and state
- Key parameters?
  - Unique biochemical markers
  - Gene and protein expression analysis
  - Secreted proteins

What to measure for potency?

- Simple identity markers may not change under conditions that affect cell function
- Need to identify **functional** biomarkers
  - e.g. Correlate with in vitro differentiation
  - e.g. Detect unacceptable behavior of cultured cells
  - e.g. Detect functional cells in complex mixture

- Develop genomic or proteomic techniques to identify functional biomarkers??

Potential Example: Cellular Therapy Product A Case study: Is a bioassay necessary?

- **T-cell product: Tumor Infiltrating Lymphocytes (TIL)**
- Potential Potency Assay Matrix:
  - Viable cell number
  - Phenotype characterization (e.g. Flow cytometry)
  - Tumor specific cytotoxicity assay
    - Without CTL experiment: do not know the amount of tumor specific T-cells in complex mixture of expanding T-cells
  - Functional biomarker and/or correlation studies

Summary

- **Potency Measurements**
  - **Directly: Biological assay**
  - **Indirectly: Surrogate assay(s) directly correlated to biological activity**
  - **One of many assays that measure product quality**
- **Progressive assay refinement**
  - **Start Potency Assay Development Early!**
    - Recognize challenges to meeting requirements
    - Evaluate more than one assay
    - Collect correlation data
- **A well characterized product is important when interpreting clinical data!**

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Obtaining Information from CBER

- http://www.fda.gov/cber/publications.htm
  - Guidance Documents
  - ICH Guidelines
- Email:
- Manufacturers assistance:
- MATT@CBER.FDA.GOV
- Consumers:OCTMA@CBER.FDA.GOV