Apertura GENE THERAPY

#2237

Establishing scalable TfR1 CapX manufacturing platform to accelerate multiple CNS gene therapy programs

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Acknowledgments

We want to recognize our collaborators at the Broad, Viralgen, and at Finding Hope for FRIZZLE (FRRS1L).

Finding Hope for FRIZZLE (FRRS1L)

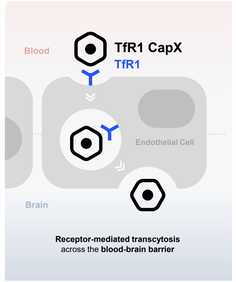
General references

- Guidance for Industry, Preclinical Assessment of Investigational Cellular and Gene Therapy Products. FDA (November 2013)
- Qin Huang et al. An AAV capsid reprogrammed to bind human transferrin receptor mediates brain-wide gene delivery. Science 384, 1220-1227 (2024).

Introduction

The clinical translation of novel AAV capsids is frequently constrained not by biological performance, but by Chemistry, Manufacturing, and Controls (CMC) readiness, including scalability, comparability, and access to clinical-grade starting materials. These factors can become rate-limiting for IND submission when nonclinical and clinical manufacturing approaches diverge, when scale-up introduces unplanned changes to process or control strategy, or when critical starting materials are not available in forms suitable for clinical manufacturing.

Human Transferrin Receptor and TfR1 CapX™



Well-Understood BBB Receptor

TfR1 is a well-studied receptor **highly expressed on the BBB**. The receptor cycles rapidly and is expressed consistently throughout life. There are no known SNPs in the critical regions for TfR1 CapX binding.

Efficent Brain-Wide Delivery

All brain cells are **within 20µm of a blood vessel across mammalian species.** RMT via endothelial cells enables efficient, brain-wide CNS delivery, making IV-delivery an ideal route of administration.

Scalable Across Species

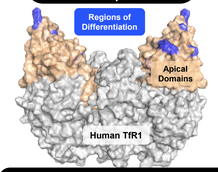
IV administration avoids diffusion scaling challenges seen with intraparenchymal and intrathecal injections. This facilitates direct translational relevance from preclinical models (e.g., hTFR1 mouse) to humans.

Validated Clinical Target

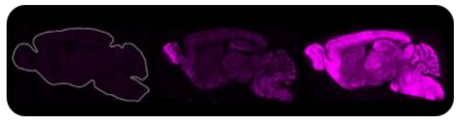
Clinical efficacy has been demonstrated by studies of TfR1-binding antibodies from multiple companies. JCR, Denali, and Roche all have shown promising clinical data with their TfR1-binding antibody programs, validating our MOA in humans.

Pardridge, W. M. (2023); Duvernoy, H. et al (2002); Galvan, A. et al. (2021); NCT03568175; NCT06181136; NCT05661125

TfR1 CapX Is Uniquely hTfR1 Specific



TfR1 CapX Demonstrates Superior Biodistribution vs Other Vectors



Compared to AAV9 and the first-generation BI-hTFR1 capsid, TfR1 CapX demonstrates transduction of **>80% of neurons** at low dose of 2E12 vg/kg, superior **175-fold brain enrichment** relative to AAV9, and maintains a highly selective **1:1.7 brain-to-liver sequestration ratio**.

Payload	ssAAV-CAG-NLS-mScarlet-2A-luc-WPRE-pA
Dose	2.0 x 10^{12} vg/kg
Readout	21 days post injection
Age of Mice	10 – 16 weeks (adult)

	AAV9	BI-hTFR1	TfR1 CapX
Receptor	Galactose AAVR	hTfR1, Galactose, AAVR	
Species			
Dose (vg/kg)		2E12	
Brain Delivery (vg/dg)	0.02	1.43	2.80
Liver Sequestration (vg/dg)	14.38	21.01	4.74
Brain-to-Liver Ratio (1 vg : vg)	1:899	1:14.7	1:1.7
Brain enrichment over AAV9	1	89.4	175
Cortical neuron transduction (%)	N/A	15.0%	82.2%

Model utility for evaluating TfR1 CapX

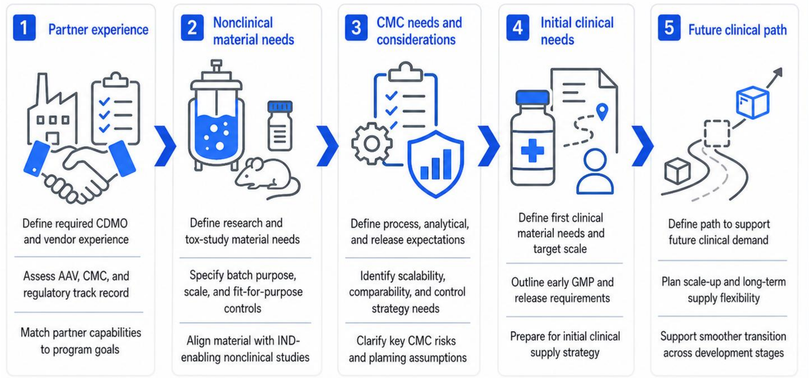
Evaluation Method	Expresses hTfR1	Biological Rationale	Efficacy	Dose Response	Distribution	Safety	Immunogenicity
Human cell line models	✓	✓	⊘	⊘	⊘	⊘	⊘
Disease mice model	⊘	✓	✓	✓	⊘	⊘	⊘
hTfR1 KI mice	✓	⊘	⊘	✓	✓	✓	✓
Disease x hTfR1 KI mice	✓	✓	✓	✓	✓	✓	✓
NHP	⊘	⊘	⊘	⊘	⊘	⊘	✓
Clinical trials	✓	✓	✓	✓	⊘	✓	✓

Defining Program Needs Early is Critical for Success

TfR1 CapX is a human transferrin receptor–targeted AAV capsid being advanced across multiple CNS gene therapy programs.

Here, we describe a manufacturability-focused framework centered on early CDMO selection and platform manufacturing, drawing on experience from two independent TfR1 CapX programs: a pediatric FRRS1L gene therapy program sponsored by a parent-led patient advocacy foundation and a prion disease program (PRNP) led by collaborating investigators.

In both cases, Viralgen was selected to support process development, scale-up, and IND-enabling manufacturing activities.



Manufacturing Representative Virus at Multiple Scales

For the TfR1 CapX/FRRS1L program, Viralgen manufactured a 50 L toxicology batch using their platform process that was representative of the planned clinical manufacturing process and supported its use in GLP safety and biodistribution studies. This tox-scale manufacturing experience helped reduce comparability risk between nonclinical and clinical material, informed IND-enabling CMC planning, and supported execution of the subsequent 500 L clinical-scale manufacturing lot. The 500 L clinical-scale manufacturing run was completed successfully, further demonstrating alignment between the nonclinical and clinical manufacturing strategies and supporting the feasibility of advancing the TfR1 CapX/FRRS1L program toward clinical use.

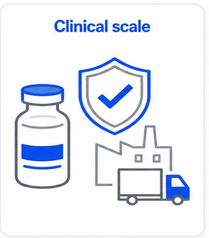
In parallel, Viralgen is supporting manufacturing for a TfR1 CapX-based PRNP program, providing an independent example of the capsid's compatibility with Viralgen's platform process and laying the groundwork for a shared manufacturing framework across indications.

Virus produced at small-scale feasibility batches can have multiple uses in a development program:

- Demonstrates payload/capsid performance in the manufacturer's platform process
- Supports early projection of future scaled yields and process fit
- Generates material for assay development and optimization
- Enables early in vitro and in vivo proof-of-concept studies

Virus produced in 50 liter-scale batches can have multiple uses in a development program:

- Demonstrates payload/capsid performance at a larger, representative manufacturing scale
- Provides material for critical IND-enabling studies, including biodistribution and toxicology
- Supports delivery-device compatibility and in-use stability assessments
- Enables stability studies and generation of reference material for assay qualification
- Reduces comparability risk between nonclinical and planned clinical material



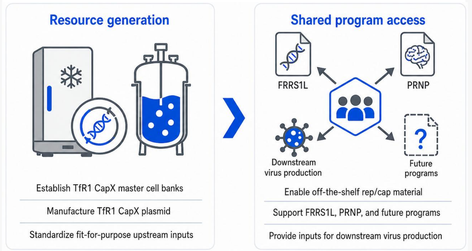
Virus produced at clinical scale can serve multiple uses in a development program:

- Provides GMP material for the initial clinical trial
- Supports planning for future clinical supply needs
- Generates stability information to inform future manufactured lots
- Provides reference material and retained samples for comparison with future lots
- Supports release testing, clinical supply strategy, and long-term manufacturing continuity

TfR1 CapX Rep/Cap is an Enabling Resource

Building on these experiences, a collaboration was established with Viralgen to generate TfR1 CapX master cell banks and plasmid to enable off-the-shelf, clinical-grade TfR1 CapX plasmid supply for downstream virus production, addressing a common bottleneck for emerging programs by improving access to fit-for-purpose starting materials.

By standardizing and de-risking a critical upstream input for clinical manufacturing, this model is intended to reduce redundant CMC work across programs and support more predictable regulatory and CMC timelines for smaller sponsors and rare disease foundations.



Conclusions

Collectively, these programs illustrate how early CDMO partnership, platform manufacturing, and shared starting materials can de-risk IND-enabling development for novel AAV capsids. The successful 500 L clinical-scale FRRS1L manufacturing lot demonstrates that TfR1 CapX can be incorporated into existing Viralgen platform processes and infrastructure, supporting scalability from feasibility and tox-scale manufacturing through clinical production.

This case study highlights a practical roadmap for advancing receptor-targeted AAV vectors into the clinic while maintaining manufacturing rigor, comparability, scalability, and regulatory alignment.

Call to Collaboration

No single organization can address every disease. Apertura recognizes that partnering with driven organizations can help expand our reach and hopefully drive research forward from bench to bedside. It is our intention that the tools, technologies, and know-how we develop can be leveraged by partners across patient advocacy groups, foundations, academia, as well as biotechnology & pharmaceutical companies.

Connect with us – info@aperturagtx.com