CONSIDERATIONS FOR GMP MANUFACTURING OF VIRAL VECTORS FOR GENE THERAPY

Nick Conley, PhD
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Overview

- Current Good Manufacturing Practices (cGMPs)
- IND Module 3
- FDA Draft Guidance for Industry – Human Gene Therapy for Rare Diseases
- Precedent for AAV9 Gene Therapy Delivered Intrathecally in Peds
- Manufacturing of Recombinant Adeno-Associated Viral (rAAV) Vectors
- R&D vs. Scalable Manufacturing Approaches
- Critical Quality Attributes (CQAs) and Release Tests
What are cGMPs?

• cGMP stands for Current Good Manufacturing Practices
• 11 Sections of 21 CFR 211: Current GMP for finished pharmaceuticals
• GMPs provide for systems that assure proper design, monitoring, and control of manufacturing processes and facilities
• Adherence to the cGMP regulations assures the identity, potency, and purity of drug products by requiring that manufacturers of medications adequately control manufacturing operations
• Two primary ways to run afoul of FDA: Misbranding and Adulteration
• FDA doesn’t have to prove that your drug is adulterated. If you don’t follow GMPs, they can conclude that your drug is adulterated.
• Failure to follow GMPs can result in statutory, regulatory, and administrative sanctions
  • 483 observations
  • Warning letters
  • Consent decree, recalls, loss of marketing approval
  • Ban from working in industry
  • Fines
  • Prison
10 Basic Principles of GMPs

• Personnel are capable/qualified to perform assigned duties
• Ingredients used in manufacturing have their purported or expected qualities*
• Process validation ensures procedures used will consistently result in product with the expected qualities
• Production environment is suitable for intended purpose
• Finished product has its purported characteristics with end-product testing, effective QC checks, or combination of both
• Finished product retains its characteristics until its labeled expiration date
• Processes are always conducted under control, and as specified
• Prevention of product contamination, cross-contamination and mix-ups
• Adequate records and procedures for thorough investigation of product failures
• Separation of functions/decisions of production and quality control

*with the exception of MCB/WCB, no raw material/excipient release testing required until Phase 2

Key reference: Guidance for Industry – CGMP for Phase 1 Investigational Drugs
https://www.fda.gov/media/70975/download
Module 3 of an IND Application Describes the Chemistry, Manufacturing, and Controls (CMC)

IND Application Components

- Nonclinical data (pharmacology, ADME, toxicology)
- Chemistry, Manufacturing, and Control (CMC)
- Investigator’s Brochure
- Protocol(s)
- General investigational plan
- Previous human experience
- Other relevant information

CTD Pyramid with IND Modules 1-5

Guidance for Industry -- Human Gene Therapy for Rare Diseases

Draft Guidance
FDA CBER
July 2018

“This guidance provides recommendations to stakeholders developing a human gene therapy (GT) product intended to treat a rare disease in adult and/or pediatric patients regarding the manufacturing, preclinical, and clinical trial design issues for all phases of the clinical development program. Such information is intended to assist sponsors in designing clinical development programs for such products, where there may be limited study population size and potential feasibility and safety issues, as well as issues relating to the interpretability of bioactivity/efficacy outcomes that may be unique to rare diseases or to the nature of the GT product itself.”

• Rare disease: disorder or condition that affects <200,000 people in the US
• Nearly 7,000 rare diseases affect >25 mln Americans
• ~80% caused by single-gene defect and half affect children

Reference: https://www.fda.gov/media/113807/download
“Smaller study populations may result in the need for fewer manufacturing runs, which can make it difficult to establish the critical process parameters (CPP) necessary for ensuring critical quality attributes (CQA). However, demonstrating process control to ensure a consistent product with predefined CQA for potency, identity and purity is required to demonstrate compliance with licensure and regulatory requirements.³

These factors make it even more critical that a sponsor of a gene therapy (GT) product for a rare disease establish a well-controlled manufacturing process along with suitable analytical assays to assess product CQA as early in development as possible, optimally before administration of the GT product to the first subject. Importantly, as the phase 1 study may provide evidence of safety and effectiveness, characterization of product CQA and manufacturing CPP should be implemented during early clinical development…”


**Translation from FDA speak:** we know that what you’re trying to do is difficult, but you are bound by the same requirements (i.e. federal regulations) that apply to any other investigational drug.
Precedent for AAV9 Gene Therapy Delivered Intrathecally in Ped.

Trial: https://clinicaltrials.gov/ct2/show/NCT02362438

Manufacturing of Recombinant Adeno-Associated Viral Vectors

doi:10.1016/j.omtm.2018.02.005

**Virus Production**

AAV vectors were produced using methods developed by the University of North Carolina (UNC) Vector Core facility, as described.\(^{42}\) The purified AAV was dialyzed in PBS supplemented with 5% D-Sorbitol and an additional 212 mM NaCl (350 mM NaCl total). Vector was titered by qPCR\(^{43}\) and confirmed by PAGE and silver stain. The recombinant vectors in these studies were sc vectors, except for the ss AAV/CMV-GAN vector. The vectors were packaged in AAV2 for fibroblast studies and in AAV9 for Lec2 and animal studies.


<table>
<thead>
<tr>
<th>Center, location</th>
<th>Production</th>
<th>Cells and platform</th>
<th>Purification</th>
<th>Serotypes</th>
<th>Removal empties</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Powell Gene Therapy, Human Applications Laboratory, University of Florida, Gainesville, FL</td>
<td>2-plasmid Transfection (CaPO4)</td>
<td>HEK293</td>
<td>Cell harvest microfluidization or acidic flocculation and lysis, Benzonase, Heparin AC, IEC, SP, POROS, PS, HA, Hollow fiber tangential flow filtration (TFF)</td>
<td>1, 2 (Y444, 500, 730P), 9</td>
<td>No</td>
<td>14, 48, 51, 56, 63–70</td>
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<td>Avigen Incorporation, Alameda, CA</td>
<td>3-plasmid Transfection (CaPO4)</td>
<td>HEK293</td>
<td>Cell harvest, PEG precipitation, CsCl gradient, Dialysis and concentration</td>
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<td>Yes</td>
<td>61, 78–80</td>
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<tr>
<td>St Jude Children Hospital Children’s GMP, Memphis, TN</td>
<td>2-plasmid Transfection (CaPO4)</td>
<td>293-T CellSTACKS</td>
<td>Cell harvest microfluidization, Benzonase, Sephracyl, Poros 50HQ, Sephracyl, TFF</td>
<td>8</td>
<td>No</td>
<td>8, 50, 81, 82</td>
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<tr>
<td>Ketter Gene Therapy Core Facility, Weill Cornell Medical College, New York, NY</td>
<td>2-plasmid Transfection (CaPO4)</td>
<td>293-T CellSTACKS</td>
<td>Cell harvest freeze-thaws, Iodixanol, Heparin affinity, Dialysis</td>
<td>2, rh10</td>
<td>Yes</td>
<td>83–86</td>
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<tr>
<td>Neurologix, Ft Lee, NJ</td>
<td>Transfection</td>
<td>HEK293</td>
<td>Heparin affinity chromatography</td>
<td>2</td>
<td>Yes</td>
<td>87, 88</td>
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<td>Asklepios BioPharmaceuticals and University of North Carolina, Vector Core, Chapel Hill, NC</td>
<td>3-plasmid Transfection (PELmax)</td>
<td>Suspension Flasks and WAVE Bioreactors</td>
<td>Cell harvest, Sonication, Benzonase, Clarification, Iodixanol gradient, Anion exchange chromatography, Dialfiltration</td>
<td>2, 2.5, 3, 8, 9</td>
<td>Yes</td>
<td>15, 89–91</td>
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<tr>
<td>Harvard Gene Therapy Initiative, Boston, MA</td>
<td>Transfection (CaPO4)</td>
<td>HEK293 CF-10</td>
<td>Freeze/thaw, Benzonase, Detergent, Iodixanol, Chromatography</td>
<td>1, 2</td>
<td>Yes</td>
<td>92</td>
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<tr>
<td>Zaregene Inc, San Diego, CA</td>
<td>Transfection (CaPO4)</td>
<td>HEK293</td>
<td>Heparin Affinity</td>
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<td>No</td>
<td>93</td>
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<tr>
<td>Targeted Genetics Corporation, Seattle, WA</td>
<td>wtAds Infection</td>
<td>HEK293 Producer Line</td>
<td>Depth filtration, Benzonase, Ion exchange, UF/TFF, Chrom step, Heat inactivation, Chrom step, Nanofiltration/Polishing Formulation</td>
<td>1, 2</td>
<td>No</td>
<td>94</td>
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<tr>
<td>AGTC, FL at SABC, CA</td>
<td>HSVInfection</td>
<td>sBHK WAVE bioreactors</td>
<td>Detergent lysis, Benzonase, Depth filtration, TFF, Ion Exchange chromatography, Affinity chromatography, TFF</td>
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<td>No</td>
<td>38, 41, 42, 95, 96</td>
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<td>InQure/AMT</td>
<td>Baculovirus Infection</td>
<td>s9 WAVE bioreactors</td>
<td>Proprietary</td>
<td>1</td>
<td>No</td>
<td>5</td>
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</tbody>
</table>

The information in the table was assembled utilizing information gathered in detailed references or from general websites.

rAAV Manufacturing Process (by Transfection)

Generate GMP plasmids and MCB/WCB
- Establishes clonality of cell line with full characterization

HEK293 cell culture and transfection
- Generates rAAV

Cell harvest
- Collects rAAV-containing cells

Cell lysis
- Releases rAAV particles

Naked DNA digestion
- Removes unencapsidated DNA

Density Gradient Centrifugation & Chromatography
- Removes empty capsids and process impurities

Concentration, buffer exchange, sterile filtration, and filling
- Generates sterile drug product

Generates rAAV
Collects rAAV-containing cells
Releases rAAV particles
Removes unencapsidated DNA
Removes empty capsids and process impurities
Generating sterile drug product

# R&D vs. Scalable/GMP Manufacturing Approaches

<table>
<thead>
<tr>
<th>Process</th>
<th>R&amp;D Approach</th>
<th>Scalable/GMP Approach</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture/amplification</td>
<td>Plate from lab buddy’s last HEK293 passage &amp; plasmid from your undergrad’s last Maxiprep</td>
<td>GMP plasmid + MCB/WCB</td>
</tr>
<tr>
<td>Culture/amplification</td>
<td>Tissue culture flask</td>
<td>HEK293 Adaptation + Bioreactor</td>
</tr>
<tr>
<td>Clarification</td>
<td>Centrifuge</td>
<td>Depth filters</td>
</tr>
<tr>
<td>Dialysis, concentration, buffer exchange</td>
<td>Dialysis tubing or centrifugal MWCO filters</td>
<td>Tangential flow filtration membranes or hollow fiber</td>
</tr>
<tr>
<td>Chromatography</td>
<td>GE Akta Pure or equivalent</td>
<td>Chromatography skid</td>
</tr>
</tbody>
</table>
rAAV Manufacturing Challenges

- Maximizing cell density and transfection efficiency in bioreactor
- Removal of process impurities, especially host cell DNA and proteins
- Removal of empty AAV capsids
- Minimizing purification losses
- Stability
- Developing phase-appropriate analytical methods for identity, potency, and purity
What CQAs & release tests are required for a rAAV gene therapy delivered intrathecally?

- Appearance, USP<790>
- Ratio of full capsids (by qPCR) to infectious particles (TCID$_{50}$)
  - Viral genome titer by qPCR assay
  - Infectious titer by infectious center assay or fluorescence cell assay (potency)
- Identity
  - Whole-genome sequencing and assembly of all extractable DNA
  - AAV-specific identity test
- Expression/activity test for therapeutic gene
- Residual host-cell protein
- Residual host-cell DNA
- Elemental impurities, if justified, USP<232>
- Bacterial endotoxins, USP<85>
- Particulate Matter in Injections, USP<788>
- pH, USP<791>
- Container content for injections, USP<697>
- Sterility, USP<71>
- ICH stability studies
Q&A