Apertura GENE THERAPY

#1900

Revolutionizing Neurometabolic Gene Therapy: The TfR1 CapX Platform and a Primate-Free Regulatory Pathway

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Acknowledgments

We are grateful for the support of our partners at LabCorp, the PAS team at Advanced Cell Diagnostics, Ben Deverman, Sonia Vallabh, and the entire team at Apertura.

We thank the countless public servants at the FDA, past and present, who have devoted their careers to developing the scientifically sound, humane, and forward-looking guidance for industry.

References

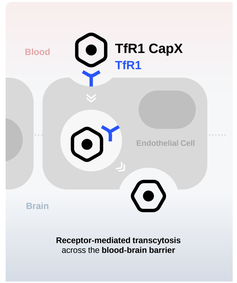
- Guidance for Industry, Preclinical Assessment of Investigational Cellular and Gene Therapy Products. FDA (November 2013)
- Qin Huang et al. An AAV capsid reprogrammed to bind human transferrin receptor mediates brain-wide gene delivery. Science 384, 1220-1227 (2024).

Introduction

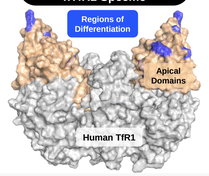
Neurometabolic disorders represent a diverse group of conditions with severe CNS manifestations, many of which are amenable to gene therapy. However, effective AAV-based CNS delivery remains a major translational challenge, particularly for large or systemically progressive disorders requiring broad brain distribution. TfR1 CapX, an engineered human transferrin receptor-targeting AAV capsid, has demonstrated high-efficiency BBB penetration and robust CNS transduction after intravenous administration, positioning it as a versatile platform for neurometabolic gene therapies.

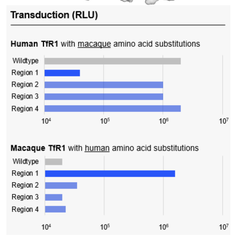
Finalized and draft FDA guidance as well as INTERACT feedback that Apertura Gene Therapy has recently received support the use of alternative models when scientifically justified, particularly in cases where non-human primate (NHP) studies lack pharmacological relevance. Here we leverage our regulatory experience in genetic epilepsies & neurodegenerative disease, preclinical data, and direct FDA engagement, and we outline a streamlined pathway for developing neurometabolic gene therapies with TfR1 CapX—one that does not require NHP studies.

Human Transferrin Receptor and TfR1 CapX



TfR1 CapX Uniquely hTfR1 Specific





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

Advantages of Avoiding NHPs in Preclinical Development

Accelerated Timeline: Faster transition from preclinical studies to IND allowance.

Cost Efficiency: Reduced expenses for animal studies and preclinical manufacturing.

Higher Experimental Throughput: More animals can be dosed when using mice, increasing statistical power.

Improved Resource Utilization:

- Mice have a stable, reliable supply chain, unlike NHPs.
- Mouse studies are easier to contract, increasing study slot availability.
- Supports the 3R principles (Replacement, Reduction, Refinement) by minimizing the use of higher-order species.

Development of a Streamlined and Evidence-Based Preclinical and Regulatory Roadmap

Regulatory strategy drives preclinical development: Early regulatory engagement through FDA INTERACT

Animal Species Selection

The human transferrin receptor knock-in (B-hTfR1) mouse model, instead of non-human primates (NHPs), can be used as the definitive model for the evaluation of biodistribution, safety, and toxicology.

Dose Range Finding

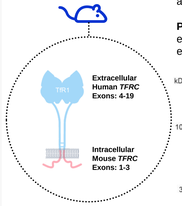
Dose range and initial clinical dose for FIH studies can be determined by outcomes and distribution from a surrogate capsid in the mouse disease model and the hTFR1 KI mouse model, compared to the distribution of the TfR1 CapX capsid in the hTFR1 KI mouse model.

- **NHP studies conducted at Apertura have confirmed that, as expected from our mechanistic understanding of TfR1 CapX, this capsid and other hTfR1-capsids are not enriched in the CNS of NHPs.** We have received FDA INTERACT feedback and alignment that NHPs are pharmacologically irrelevant for TfR1 CapX studies.
- **Apertura is developing gene therapies for genetic epilepsy in tuberous sclerosis complex (TSC) using TfR1 CapX for broad CNS delivery.** The therapy targets TSC1/TSC2 mutations, with a focus on both pediatric and adult patients, in collaboration with the TSC Alliance.
- **The Broad Institute is developing a gene therapy for prion disease, also utilizing TfR1 CapX, to reduce PRNP expression and slow neurodegeneration.** Dr. Sonia Vallabh leads the program, with Apertura advising on preclinical development, regulatory strategy, and CMC.
- **The FDA supports using the TfR1 CapX capsid and the humanized TfR1 knock-in mouse model for distribution and safety evaluation,** eliminating the need for NHP data for IND submission in both the TSC and PRNP programs. The agency has also endorsed the surrogate capsid strategy for dose range finding.

FDA-Endorsed Surrogate Capsid Approach Enables Efficient and Scalable Preclinical Development

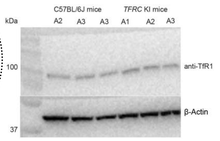
***TFRC* KI mice**

B-hTFR1 mice (Biocytogen #110861)

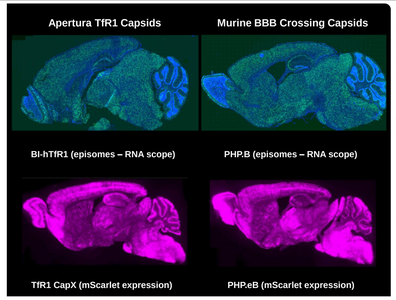


Human-Relevant Expression: B-hTFR1 mice express a chimeric transferrin receptor with human extracellular domains, enabling accurate biodistribution and toxicology assessments for TfR1 CapX gene therapies.

Physiologically Comparable: TfR1 expression in B-hTFR1 mice mirrors endogenous levels in the background strain.



Similar expression of TFRC between WT and KI



Advantages of Surrogate Capsids

- Eliminates additional breeding.
- Leverages existing data for future indications.
- Broad applicability across multiple gene therapy targets

Mouse BBB-Crossing Capsids

- Developed by Dr. Ben Deverman (Deverman et al., 2016), **PHP.B and PHP.eB capsids cross the BBB upon IV administration**, enabling robust transduction of astrocytes and neurons in LY6A-expressing mice. **Capsid BI-28** (Huang et al., 2023), leveraging the LY6C receptor, expands applicability across most mouse strains.
- **RMT Mechanism:** Both capsid families utilize receptor-mediated transcytosis (RMT) for BBB penetration.

	Payload Feasibility	Surrogate Capsid Evaluation	Mouse Model Correlation	Capsid Correlation	Safety, Distribution & Toxicology Study
	Proof of concept of payload	Correlation of PHP.B between diseased vs. non-diseased mice	Evaluation of PHP.B in hTfR1 KI mice compared to non-disease mice	Assess correlation of PHP.B and TfR1 CapX in hTfR1 KI mice	TfR1 CapX dose equivalency between hTfR1 KI mice and humans
PROCESS	A: TSC1 Disease Mice + PHP.B + Therapeutic Gene	B: Non-Disease Mice + PHP.B + Therapeutic Gene	C: hTfR KI Mice (non-disease mice) + PHP.B + Reporter Transgene	D: hTfR KI Mice (non-disease mice) + TfR1 CapX + Reporter Transgene	E: hTfR1 KI Mouse ≈ Human Patient + TfR1 CapX + Therapeutic Payload
OUTCOME	A	A ∝ B	A ∝ B ∝ C	A ∝ B ∝ C ∝ D	D = E
ANALYSIS	Assessments by immunofluorescence & RNAscope (spatial resolution) and ddPCR analysis (bulk tissue measurement)				

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	✓	✓	✓	✓	⊘	✓	✓

Example Dose Extrapolation - illustrative only

POC Study (Surrogate Capsid)

PHP.B + Therapeutic Payload → Disease Mice / Non-Disease Mice

Dose (vg/kg)	Effect on Disease Outcomes	
4.0 x 10^{12}	+/-	Slight improvement
2.0 x 10^{13}	++	Robust improvement: Minimum Effective Dose
1.0 x 10^{14}	+++	Robust improvement

✓ **MED: 2.0 x 10^{13} vg/kg, with PHP.B**
✓ **Comparable PHP.B delivery to disease and non-disease mice**

Capsid Comparison Reporter Study

PHP.B + Reporter; TfR1 CapX + Reporter → hTfR1 KI Mice

Dose (vg/kg)	CNS Transduction	
	PHP.B	TfR1 CapX
4.0 x 10^{12}	+	++
2.0 x 10^{13}	++	+++
1.0 x 10^{14}	+++	++++

✓ **TfR1 CapX is 5x more potent than PHP.B**
✓ **Comparable PHP.B delivery to disease mice, non-disease mice, and hTfR1 mice**

Min Effective Dose

2.0x10^{13} vg/kg, PHP.B

Example Potency: TfR1 CapX = 5x PHP.B

Capsid Correlation (Reporter Study)

2.0×10^{13} vg/kg, PHP.B $\times \frac{1 \text{ TfR1 CapX}}{5 \text{ PHP.B}} = 4.0 \times 10^{12}$ vg/kg, TfR1 CapX

Drug Candidate Toxicology Study

Drug Candidate (TfR1 CapX + Therapeutic Payload) → hTfR1 KI Mouse (1:1) ≈ Human Patient

Dose (vg/kg)	Toxicological Assessment	
4.0 x 10^{12}	✓	Well tolerated
2.0 x 10^{13}	✓	Well tolerated
1.0 x 10^{14}	✓	Well tolerated - NOAEL
5.0 x 10^{14}	⊠	Treatment-related toxicological findings

✓ **Drug candidate NOAEL: 1.0 x 10^{14} vg/kg**
✓ **CapX dosing: hTfR1 KI mice = humans**

Dose Escalation in Patients

✓ 4.0 x 10^{12} vg/kg Drug Candidate
✓ 2.0 x 10^{13} vg/kg Drug Candidate
✓ 1.0 x 10^{14} vg/kg Drug Candidate

This data is for illustrative purposes only! It is critical that all genomic titers be conducted using a unified titer assay in order to make these extrapolations. See poster #1320 for particular considerations.

Conclusions

TfR1 CapX achieves widespread CNS transduction following intravenous administration in human transferrin receptor knock-in (B-hTfR1) mice, a commercially available model that provides robust pharmacokinetic and safety data. INTERACT feedback confirmed the pharmacological irrelevance of NHPs, as TfR1 CapX does not efficiently bind NHP transferrin receptors. These findings, aligned with prior regulatory decisions, eliminate the need for large-scale NHP toxicology studies and significantly reduce costs and timelines.

TfR1 CapX represents a scalable and regulatory-aligned platform for neurometabolic gene therapies, allowing for efficient clinical translation without reliance on NHP models. With a clear FDA-supported preclinical pathway, robust CNS delivery, and established translational feasibility, TfR1 CapX is well-positioned to advance next-generation gene therapies for neurometabolic disorders.

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