Apertura GENE THERAPY

#1519

Defining pharmacologically relevant animal models for characterizing human transferrin receptor-binding AAVs

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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.

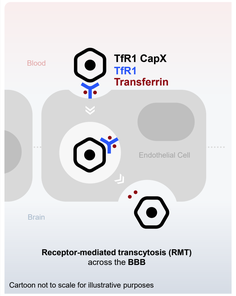
General references

- 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

Understanding the tissue distribution and safety outcomes associated with novel engineered adeno-associated viruses (AAVs) is essential to inform whether and how these AAVs can be applied in human gene therapy. In accordance with regulatory guidance for the development of cell and gene therapies, distribution and safety evaluations should make use of a pharmacologically relevant model system where the gene therapy product is expected to be biologically active. We have developed AAVs targeting human transferrin receptor (hTfR1), where in vitro experiments demonstrate these hTfR1-binding capsids are specific to human TfR1 and do not engage mouse or non-human primate (NHP) TfR1. Accordingly, animal models that do not express hTfR1 would not be expected to faithfully model the distribution and safety of an hTfR1-bnding capsid. However, empirical in vivo assessments are needed to demonstrate pharmacological irrelevance of such models and to advocate for the use of alternative hTfR1-expressing model systems in regulatory discussions and for therapeutic development.

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

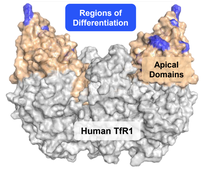
IV administration avoids diffusion scaling challenges seen with intraparenchymal and intrathecal injections. This facilitates direct translational relevance from preclinical models (e.g., hTFR1 mouse) to humans.

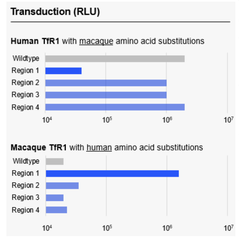
Validated Clinical Target

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





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.

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	✓	✓	✓	✓	⊘	✓	✓

TfR1-binding capsid distribution evaluation in NHPs

Goal Evaluate distribution of hTfR1-binding capsids in non-human primate (NHP) tissue after being administered individually or co-administered with comparator capsids

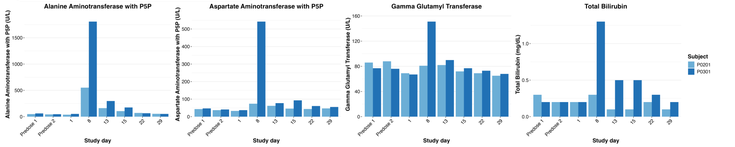
Study details

- **Subjects:** 2 female cynomolgus macaques
- **Age at dosing:** 28-29 months
- **Route of administration:** Intravenous
- **Capsids and dose levels:**
  - Single capsid (P0201): ~1E14 vg/kg BI-hTFR1
  - Multi-capsid (P0301): ~1E14 vg/kg BI-hTFR1, ~7.2E12 vg/kg AAV9, ~1.3E14 vg/kg Comparator 2 (data not shown)
- **Transgene:** CEG-hGBAv[X]-HA-WPRE
- **Readouts:** Distribution evaluation by *in situ* hybridization (ISH), hematology, clinical chemistry, histopathology

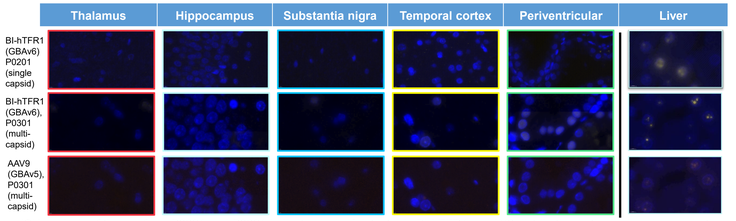
Distribution evaluation by ISH

Sample	Tissue	Region of Interest	AAV9 ISH		BI-hTFR1 ISH	
			GBAv5 Score	GBAv5 %Positive Cells	GBAv6 Score	GBAv6 %Positive Cells
Negative Control	NHP Cortex	Whole tissue	0	0%	0	0%
Single Capsid (P0201)	NHP Brain	Thalamus	N/A	N/A	0	0%
		Hippocampus	N/A	N/A	0	0%
		Substantia nigra	N/A	N/A	0	0%
		Temporal cortex	N/A	N/A	0	0%
		Periventricular regions	N/A	N/A	0	0%
	Liver	Whole tissue	N/A	N/A	1-2	76-99%
Multi Capsid (P0301)	NHP Brain	Thalamus	0	0%	0	0%
		Hippocampus	0	0%	0	0%
		Substantia nigra	0	0%	0	0%
		Temporal cortex	0	0%	0	0%
		Periventricular regions	0	0%	0	0%
	Liver	Whole tissue	1	51-75%	1-2	76-99%

Hematology and clinical chemistry



- Based on clinical chemistry, subject P0201 showed transient, mildly to moderately increased ALT activity on days 8 and 15 post-dosing. In comparison, subject P0301 showed mildly to markedly increased AST, ALT, GGT, and total bilirubin on days 8 and 15 of dosing. These stronger impacts on clinical chemistry parameters are consistent with the 3-fold higher total dose delivered to subject P0301.
- No test article-related effects on hematology parameters were observed for either subject.



- ISH analysis of tissues from P0201 and P0301 indicates lack of detectable BI-hTFR1 or AAV9 distribution in the brain, whereas both capsids are observed to distribute to the liver as anticipated. These results are consistent with our expectation that BI-hTFR1 would be unable to distribute to the brain after intravenous delivery due to lack of expression of the cognate human receptor.
- In a parallel study, we dosed NHPs with a capsid variant library composed of BI-hTFR1 and a second-generation hTFR1-binding capsid, TfR1 CapX, alongside a variety of comparator capsids such as AAV9 (data not shown). We used sequencing to quantify the enrichment of capsid variants in CNS and peripheral tissues. Consistent with our ISH analyses, we found engineered hTFR1-binding capsids do not show enhanced CNS distribution relative to AAV9 in NHPs due to their lack of human TfR1 expression.

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
4.0×10^{12}	+/- Slight improvement
2.0×10^{13}	++ Robust improvement: Minimum Effective Dose
1.0×10^{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 — hTfR1 KI Mice — TfR1 CapX + Reporter

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

2.0x10^{13} vg/kg, PHP.B $\frac{1\ \text{TfR1 CapX}}{5\ \text{PHP.B}}$ = **4.0x10^{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	
4.0×10^{12}	✓	Well tolerated
2.0×10^{13}	✓	Well tolerated
1.0×10^{14}	✓	Well tolerated - NOAEL
5.0×10^{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 to make these extrapolations.

Preclinical development with TfR1 CapX in humanized TfR1 knock-in mice

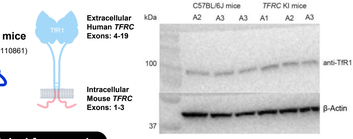
Mouse strain

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.

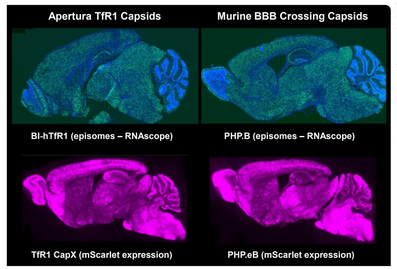
Physiologically Comparable: TfR1 expression in B-hTFR1 mice mirrors endogenous levels in the background strain.

Regulatory alignment: The FDA supports using the TfR1 CapX capsid and the humanized TfR1 knock-in mouse as the exclusive model for distribution and safety evaluation across multiple Apertura-supported programs.

B-hTFR1 mice
(Biocytogen #110861)



Capsid bridging



Dose ranging: 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.

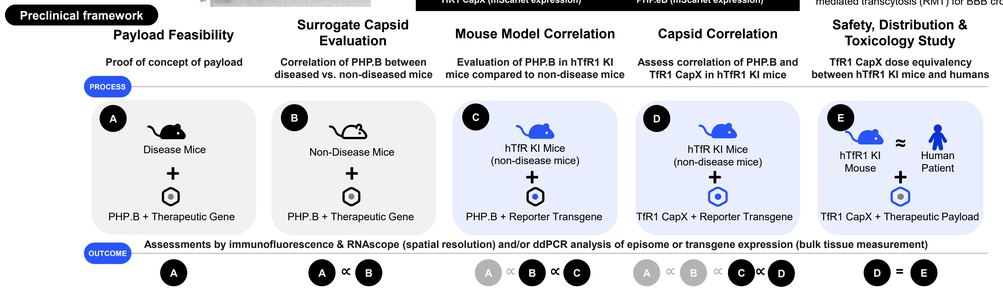
Advantages of Surrogate Capsids

- 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 crossing.

Preclinical framework



Conclusions

- Dosing of 1E14 vg/kg BI-hTFR1 in NHPs was well-tolerated, leading to transiently elevated liver transaminase levels that resolved over the course of the study.
- As anticipated, we did not observe enhanced CNS distribution of BI-hTFR1 compared to AAV9 through ISH. We observed consistent results in our capsid variant library profiling (data not shown), where neither BI-hTFR1 nor TfR1 CapX were enriched in CNS tissues relative to AAV9.
- As NHPs lack hTfR1 expression, they provide a limited view on distribution and safety of our hTfR1-binding capsids and motivate characterization in models expressing hTfR1. Empirical characterization of hTfR1-binding capsid distribution in NHPs was critical for regulatory alignment on the use of pharmacologically relevant models expressing human receptors, such as humanized TfR1 knock-in mice, for the development of TfR1 CapX gene therapies.
- **The FDA has endorsed the use of hTfR1 knock-in mice as the exclusive species for safety and distribution evaluations across multiple programs, with no requirement for additional NHP studies. These NHP distribution data can be generalized and leveraged for the development of other TfR1 CapX-based therapies without the need for additional NHP studies.**

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