



ASGCT CLINICAL TRIALS TRAINING COURSE May 17-18, 2010

CMC Requirements for Early Phase Gene and Cell Therapy Clinical Trials

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Before You Begin Manufacturing...

Guidance for Review Staff and Sponsors: Content and Review of Chemistry, Manufacturing, and Control (CMC) Information for:

- ❖ Human Gene Therapy Investigational New Drug Applications (INDs)
- ❖ Human Somatic Cell Therapy Investigational New Drug Applications (INDs)
- ❖ Validation of Growth-Based Rapid Microbiological Methods for Sterility Testing of Cellular and Gene Therapy Products (2008)
- ❖ Potency Tests for Cellular and Gene Therapy Products Draft Guidance (2008)

<http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/default.htm>

Product Safety and Quality

- **Components used in Product Manufacture**
- **Final Product Testing and Characterization**
- **Control of Manufacturing Process**
 - cGMP Practices
 - In process controls

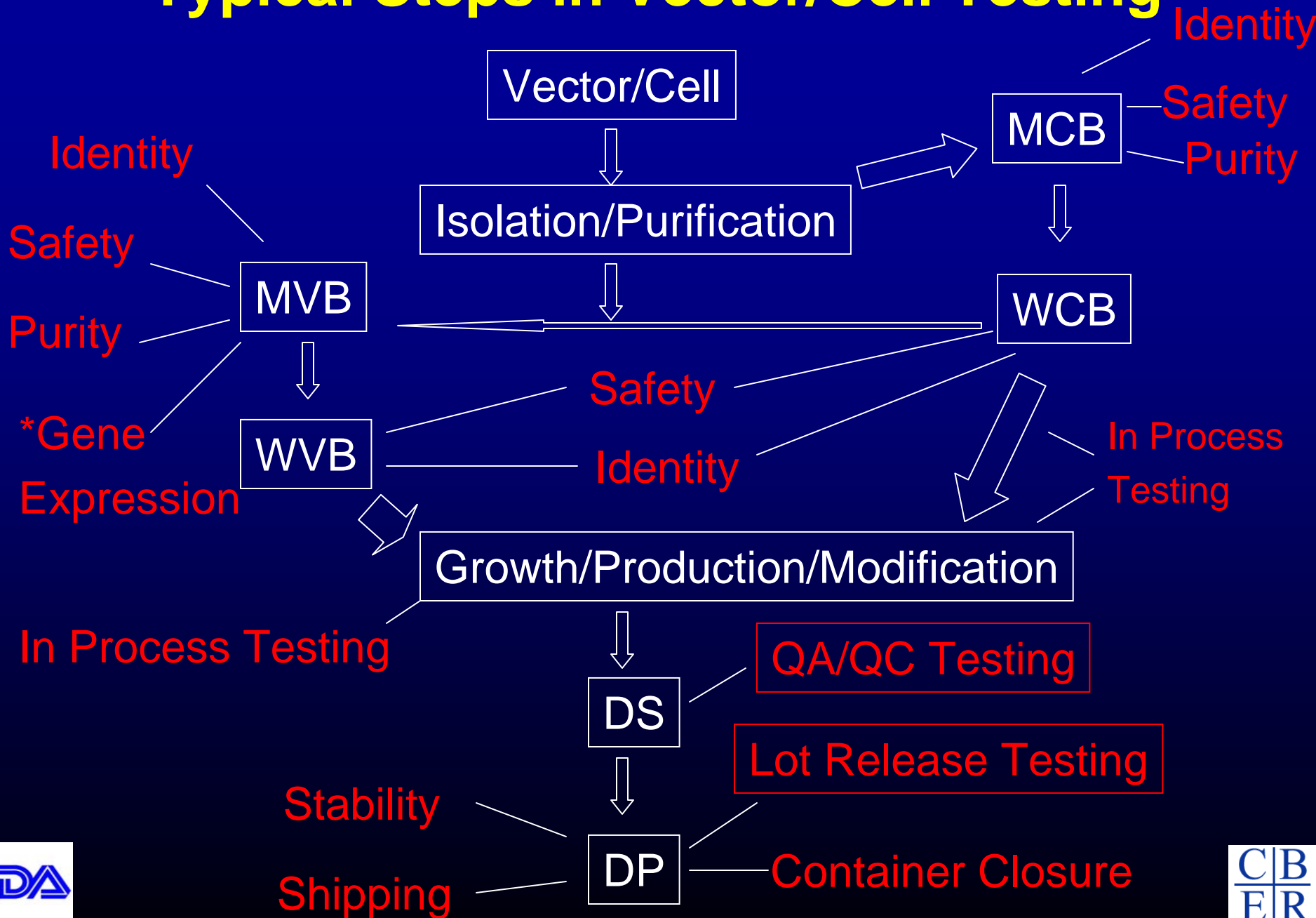
Step-wise Approach to Product Characterization

- **For phase I submission, product safety is the focus of the CMC assessment**
 - Microbial contamination, freedom from adventitious agents, other assessments
 - Characterization as directly related to safety will need to be documented
 - At a minimum, basic product characterization is expected
- **Expectations for Assurance of product quality increase with clinical development**
 - Characterization (CFR 610)
 - cGMPs (CFR 210, 211)

CMC Information-How much is Enough?

- **Important to describe your manufacturing process in enough detail for FDA to assess safety**
 - If safety tests done by contract lab, submit SOPs and available information on assay sensitivity & specificity. If regulatory file available submit cross reference
 - Flow charts with narrative
- **When cross-referencing manufacturing information, be specific in what you are referencing.**

Typical Steps in Vector/Cell Testing



Product Characterization Expectations

GENE THERAPY PRODUCTS

Vectors:

1. Source (original source/isolate)
2. Derivation
 - Construction Method
 - Vector Diagram
 - Gene Inserts
 - Selection Markers
 - Sequencing
 - Identity Tests
3. Production and Purification
 - Host Cells (MCB/WCB)
 - Selection Procedure
 - Growth Medium
 - Reagents

CELL THERAPY PRODUCTS

1. Source (Tissue and cell type)
2. Mobilization protocol
3. Autologous Cells
 - Sterility & Endotoxin Testing
 - Identity & Purity Tests
 - Viability testing
 - Reagents/Cytokines/Growth factors/MoAb
 - Passages
 - Ex vivo Modifications
 - Feeder cells
4. Allogenic Cells (*All the above +*)
 - Donor Screening
 - Donor Testing
 - Cell Banking (MCB/WCB)

Manufacturing Reagent Qualification

- **Tabulation of reagents used**
 - Final concentration
 - Vendor
 - Source (human, bovine, etc.)
 - Licensed product, clinical grade, reagent grade
- **Certificates of Analysis**
- **Cross reference letter**
- **Qualification program**
 - Safety testing and quality assessment

Donor Eligibility-Screening (21CFR1271)

- **Allogeneic**
 - Donor screening is medical history interview, physical assessment and medical record review
 - Risk-factors for, and clinical evidence of relevant communicable disease agents or diseases
 - Communicable disease risk associated with xenotransplantation

Donor Eligibility-Testing

(21CFR1271)

- **Allogeneic**
 - HIV 1&2, HBV, HCV, CMV, Treponema pallidum
 - For viable, leukocyte-rich cells/tissues, additional test for HTLV I & II
 - For reproductive cells/tissues, additional tests for Chlamydia trachomatis and Neisseria gonorrhoea
 - FDA licensed or approved kits or description of test methods, controls, sensitivity
 - Testing on donor mothers for cord blood or maternally derived tissue (not banked)
- **Autologous**
 - DE testing recommended, not required
 - Determine if cell culture methods could propagate viruses

Master Cell Banks & Working Cell Banks

(ICH Q5D)



- *MCB (Master Cell Bank)*—An aliquot of a single pool of cells prepared from the selected cell clone under defined conditions. The MCB is used to derive working cell banks
- *WCB (Working Cell Bank)*—The Working Cell Bank is prepared from aliquots of a homogeneous suspension of cells obtained from culturing the MCB under defined culture conditions.
- A single-tiered banking system consisting only of the MCB but no WCB's could be used in principle, for example, if relatively few containers were needed each year to produce the desired product.

MVB/MCB Testing

<u>TESTS</u>	<u>SPECIFICATION</u>	<u>MVB</u>	<u>MCB</u>
Identity	Product Specific		
Sterility	No Growth		
Mycoplasma	Negative		
<i>Assay for Viruses:</i>			
In vitro AVA assays			
In vivo AVA assays			
Cell line specific virus assays	Negative		
Purity	Product Specific		
Genetic Stability	Product Specific		
Viability			
Replication Competent Virus (RCV)	Negative ⁺		
Gene Expression	Positive		

* Free from avoidable extraneous materials

Tests for Specific Viral Agents in MCB/MVB

	MVB	MCB
Human Viruses: HIV 1 & 2 Hepatitis B Virus Hepatitis C Virus HTLV-1&2 Epstein Barr Virus (EBV) Cytomegalovirus (CMV) Human Herpes Virus (HHV) 6 & 8 Human Parvovirus B19		
Bovine viruses*:		
Porcine Viruses*: Porcine Parvovirus	YES	YES
Production Cell Specific Viruses*:		

* Depending on growth medium/reagents/cells

WVB/WCB Characterizations

Recommendations for a Two Tiered Cell Bank

- Derivation History
- Passage Number
- Genetic Stability
- In vitro adventitious viral agent testing
- Replication competent virus (WVB)*
- Bacterial and fungal sterility
- Mycoplasma
- Limited identity testing (e.g., Southern blot)

* Product Specific

Product Characterization *(CFR Specifications)*

Product should be characterized with reference to its:

- ❖ Safety (610.11, 610.12, 610.30, 610.40)
 - Sterility (bacterial and fungal sterility)
 - Endotoxin
 - Mycoplasma
 - Tests for opportunistic viruses
- ❖ Identity (610.14)
 - Specific test to distinguish it from others
- ❖ Purity (610.13)
 - Free of extraneous materials
- ❖ Constituent Materials (610.15)
 - Ingredients, Preservatives, Diluents, Adjuvants, Excipients
- ❖ Potency (610.10)
 - Assay for biological function

Sterility Testing Expectations

Required for MCB/MVB, WCB/WVB, DS/DP

- **What to test (Q5D/21CFR610.12):**
 - 1% of the total number but not less than two containers of the MCB and WCB
 - DP testing: 10% of the final containers. (If less than 20 – test 2)
 - One repeat test is acceptable under 610.12
- **When to test (Human Somatic Cell Guidance):**
 - In process testing: 48 to 72 hours prior to final harvest or after the last re-feeding of the cell cultures
 - Cellular Products: If final product is frozen, Test before freezing*
- **How to test:**
 - CFR 610.12 defined methods
 - Rapid alternative assays to CFR methods may be acceptable with supporting data (Draft Guidance 2008)

Sterility: Release Testing for Non-Cryopreserved Cells

Regulation

- 14 day sterility assay on final product

Approach

- Sample 72-48 hours or after last manipulation-release on “no growth”
- Results of Gram stain available-release on negative result
- Sample final product & initiate 14 day sterility culture

Sterility: Release Testing for Non-Cryopreserved Cells -2

Following information is required:

- in process testing results
 - sterility of cell culture reagents
 - sterility of any diluent used
 - Sterility results must be obtained by CFR or equivalent assay
- A suitable remedial measure needs to be in place in case of post inoculation culture positivity

Endotoxin / Pyrogenicity Testing and Acceptance Limits for Lot Release

For Cell and Gene Therapy Products:

Endotoxin Assays

- Qualified for early phase clinical trials
- Validated prior to Licensure
- Parenteral drugs ≤ 5 EU/kg body weight/dose
- Intrathecally administered drugs ≤ 0.2 EU/kg body weight/dose
- Rabbit Pyrogenicity Test (21 CFR 610.13 (b))
 - Required for GT/CT products (prior to Licensure)*

** Unless LAL test is shown to provide equal or greater assurances of safety (see 21 CFR 610.9).*

IDENTITY ASSAYS

Identity testing is required to distinguish the product from other products produced in the same facility

Vector

(Recommended Assays)

1. Restriction mapping
2. Sequence analysis (MVB) *
Full sequence for vectors <40KB
Vectors >40kb:
sequence inserts,
flanking regions (500 bp either side)
modified regions

Description of unexpected sequences

Do not submit only raw data

Cells

(Recommended Assay Examples)

1. RFLP Studies/Isoenzyme analysis
2. Assays for Cell Surface Markers
3. Gene Signature

Identity tests may not be able to distinguish Patient specific cellular products

PURITY EXPECTATIONS (DP) (ICH Q3A)

Product purity can be defined as freedom from extraneous material, except that which is unavoidable in the manufacturing process (21 CFR 610.13)

Organic impurities (process- and drug-related)

- Host cell DNA content (< 10 ng/dose)
 - Size of host cell DNA (< 200bp)
- Host Cell Protein Content
- Live Dead cell ratio
- Residual Benzonase
- Residual culture media components
- Particle to PFU ratio (*Product specific*)

Inorganic impurities

- Filter aids, charcoal, column resins, Magnetic beads
- Inorganic salts
- Heavy metals or other residual metals

Residual solvents

Acceptance criteria should be based on pharmacopoeial standards or known safety data (ICH Q6A).

Approaches for Potency Measurements

- **Direct measure of biological activity**
 - In vivo or in vitro assay
- **Indirect measure of biological activity**
 - Analytical assay methods: non-bioassay method directly correlated to a unique and specific activity of the product
- **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

Stability Determination

21 CFR 312.23(a)(7)(ii)

ICH Q5C

- Stability testing required in all phases of the IND
- Recommend a stability protocol and data for both:
 - In-process material
 - Final product
- Stability protocol should measure:
 - Product sterility
 - Identity
 - Purity
 - Potency
- Expiration dating should be based on real-time/real-temperature data

Stability Determination- 2

For Each test conducted, describe:

- Test method,
- Sampling time points (include a zero-time point)
- Testing temperature
- Justification of the Stability determining Assays

Product Tracking and Labeling

Product labeling requirements: *21CFR 312.6(a)*

- An investigational product must contain the following statement
“Caution: New Drug – Limited by Federal Law to Investigational Use”
- Date of product manufacture, storage conditions, expiration date

Autologous/Patient specific Cellular Products:

- Identify and Track from collection to administration of the product
 - In process segregation:
 - Product is segregated from other products in incubators, hoods, and cryopreservation units
 - Product label should include:
 - Product name, and two unique patient identifiers

Product Specific Lot Release Tests

Vectors:

- Viral Particles/Genomic Units to Infectious Units ratio
- Non Replicating Viral Products:
 - Replication Competent Virus testing
- Replication Competent Viral Products:
 - In vitro virus neutralization prior to adventitious agent testing
 - Parallel culture assays when unable to neutralize
 - Assays for selective replication (oncolytics)

Cells:

- Tumorigenicity Assays (Stem cell products)

Lot Release Specifications and Acceptance Criteria

- Specifications are the quality standards (i.e., tests, analytical procedures, and acceptance criteria) that confirm the quality of products and other materials used in the production of a product
- Acceptance criteria are numerical limits, ranges, or other criteria for the tests described

Current Good Manufacturing Practices (cGMP)

Definition

- A set of current, scientifically sound methods, practices or principles that are implemented and documented during product development and production to ensure consistent manufacture of safe, pure and potent products
- Increase in control of the manufacturing process with clinical trial advancement
- Full compliance with cGMP is not required for early phase clinical trials

**Guidance for Industry: CGMP for Phase 1
Investigational Drugs**

GMPs: Early Clinical Phase Critical Questions

- Is the process reproducible?
- Appropriate testing at critical steps?
- Quality of the raw and source materials adequately controlled?
- Are the records and record keeping systems adequate?

QC: Establish Early in Product Development

- **Quality (QC) Program**
 - Ideal - separate unit with ultimate reporting to sponsor
 - Responsibility and authority to accept or reject materials, procedures and specifications
 - Designed to prevent, detect, and correct deviations and failures

Summary

- **Step-wise Approach to Regulatory Requirements**
 - Safety testing
 - Product characterization
- **Control of Manufacturing Process**
 - Describe ALL procedures/Test methods used in the product manufacture
 - Provide Sensitivity and Specificity of the test methods
 - Set Acceptance limits with Rationale for the limits
 - Lot to Lot consistency assessment
- **cGMP Practices**

Ensure a Safe and Quality Product

References & Contact Information

- **References for the Regulatory Process for OCTGT**
 - <http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/OtherRecommendationsforManufacturers/ucm094338.htm>
- **OCTGT Regulatory Issues**
 - Dr. Patrick Riggins (Branch Chief RPM)
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