Apertura
GENE THERAPY

FDA-Aligned Strategy for AAV-Based TSC1 Gene Therapy: Advancing Preclinical Development Without Non-Human Primates

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ASGCT 28th Annual Meeting – May 2025

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1. Apertura Gene Therapy
2. Tuberous sclerosis complex subunit 1 (TSC1)
3. TSC1 Regulatory Strategy
4. Closing

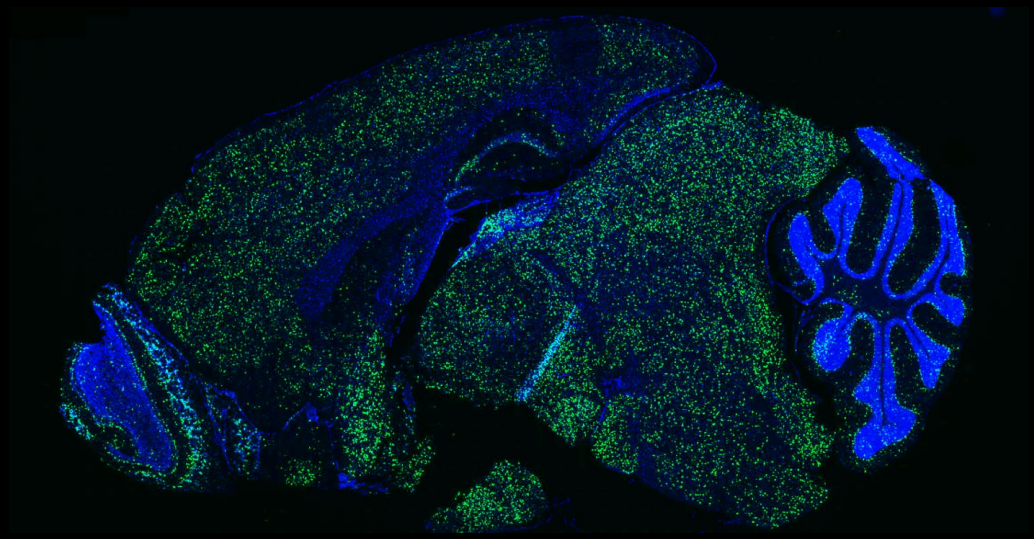
Widening the aperture of gene therapy through receptor-targeted engineering.

Novel, first-in-class family of BBB crossing capsids

- BBB-crossing targeting human-specific receptor
- FDA support for preclinical path

Modular NAb evasion capsid engineering platform

- Up to 50% reduction in NAb binding relative to natural serotypes
- Capsid tropism maintained after evasion modification



Broad CNS biodistribution via IV administration shows successful cell transduction of **payload** using our hTfR1 capsids.

Payload	CEG-h*GBA*-HA-WPRE
Mouse model	hTfR1 knock-in (Biocytogen)
Age of mice	14 weeks
Route of administration	Intravenous (tail vein)

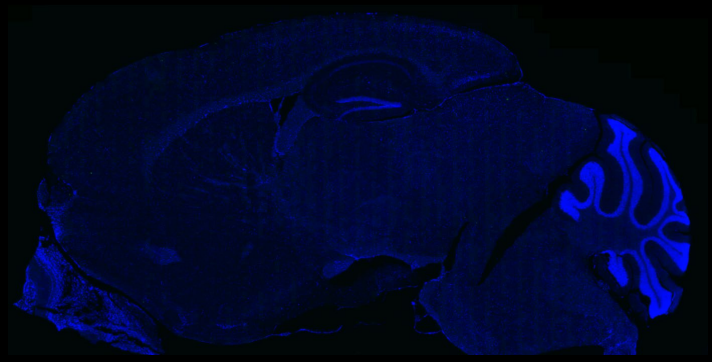
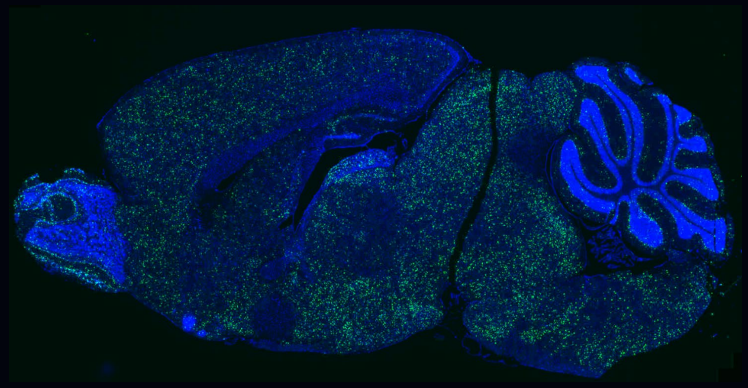
TfR1 capsids offer best-in-class BBB-crossing capabilities.

Payload	CEG-h*GBA*-HA-WPRE
Mouse model	hTfR1 knock-in (Biocytogen)
Age of mice	14 weeks
ROA	Intravenous (tail vein)

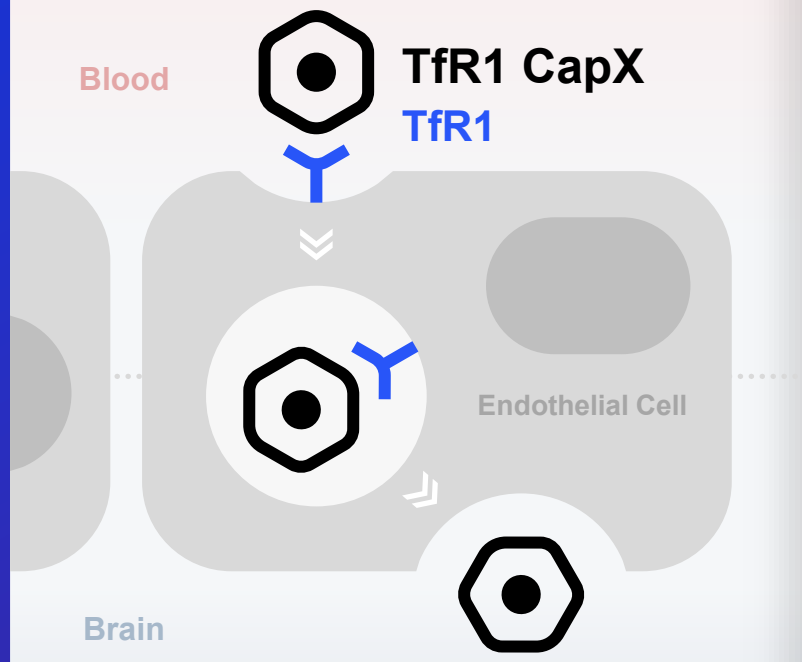
Science JOURNALS | AAAS

Qin Huang *et al*. An AAV capsid reprogrammed to bind human transferrin receptor mediates brain-wide gene delivery. *Science* **384**,1220-1227(2024)

hGBA - successful cell transduction
DAPI - nucleus

	AAV9 Capsid	hTfR1 Capsids
		
CNS penetrance	Limited BBB crossing	High BBB crossing
Deep brain structure access	Limited, requires direct injection	High-value, difficult to reach brain regions
Dosing	High doses required	Opportunity for lower dosing
Liver biodistribution	High transduction	Capable of very low transduction
Optimal CNS delivery	Direct injection to brain or CSF delivery	IV delivery
Manufacturability	Commercially manufacturable	Comparable to AAV9

Human transferrin receptor 1 (TfR1) is well-validated as a valuable brain target on the blood brain barrier (BBB).



Receptor-mediated transcytosis across the **blood-brain barrier**

Well-Understood BBB Receptor

TfR1 is a well-studied receptor **highly expressed on the BBB**. The receptor cycles rapidly with a highly conserved sequence that is expressed throughout life.

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., B-hTFR1 mouse) to humans.

Efficent Brain-Wide Delivery

Because as 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-administered viral delivery an ideal route of administration.

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

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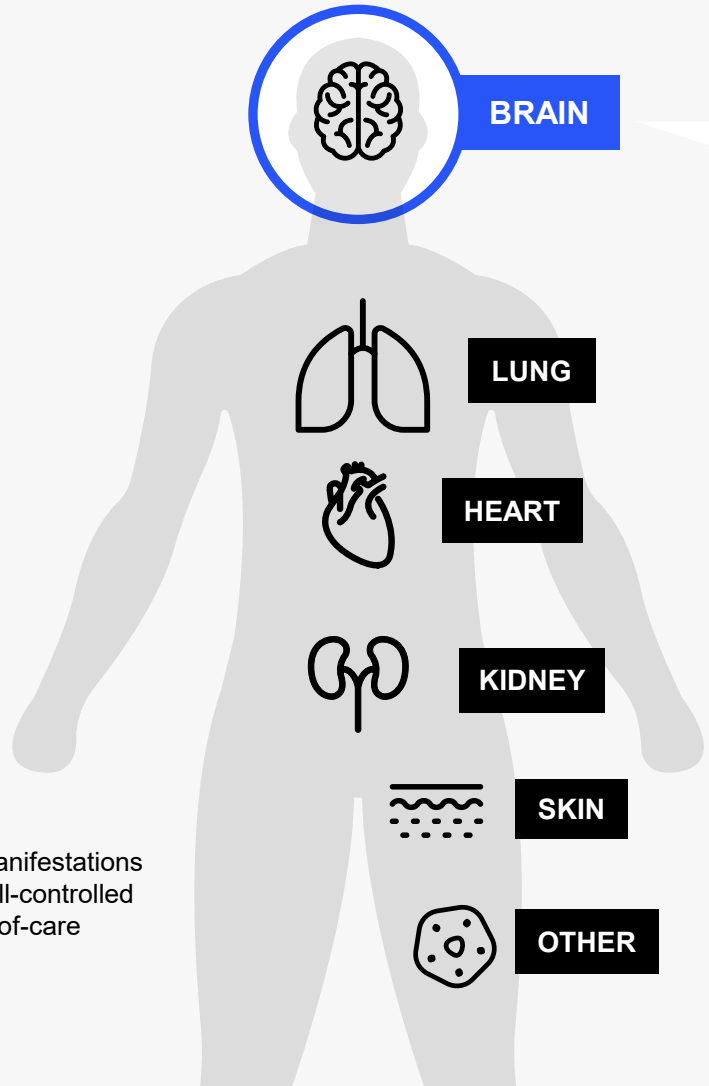
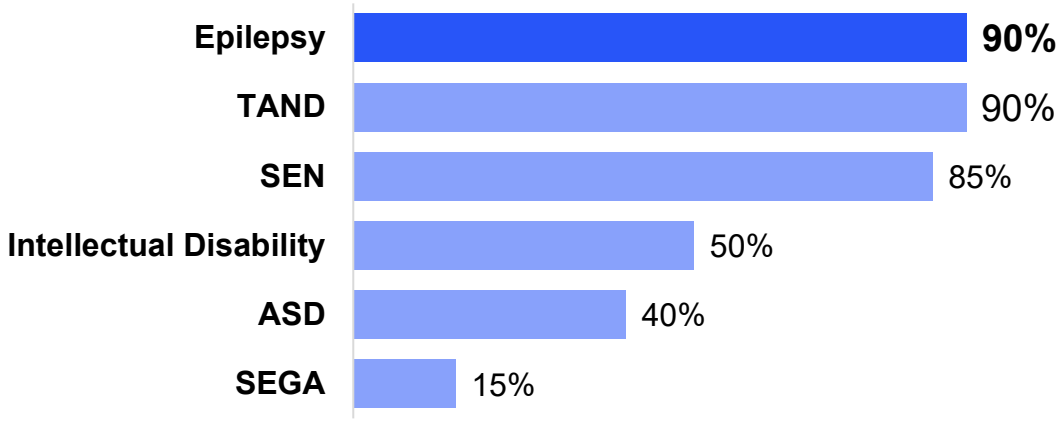
TSC is a systemic disorder with epilepsy and neuropsychiatric conditions presenting in 90% of patients.



Non-CNS manifestations generally well-controlled by standard-of-care



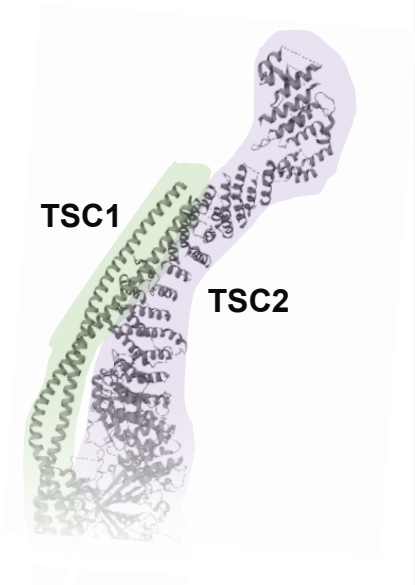
TAND = TSC-Associated Neuropsychiatric Disorders
SEN = Subependymal Nodules
ASD = Autism Spectrum Disorder
SEGA = Subependymal Giant Cell Astrocytoma

Mowrey, K. et al (2020); Curatolo, P. et al (2012); Chu-Shore, C. J. et al (2010); Moavero, R. et al (2010)

TSC

Pathway offers opportunity to intervene upstream of CNS disease manifestations.

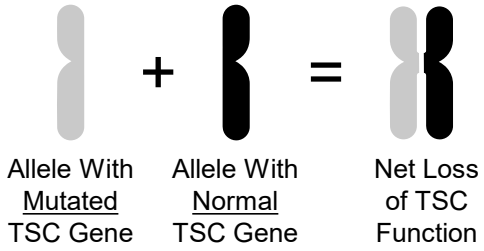
Structure



TSC1/2 Complex

Genetics

- **Most cases** (~70%) are **spontaneous**, with only a minority (~30%) inherited
- **Haploinsufficiency, loss-of-function mutation**: 50% level of TSC function is insufficient
- **Autosomal dominant**: when inherited, single copy of the mutated TSC gene from one parent is enough to cause the disorder:



Mode of Action

TSC plays a **negative regulatory role** in the mTOR pathway:

Normal Pathway

Loss of TSC function causes mTOR and downstream pathways to be **hyperactive**:

Dysfunctional Pathway

TSC Alliance, The Voice of the Patient: A Report from the Tuberous Sclerosis Alliance's Externally-Led Patient-Focused Drug Development Meeting (2017); Zeng, L. H. et al (2008)

TSC

Gene therapy addresses the root cause of TSC.

Why AAV Gene Therapy?

Genetic etiology

- Monogenic disorder with too many mutations for sequence-specific modalities like ASOs and gene editors

Clinically relevant

- Established phenotypic benefit in literature
- Non-focal nature of seizures requires broad CNS biodistribution
- Potential benefit for both CNS and the periphery

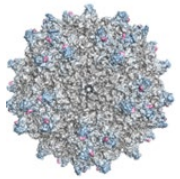
Stable & sustained expression

- Targets non-dividing, post-mitotic CNS cells: potentially a single-treatment therapy
- No observable risk of gene overexpression

Feasible delivery

- TSC1 cDNA fits into AAV

AGTX-1111: TSC1 Gene Therapy



TfR1 CapX

TSC1 Transgene Cassette

Broad CNS distribution

- Extensive neuron and astrocyte transduction in brain

Potential safety & efficacy upsides

- Liver detargeting
- Potential to impact other debilitating symptoms (TAND)
- Potential for lower dosing
- Potential for lower manufacturing costs

Minimally invasive

- IV administration is attractive for patients and caregivers
- Supportive of expanding patient access to gene therapies

Bartel, M. A. et al (2012); Hammond, S. L. et al (2017); Fuentes, C. M. et al (2018)

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

Indication Classification

Epilepsy associated with TSC1 mutations can be classified as a neurodegenerative disorder and the “Human Gene Therapy for Neurodegenerative Diseases” FDA Guidance (2022) can guide development of TSC gene therapies.

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 of AGTX-1111.

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 TSC1 mouse disease model and the B-hTFR1 KI mouse model, compared to the distribution of the TfR1 CapX capsid in the B-hTFR1 KI mouse model.

Potency Assay

A cell-based ELISA assay using a TSC1 knockout cell line to measure total and phosphorylated p70 S6 kinase (P70S6K), a downstream target of mTOR, can serve as a potency assay for AGTX-1111.

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Potency Assay

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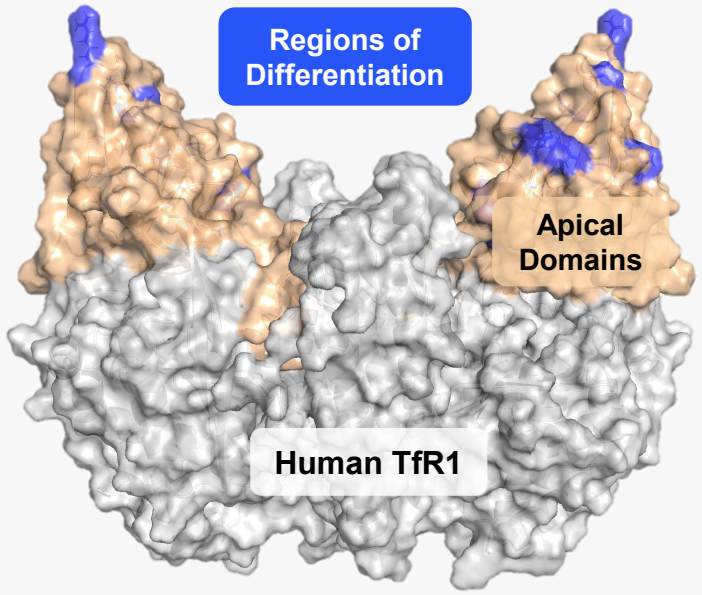
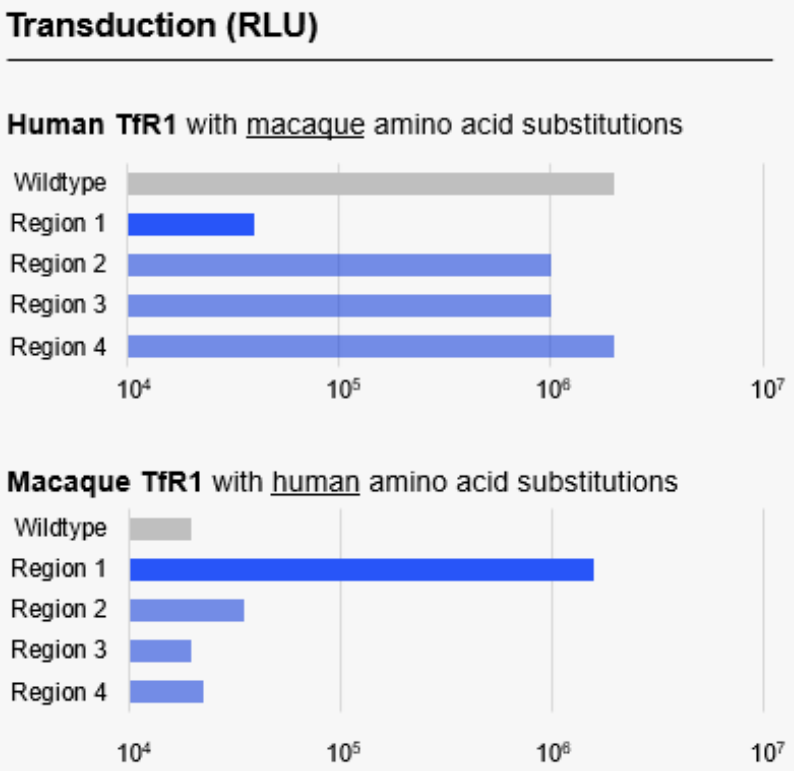
Apertura has clear guidance from FDA on TfR1 CapX preclinical development.

- **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.
- **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.

PR Newswire. *Apertura Gene Therapy Supports the Broad Institute in Development of Gene Therapy for Prion Disease Using Engineered AAV Capsid Targeting TfR1 for CNS Delivery*. Feb 2025

Successful targeting of human TfR1 revealed key amino acid differences in the apical domain compared to NHP TfR1.





NHP studies conducted at Apertura have confirmed that TfR1 CapX and other hTFR1-binding capsids are not enriched in the CNS of NHPs.

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

Guidance for Industry, Preclinical Assessment of Investigational Cellular and Gene Therapy Products. FDA (November 2013)

B-hTFR1 mice are an ideal tool for *in vivo* evaluation of hTfR1 capsids.

B-hTFR1 Mouse: A Pharmacologically Relevant Model

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

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

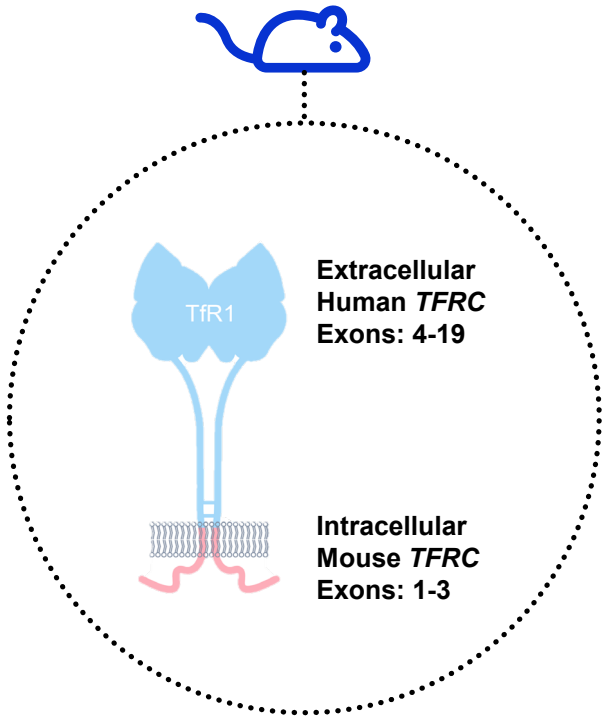
CNS Targeting Validation: Demonstrates enhanced TfR1 capsid distribution to the CNS due to human TfR1 expression.

Regulatory & Industry Precedent:

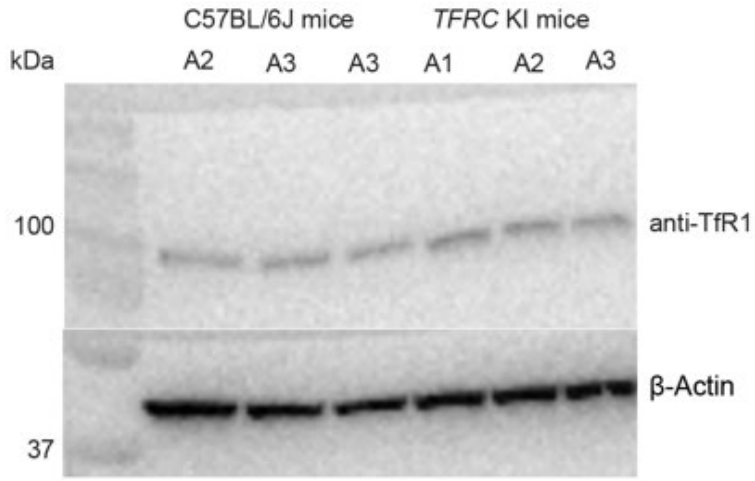
- **FDA-Endorsed Approach**
- **Industry Validation**: Similar successful strategies used with hTfR1 transgenic mice

TFRC KI mice

B-hTFR1 mice (Biocytogen #110861)



Similar expression of TFRC between WT and KI



Qin Huang et al. An AAV capsid reprogrammed to bind human transferrin receptor mediates brain-wide gene delivery. *Science* **384**, 1220-1227 (2024).

Proven Use of TfR1 Binding and Humanized TfR1 Mice in Toxicology & Distribution Studies

Company	Drug/Lead Name	Indication	NTC# (Clinical Trial) or Approval Year	Usage of TfR1 mouse strain
JCR	Pabinafusp alfa (JR-141)	Mucopolysaccharidosis II (Hunter Syndrome)	Approved in Japan in 2021; Phase I/II: NCT03128593; Phase II/III: NCT03568175	Evaluated brain uptake, distribution, and pharmacodynamics of JR-141 via TfR1-mediated transcytosis across the blood-brain barrier.
Denali	ETV:SGSH (DNL126). Fusion protein of the SGSH enzyme and TFR1 Fc fragment	Mucopolysaccharidosis IIIA (MPS IIIA)	Phase I/II: NCT06181136	Evaluated brain uptake, distribution, and efficacy of ETV:SGSH via TfR1-mediated transcytosis across the BBB.
Regeneron	TfR1-targeted AAVs (AAV9 with TfR1-targeted Fab)	Neurological indications	Clinical trials not yet initiated	Humanized TFR1 accurately translated CNS distribution in NHPs (ASGCT 2024)
Roche	Trontinemab (RG6102), a bispecific antibody targeting Aβ and TfR1	Alzheimer's disease (AD)	Phase Ib/IIa: NCT04639050	Evaluated brain uptake and efficacy of a trontinemab surrogate that binds to mouse TfR1.
Arrowhead	RNAi therapeutics conjugated to ligands that bind to TfR1	Neurological indications	Clinical trials not yet initiated	Reported favorable safety profile in rodents.

- PMDA Report on the Deliberation Results. Izcargo for I.V. Infusion 10mg. URL: https://www.pmda.go.jp/files/000242410.pdf.
- Arguello, A. ETV:SGSH, a brain-penetrant enzyme transport vehicle for SGSH, corrects heparan sulfate accumulation, lysosomal lipid storage and inflammation in MPS IIIA mouse brain. URL: https://www.denalitherapeutics.com/sites/default/files/2023-02/WORLDS2023_Transport_Vehicle_MPSIIIA.pdf.
- Nambiar, K. Antibody-Based AAV Retargeting to Transferrin Receptor Mediates Efficient Blood Brain Barrier Crossing and In Vivo Gene Delivery to the CNS in Mice and Non-Human Primates. Abstract #118, ASGCT Conference 2024.
- Kulic, L. et al. Translation of RG6102, an amyloid-targeting therapy with superior brain penetration properties, to the clinic. URL: https://medically.roche.com/content/dam/pdmahub/restricted/neurology/adpd-2021/ADPD-2021-presentation-kulic-translation-of-RG6102-an-amyloid-targeting-therapy.pdf
- Arrowhead Pharmaceuticals Corporate Overview. URL: https://ir.arrowheadpharma.com/static-files/25007351-232f-400a-bc0e-0fd4e2906fe8

Disease × TfR1 KI Mouse Model Enables Direct TfR1 CapX Assessment in POC Studies

Evaluation Method	Expresses hTfR1	Biological Rationale	Efficacy	Dose Response	Distribution	Safety	Immunogenicity
Disease x hTfR1 KI mice	✓	✓	✓	✓	✓	✓	✓

- Crossing the disease model with hTFR1 KI mice enables direct evaluation of TfR1 CapX-delivered therapies in a relevant biological context.
- Allows for dose-response assessment by testing multiple doses and their impact on disease outcomes.

Challenges & Considerations:

Time Investment: ~9–12 months to establish crossed mice.

Cost: Breeding and model characterization require significant resources.

Model-Specific Limitations: A unique cross is needed for each disease model.

FDA-endorsed surrogate capsid approach for efficient preclinical development

Regulatory Alignment:

- FDA endorsed the surrogate capsid approach, consistent with its published gene therapy guidance.

Advantages of Surrogate Capsids:

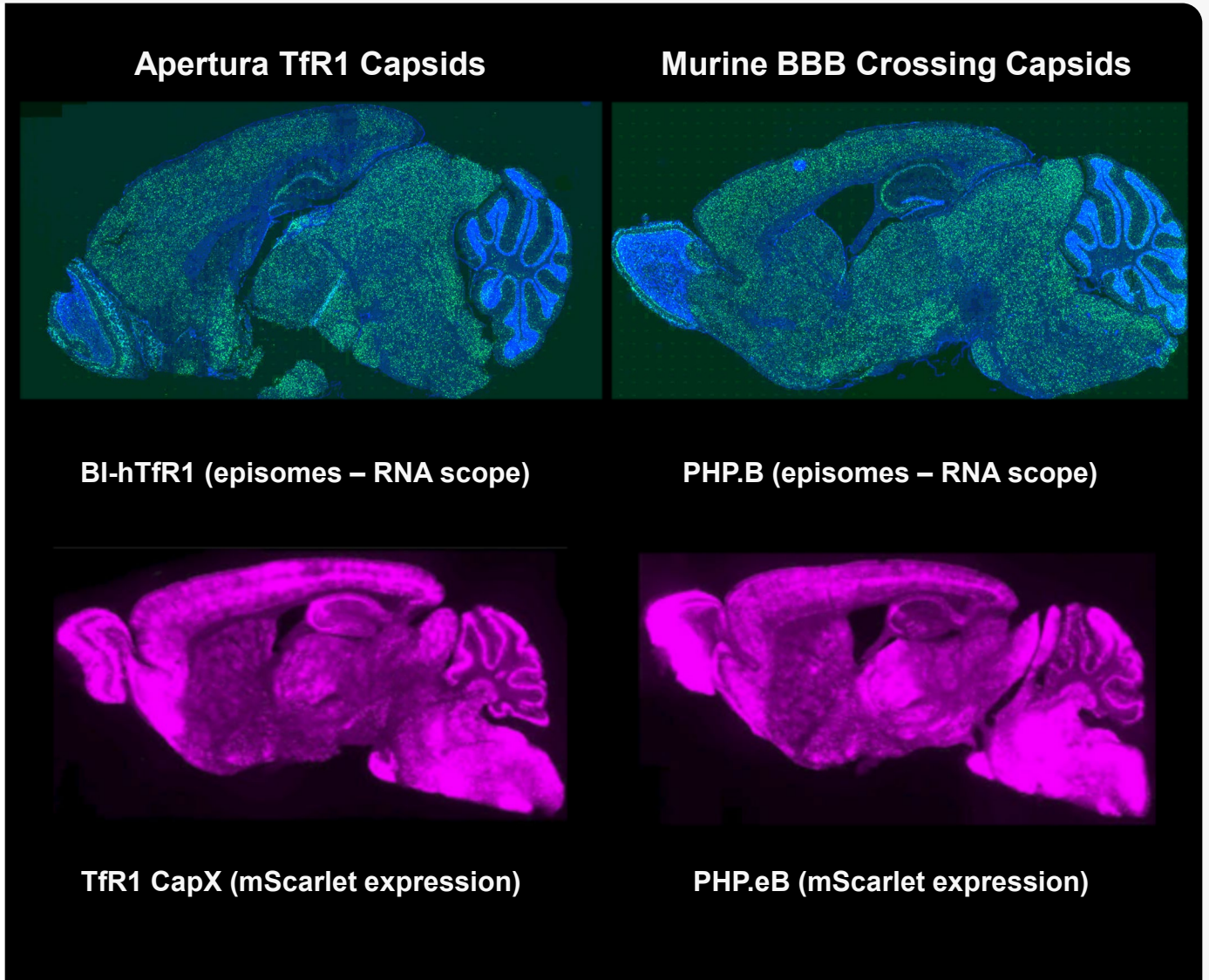
- Eliminates additional breeding.
- Leverages existing data for future indications.
- Broad applicability across multiple gene therapy targets

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.

Validated Across Models:

- Apertura: Used PHP.B and BI-28 in TSC1 preclinical studies (GFP & TSC1 payloads).
- Broad Institute: Used PHP.eB in PRNP studies (PRNP silencing payload).
- Confirmed distribution in TfR1 KI Model.



Capsid correlations between surrogate BBB crossing capsid and Apertura's TfR1 capsid would support preclinical path for efficacy and human dosing.

Goal

Draw correlations in gene delivery for Apertura hTfR1 candidate capsid (TfR1 CapX) and surrogate capsid (PHP.B) across studies.

PHP.B — Surrogate capsid, BBB crossing via receptor mediated transcytosis through LY6A

TfR1 CapX — Apertura clinical capsid, BBB crossing via receptor mediated transcytosis through human TfR1

	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 human patients
PROCESS	A TSC1 Disease Mice + PHP.B + TSC1	B Non-Disease Mice + PHP.B + TSC1	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)

Establishing capsid correlations to guide dose selection in patients – an example

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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**

+

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**

Min Effective Dose

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

Potency: TfR1 CapX = 5x PHP.B

Capsid Correlation (Reporter Study)

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

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

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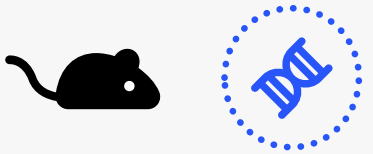
Strengthened regulatory pathway: FDA INTERACT feedback supports TfR1 CapX IND plan.



- **FDA Endorsement: Human TfR1 KI model** approved to assess **biodistribution & toxicology** to support initial **human dose selection**.
- **NHP Irrelevance Confirmed:** Preclinical data **validates the non-necessity of NHP studies** for TfR1 CapX.
- **Established Regulatory Alignment:** The **TfR1 KI mouse model** aligns with **previously approved compounds** where **rodents alone** were sufficient for regulatory approval.
- **Cross-Species Translation:** Industry data confirm **dose equivalency from small species to humans** in IV dosed TfR1-targeting therapies, supporting clinical translation.
- **Surrogate Capsid Validation:** FDA supports **PHP.B/eB as surrogates for efficacy assessment** due to similar MOA and biodistribution profiles with 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	✓	✓	✓	✓	⊘	✓	✓

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- **Apertura Gene Therapy Team**
  - **Jorge Santiago-Ortiz, PhD**
  - Christopher Davis, PhD
  - Sisi Chen, PhD
  - Ellen Horste, PhD
  - Tina Hu-Seliger, PhD
  - Hikari Sorensen, BS
  - Devanshi Shanghavi, PSM
  - Von Wiltman, BS
  - Sampada Kalan, PhD
  - Ada Zhang, MS
  - Jade Ganter, MS
  - Anthony Stabile, BS
  - Jessica Luo, BS
  - Max Seaman, MS
  - Megan Powell, BS
  - Blake Albright, PhD
  - Max Ezekiel, MS
  - Paul Puglisi, MS
  - Diego Garzón, PhD, MBA
  - Andrew Steinsapir, MBA
- **Ben Deverman, PhD**
- **TSC Alliance Preclinical Consortium**
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- **Melanie Hartsough, PhD**

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