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- FDA Draft Guidance for Industry – Human Gene Therapy for Rare Diseases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)

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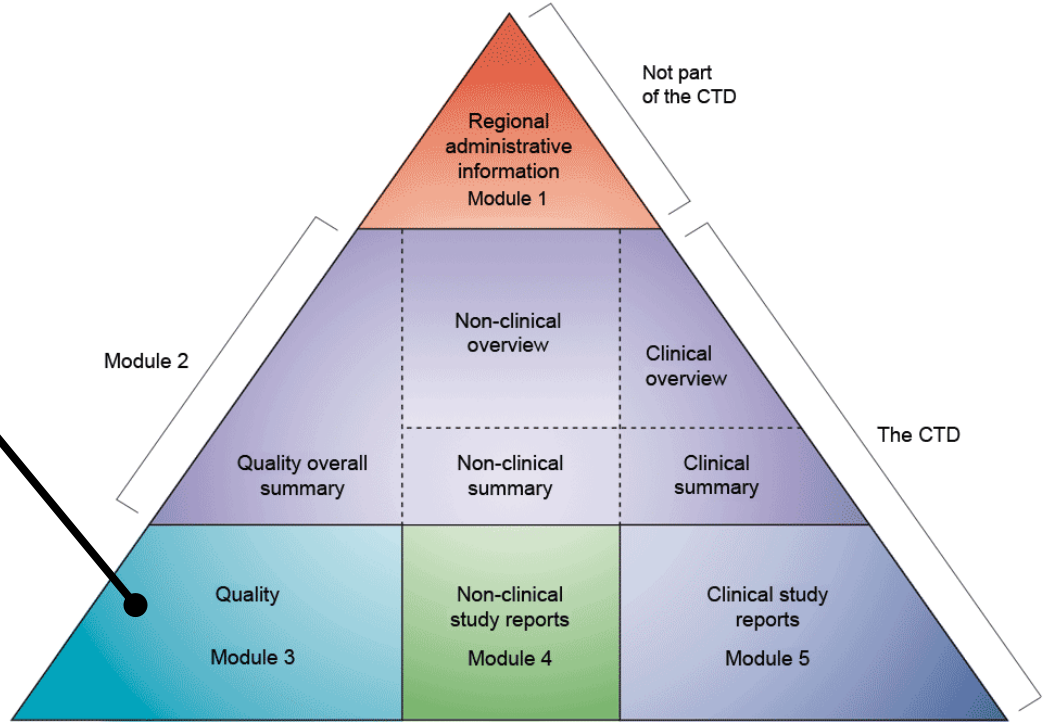
IND Application Components

- 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



Reference: Impact Pharma Services, http://www.impactpharma.com/blog/ready-to-submit-your-initial-ind/

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."

Reference: https://www.fda.gov/media/113807/download

- 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

Guidance for Industry -- Human Gene Therapy for Rare Diseases (cont'd)

"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…"

[3] Section 351(a)(2)(C)(i) of the PHS Act (42 U.S.C. 262(a)(2)(C)(i)); 21 CFR 601.2; 21 CFR 601.20; 21 CFR Part 610, Subpart B.

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 Peds

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Intrathecal Administration of scAAV9/JeT-GAN for the Treatment

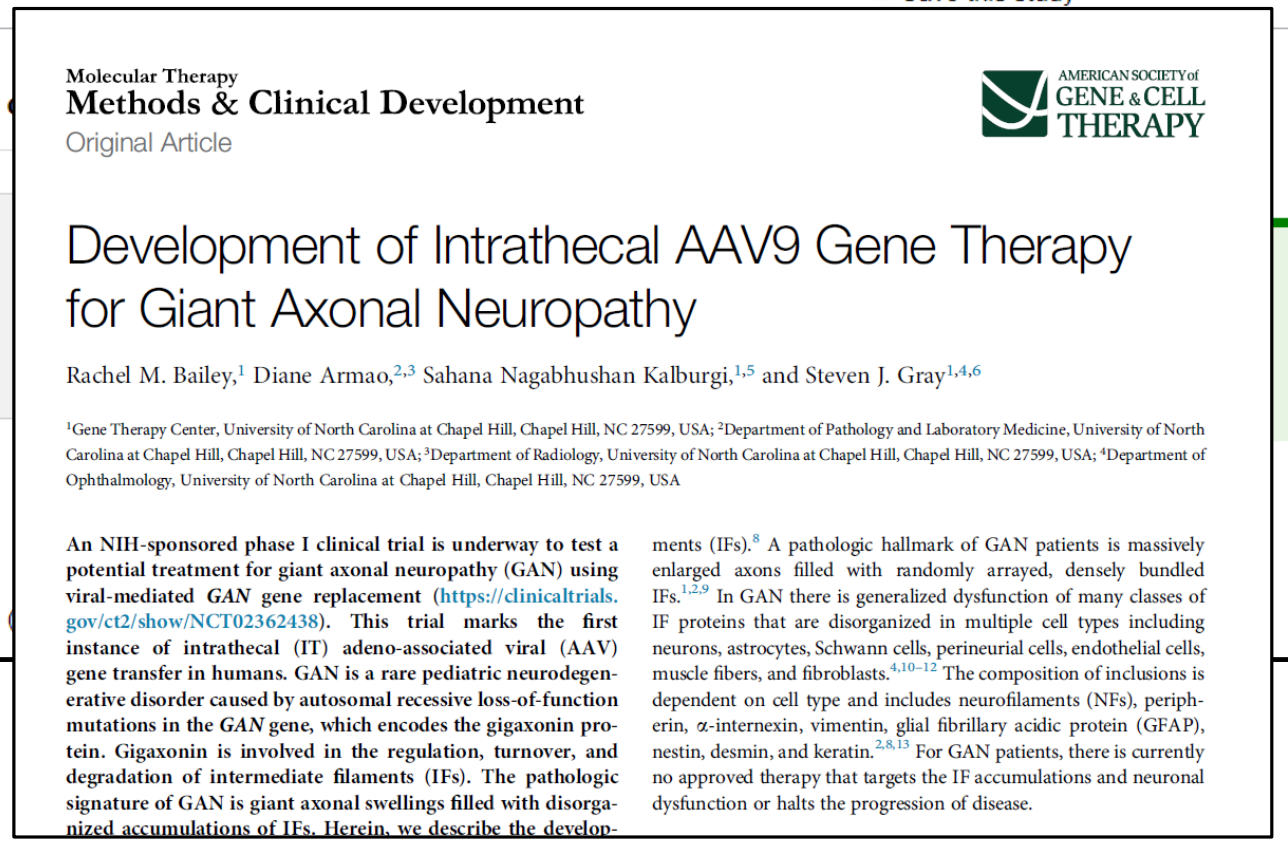
⚠ The safety and scientific validity of this study is the responsibility of the study sponsor and investigators. Listing a study does not mean it has been evaluated by the U.S. Federal Government. Know the risks and potential benefits of clinical studies and talk to your health care provider before participating. Read our disclaimer for details.

Sponsor:

National Institute of Neurological Disorders and Stroke (NINDS)

Information provided by (Responsible Party):

National Institutes of Health Clinical Center (CC) (National Institute of Neurological Disorders and Stroke (



Molecular Therapy
Methods & Clinical Development
Original Article

AMERICAN SOCIETY of GENE & CELL THERAPY

Development of Intrathecal AAV9 Gene Therapy for Giant Axonal Neuropathy

Rachel M. Bailey,[1] Diane Armao,[2,3] Sahana Nagabhushan Kalburgi,[1,5] and Steven J. Gray[1,4,6]

[1]Gene Therapy Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA; [2]Department of Pathology and Laboratory Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA; [3]Department of Radiology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA; [4]Department of Ophthalmology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

An NIH-sponsored phase I clinical trial is underway to test a potential treatment for giant axonal neuropathy (GAN) using viral-mediated *GAN* gene replacement (https://clinicaltrials.gov/ct2/show/NCT02362438). This trial marks the first instance of intrathecal (IT) adeno-associated viral (AAV) gene transfer in humans. GAN is a rare pediatric neurodegenerative disorder caused by autosomal recessive loss-of-function mutations in the *GAN* gene, which encodes the gigaxonin protein. Gigaxonin is involved in the regulation, turnover, and degradation of intermediate filaments (IFs). The pathologic signature of GAN is giant axonal swellings filled with disorganized accumulations of IFs. Herein, we describe the develop-

ments (IFs).[8] A pathologic hallmark of GAN patients is massively enlarged axons filled with randomly arrayed, densely bundled IFs.[1,2,9] In GAN there is generalized dysfunction of many classes of IF proteins that are disorganized in multiple cell types including neurons, astrocytes, Schwann cells, perineurial cells, endothelial cells, muscle fibers, and fibroblasts.[4,10–12] The composition of inclusions is dependent on cell type and includes neurofilaments (NFs), peripherin, α-internexin, vimentin, glial fibrillary acidic protein (GFAP), nestin, desmin, and keratin.[2,8,13] For GAN patients, there is currently no approved therapy that targets the IF accumulations and neuronal dysfunction or halts the progression of disease.

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

Preclinical & in vitro data: Bailey RM, Armao D, Nagabhushan Kalburgi S, Gray SJ. Development of Intrathecal AAV9 Gene Therapy for Giant Axonal Neuropathy. *Mol Ther Methods Clin Dev*. 2018;9:160–171. doi:10.1016/j.omtm.2018.02.005

Manufacturing of Recombinant Adeno-Associated Viral Vectors

Bailey RM, Armao D, Nagabhushan Kalburgi S, Gray SJ. Development of Intrathecal AAV9 Gene Therapy for Giant Axonal Neuropathy. *Mol Ther Methods Clin Dev*. 2018;9:160–171. 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.

[42] Clément, N., and Grieger, J.C. (2016). Manufacturing of recombinant adeno-associated viral vectors for clinical trials. Mol. Ther. Methods Clin. Dev. 3, 16002.

[43] Gray, S.J., Choi, V.W., Asokan, A., Haberman, R.A., McCown, T.J., and Samulski, R.J. (2011). Production of recombinant adeno-associated viral vectors and use in in vitro and in vivo administration. Curr. Protoc. Neurosci. 4, 4.17.

ble 1 Institutions and rAAV GMP manufacturing technologies

Center, location	*Production*	*Cells and platform*	*Purification*	*Serotypes*	*Removal empties*	*Reference*
Powell Gene Therapy Center, Human Applications Laboratory, University of Florida, Gainesville, FL	2-plasmid Transfection (CaPO4)	HEK293 CellSTACKS	Cell harvest microfluidization or acidic flocculation and lysis, Benzonase, Heparin AC, IEC: SP, POROS, PS, HA, Hollow fiber tangential flow filtration (TFF)	1, 2, 2(Y444, 500, 730F), 9	No	14,48,51, 56,63–70
Center for Cellular and Molecular Therapeutics, Children's Hospital of Philadelphia, PA	3 plasmid Transfection (CaPO4)	HEK293 Roller Bottles	Cell and media harvest, TFF and microfluidization, Benzonase, Poros 50HS, CsCl gradient, TFF	2	Yes	13,60, 71–77
Avigen Incorporation, Alameda, CA	3-plasmid Transfection (CaPO4)	HEK293	Cell harvest, PEG precipitation, CsCl gradient, Dialysis and concentration	2	Yes	61,78–80
St Jude Children Hospital Children's GMP, Memphis, TE	2-plasmid Transfection (CaPO4)	293-T CellSTACKS	Cell harvest microfluidization, Benzonase, Sephacryl, Poros 50HQ, Sephacryl, TFF	8	No; then Yes	8,50,81,82
Belfer Gene Therapy Core Facility, Weill Cornell Medical college, New York, NY	2-plasmid Transfection (CaPO4)	293-T CellSTACKS	Cell harvest freeze-thaws, Iodixanol, Heparin affinity, Dialysis	2, rh10	Yes	83–86
Neurologix, Ft Lee, NJ	Transfection	HEK293	Heparin affinity chromatography	2		87,88
Asklepios Bio-Pharmaceuticals and University of North Carolina, Vector Core, Chapel Hill, NC	3-plasmid Transfection (PEImax)	Suspension HEK293 Shaker flasks and WAVE Bioreactors	Cell harvest, Sonication, Benzonase, Clarification, Iodixanol gradient, Anion exchange chromatography, Diafiltration	2, 2.5, 2i8, 8, 9	Yes	15, 89–91
Harvard Gene Therapy Initiative, Boston, MA	Transfection (CaPO4)	HEK293 CF-10 flasks	Freeze/thaw, Benzonase, Detergent, Iodixanol, Chromatography	1, 2		92
Ceregene Inc, San Diego, CA	Transfection (CaPO4)	HEK293	Heparin Affinity	2	No	93
Targeted Genetics Corporation, Seattle, WA	wtAd5 Infection	HEK293 Producer HeLa Cell line WAVE and stir Tank bioreactors	Depth filtration, Benzonase, Ion exchange, UF/TFF, Chrom step, Heat inactivation, Chrom step, NanofiltrationPolishingFormulation	1, 2		94
AGTC, FL at SAFC, CA	HSV Infection	sBHK WAVE bioreactors	Detergent lysis, Benzonase, Depth filtration, TFF, Ion Exchange chromatography, Affinity chromatography, TFF	1	No	38,41,42, 95,96
UniQure/AMT	Baculovirus Infection	Sf9 WAVE bioreactors	Proprietary	1		5

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

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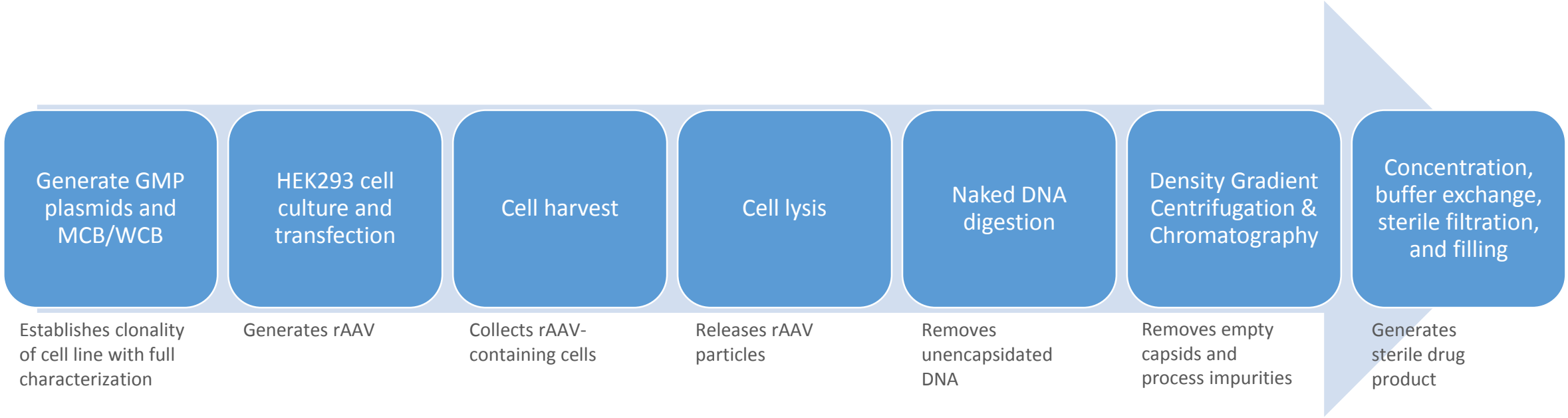
Clément, N., and Grieger, J.C. (2016). Manufacturing of recombinant adeno-associated viral vectors for clinical trials. Mol. Ther. Methods Clin. Dev. 3, 16002.

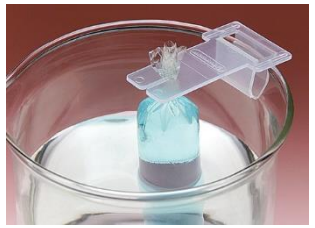
rAAV Manufacturing Process (by Transfection)



Grieger JC, Soltys SM, Samulski RJ. Production of Recombinant Adeno-associated Virus Vectors Using Suspension HEK293 Cells and Continuous Harvest of Vector From the Culture Media for GMP FIX and FLT1 Clinical Vector. *Mol Ther*. 2016;24(2):287–297. doi:10.1038/mt.2015.187

R&D vs. Scalable/GMP Manufacturing Approaches

Process	R&D Approach	Scalable/GMP Approach
Culture/amplification	Plate from lab buddy's last HEK293 passage & plasmid from your undergrad's last Maxiprep	GMP plasmid + MCB/WCB
Culture/amplification	Tissue culture flask	HEK293 Adaptation + Bioreactor
Clarification	Centrifuge	Depth filters
Dialysis, concentration, buffer exchange	Dialysis tubing or centrifugal MWCO filters	Tangential flow filtration membranes or hollow fiber
Chromatography	GE Akta Pure or equivalent	Chromatography skid



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

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Q&A