Type B, Pre-IND Meeting Briefing Package

Meeting Format: Video Conference

PRNP-CHARM-001 Gene Therapy for Treatment of Prion Disease

Product Name: PRNP-CHARM-001

Sponsor: Broad Institute

PTS #: STN PS009720

Table of Contents

Table of Contents **2**
Executive Summary **4**
List of specific questions we pose to the agency 6
List of Figures and Tables **8**
1. Application Number **9**
2. Product Name **9**
3. Product Description **10**
4. Proposed Indications **11**
5. Purpose and Objectives of the Meeting **11**
6. Proposed Agenda **11**
7. Sponsor Attendees **11**
8. History of regulatory engagement **12**
8.1 History of Development Program and Relevant Communication with FDA (Consolidated) 12
8.2 Relevant Prior Agency Communications regarding PRNP-CHARM-001 13
9. Therapeutic hypothesis, CHARM mechanism, and delivery strategy **14**
9.1 Background on prion disease and the therapeutic strategy of PrP lowering 14
9.2 Background on targeted epigenetic silencing using CHARM 20
9.3 Background on TfR1 CapX 23
10. Non-clinical product development **31**
10.1 In vivo potency and safety studies 34
10.2 Off-target analysis of PRNP-CHARM-001 42
11. Chemistry, manufacturing and controls **45**
11.1 Manufacturing plan and milestones for PRNP-CHARM-001 45
11.2 Overview of Selected Manufacturer (Provided by Viralgen) 46
11.3 Critical Starting Materials 47
11.4 General description of Viralgen drug substance (DS) manufacturing process and process controls 47
11.5 Draft drug substance (DS) testing plan 49
11.6 General Description of Viralgen Drug Product (DP) Manufacturing Process and Process Controls 50
11.7 Draft Viralgen Drug Product (DP) Testing Plan 52
11.8 Stability Plan 53
11.9 Potency Assay 53
11.10 Device compatibility 55
12. Clinical **56**
12.1 Symptomatic and pre-symptomatic patients present opposing clinical opportunities and challenges 56
12.2 Clinical plan for PRNP-CHARM-001 58
12.3 Study design and rationale 61
12.4 Clinical study draft protocols 62

13. Current Status of Product Development.....75
13.1 Manufacturing milestones..... 75
13.2 Non-clinical studies..... 76
14. Questions for the Agency and Sponsor Positions.....77
14.1 Non-clinical..... 77
14.2 CMC..... 81
14.3 Clinical.....85
Conclusion.....87
15. References..... 88
16. List of Abbreviations.....96
17. Appendices..... 100
Appendix I: Mouse Models Used in PRNP-CHARM-001 Preclinical Studies.....100
Appendix II: CDER Critical Path Innovation Meeting Minutes..... 100
Appendix III: Apertura Reference Memo Supporting PRNP-CHARM-001..... 100
Appendix IV: Apertura Gene Therapy AS-1801 NHP Study Report.....100
Appendix V: Study Report: V1SION..... 100
Appendix VI: Study Report: CABS..... 100
Appendix VII: Study Report: H3X.....100
Appendix VIII: Interim Study Report: BRAV3.....100
Appendix IX: Executed Study Protocol: PRNP-CHARM-001 Safety and Biodistribution Study (Non-GLP toxicology study).....100
Appendix X: Interim Study Report: PRNP-CHARM-001 Safety and Biodistribution Study (Non-GLP toxicology study).....100
Appendix XI: Draft Study Protocol: GLP PRNP-CHARM-001 Safety and Biodistribution Study.. 100
Appendix XII: Study Report: PRNP-CHARM-001 In Vitro Off-target Analysis.....100
Appendix XIII: Draft Study Protocol: PRNP-CHARM-001 Delivery-Device Compatibility and In-Use Stability Study..... 100
Appendix XIV: PRNP-CHARM-001 In Vitro Off-target Analysis RNA-seq Sample Information... 100

Executive Summary

This Type B Pre-IND meeting briefing package describes the development program for PRNP-CHARM-001, an investigator-initiated, one-time intravenous AAV-delivered epigenetic gene therapy for the treatment and prevention of prion disease.

Background on prion disease and the therapeutic hypothesis of PrP lowering

Prion disease is an untreatable rapidly progressive neurodegenerative disease that is typically fatal within 5 months of first symptom. Its pathogenesis is well understood: the prion protein (PrP), encoded by the prion protein gene (*PRNP*), misfolds into a protein-only pathogen which spreads across the brain, killing neurons. PrP is strictly required for disease: homozygous knockout animals are fully resistant to prion infection, and a variety of PrP-lowering drugs including antisense oligonucleotides (ASOs) and short interfering RNAs (siRNAs) both delay onset and slow progression of prion disease in animal models. Both animal and human genetic data indicate that reduced PrP expression is well tolerated. While these data provide foundational proof of concept for PrP lowering, we are motivated to develop a one-time PrP-lowering gene therapy that may prove more potent, better tolerated and deliverable to the whole brain.

Proposed therapeutic

PRNP-CHARM-001 is a therapeutic consisting of an engineered blood-brain barrier crossing capsid, TfR1 CapX, that encapsidates a *PRNP* promoter-targeted epigenetic silencer and transcriptional repressor, CHARM payload.

Our proposed therapeutic seeks to repress and irreversibly methylate DNA at the *PRNP* promoter. The investigational drug is composed of a compact zinc finger (ZF) targeting the human *PRNP* promoter, fused to an effector domain that recruits the endogenous methyltransferase DNMT3A1 already present in the cell, as well as a KRAB transcriptional repression domain. This therapy, known as PRNP-CHARM (where CHARM stands for Coupled Histone tail for Autoinhibition Release of Methyltransferase), is of a size deliverable to the CNS in a single AAV capsid. This product contains no nuclease domain; its mechanism relies on methylation-mediated silencing and transcriptional repression of the *PRNP* locus, not on DNA cleavage or editing.

Prion disease is a whole brain, neuronal disease. The capsid we will use for delivery is TfR1 CapX, an engineered AAV9-derived capsid that crosses the blood-brain barrier (BBB) via receptor-mediated transcytosis through the human transferrin receptor (TfR1), enabling broad CNS delivery to neurons following intravenous administration. TfR1 CapX achieves transduction of >80% of cortical neurons in humanized *TFRC* knock-in (*hTFRC* KI) mice at doses of ~2e12 vg/kg, approximately 50-fold below clinical precedents for AAV9. Two sponsors, Finding Hope for FRSS1L and AviadoBio, have publicly stated their intention to take TfR1 CapX encapsidated gene therapies into first-in-human dosing for CNS diseases by the end of 2026. We will monitor clinical use of this capsid, public statements and data as

they become available and assess for relevance to our program. For further information on TfR1 CapX, see Section 9.3.

Key non-clinical studies for PRNP-CHARM-001

The PRNP-CHARM-001 preclinical program encompasses transgene candidate selection, lead construct confirmation with the clinical capsid, capsid and manufacturer bridging studies, and safety/biodistribution evaluation. Because TfR1 CapX does not achieve pharmacologically relevant CNS delivery in species lacking the human Y247 TfR1 residue, all *in vivo* studies are conducted in humanized mouse models (see Appendix I: Mouse Models). We have completed *in vivo* drug candidate selection and confirmation studies, capsid and manufacturer bridging studies, and a non-GLP pilot toxicology study. A definitive GLP toxicology study in *hTFRC* KI mice is planned and we seek the Agency's feedback on our study design in this meeting.

Chemistry, manufacturing and controls strategy for PRNP-CHARM-001

Viralgen Vector Core S.L.U (San Sebastián, Spain) has been selected as the clinical manufacturer for PRNP-CHARM-001. Viralgen has manufactured material for more than 40 approved clinical trials and operates GMP-compliant facilities under Spanish Medicines Agency (AEMPS) authorization. Research lots of PRNP-CHARM-001 have been produced and used in critical non-clinical studies. The 50L toxicology batch of PRNP-CHARM-001 was vialed on May 21, 2026 and is currently undergoing release testing and stability evaluation. A 500L-scale clinical batch is planned to support the Phase 1/2 clinical study. We outline our manufacturing plan, drug substance and drug product release testing plans, stability plan, delivery-device compatibility study, as well as our proposed licensure enabling potency assay for Agency alignment in Questions 4–6 (Section 14).

Envisioned clinical trial design for PRNP-CHARM-001

Given its high potential to be protective against prion disease, we are working to swiftly advance PRNP-CHARM-001 to testing in humans in a series of investigator-initiated trials.

The key endpoints in an initial clinical study of PRNP-CHARM-001 will be 1) safety, and 2) target engagement: measuring whether the drug lowered PrP levels in the brain. It has been shown in rats that brain PrP levels can be tracked pre- and post-treatment using cerebrospinal fluid (CSF) PrP levels as a proxy.

Two populations stand to benefit from a PrP-lowering therapy: 1) symptomatic prion disease patients, and 2) carriers of high penetrance *PRNP* variants that reliably cause fatal prion disease. Symptomatic patients provide a very clear risk-benefit proposition, as they will survive only weeks to months without treatment, and would be unlikely to have any opportunity to receive a future AAV-based therapy. Pre-symptomatic patients offer an opportunity to preserve full quality of life before any symptoms strike, and also present an opportunity for safety and target engagement readouts unconfounded by disease progression.

Regulatory and Clinical Development Plan of PRNP-CHARM-001

Given the rare nature of prion disease, we hope to be forward looking in the development of PRNP-CHARM-001 and contemplate leveraging efficacy data from the proposed Phase 1/2 clinical study to support a future license application(s). We have developed a forward compatible plan for CMC and for staged qualification and validation of key product- and program-specific assays. We anticipate applying for a number of designations that would apply to prion disease as a rare, severe and life-threatening disease with no current standard of care and to PRNP-CHARM-001 as a novel gene therapy, and look forward to robust Agency engagement as the program matures.

We are requesting this meeting to align with the agency and request specific feedback necessary to ensure that we are efficiently and effectively developing this novel product. While all questions below are germane to the development of PRNP-CHARM-001 over the next six months, in addition to any topics where the Agency wishes to redirect our thinking **we wish to prioritize Question 1 (off targets; non-clinical) and Question 3 (GLP study design; non-clinical) for discussion** as they bear on the design of key upcoming studies.

List of specific questions we pose to the agency

Question 1: Off targets (Non-clinical). Does the Agency agree with the Sponsor's position that the proposed in silico, in vitro, and in vivo study designs to assess PRNP-CHARM-001 off-target activity are sufficient to proceed with an IND submission and subsequent clinical administration of the PRNP-CHARM-001 drug product?

Question 2: Dose determination (Non-clinical). Does the Agency concur with the Sponsor's proposed plan for translating dosing based on IV administration of the PRNP-CHARM-001 product in *hTFRC* KI mice, where safety and toxicology assessments, together with vector biodistribution data, will be used to establish dose range selection for the clinical program?

Question 3: GLP study design (Non-clinical). Does the Agency agree with the Sponsor's position that the proposed design of the GLP PRNP-CHARM-001 safety and biodistribution study is sufficient to generate the necessary data to proceed with an IND submission and initial clinical administration of the PRNP-CHARM-001 product?

Question 4: Manufacturing plan (CMC). Does the Agency agree that the proposed drug product manufacturing plan, stability testing plan, and draft release testing plan are appropriate for the PRNP-CHARM-001 product to be used in the program's initial Phase I/II clinical trial?

Question 5: Device compatibility and in-use stability (CMC). Does the Agency agree that the proposed delivery-device compatibility and in-use stability study is appropriate for initial clinical administration of the PRNP-CHARM-001 product?

Question 6: Potency assay (CMC). We propose to use RT-qPCR-based quantification of *PRNP* RNA lowering in human HEK293T cells as a potency assay for release criteria. Does

the Agency agree that the proposed potency assay will be adequate to support licensure-enabling studies?

Question 7: Study populations (Clinical). Does the Agency agree with the Sponsor's position that symptomatic prion disease patients and presymptomatic individuals at risk of genetic prion disease are separate populations and can be addressed in separate first-in-human studies?

Question 8: Clinical trial design (Clinical). Does the Agency agree that the clinical plan overview for first-in-human studies of PRNP-CHARM-001 in symptomatic prion disease patients and individuals at risk for presymptomatic genetic prion disease is reasonable, including general trial structure, staging of trial arms, and endpoints?

Question 9: Regulatory and clinical development path (Clinical). Does the Agency concur with our proposed regulatory and clinical development engagement plan and staging of application for relevant designations for the PRNP-CHARM-001 gene therapy program?

List of Figures and Tables

List of Figures

Figure 1. Schematic representation of PRNP-CHARM-001
Figure 2. The pathway of prion disease
Figure 3. Published genetic and pharmacologic proofs-of-concept validating PrP lowering as a therapeutic hypothesis in prion disease
Figure 4. Treatment time point is a key determinant of efficacy of PrP-lowering therapy
Figure 5: CHARM: Coupled Histone tail for Autoinhibition Release of Methyltransferase
Figure 6: CHARM methylates the *Prnp* promoter, dose-responsively and durably lowers brain PrP, and extends survival of prion-infected mice
Figure 7: TfR1 CapX more efficiently transduces and crosses human brain endothelial cell monolayers in vitro by binding the apical domain of human TfR1
Figure 8: TfR1 CapX efficiently delivers genes to neurons in the CNS of *hTFRC* KI mice
Figure 9: TfR1 CapX exhibits enhanced tropism to the CNS of *hTFRC* KI mice
Figure 10: The enhanced binding and transduction phenotype of BI-hTFR1 is specific to human TfR1
Figure 11: Binding of the TfR1 CapX capsid to the apical domain of human TfR1 depends on the Y247 residue that is unique to humans.
Figure 12: Lowering of PrP in the brain by human PRNP ZF-CHARM
Figure 13: Comparison of CHARM-mediated *Prnp* silencing delivered with AAV-PHP.eB or TfR1 CapX.
Figure 14. Brain *Prnp* silencing as a function of viral genomes/diploid genomes.
Figure 15. Comparison of candidate constructs in a biologically relevant mouse model.
Figure 16. General Flow Diagram of the Viralgen Manufacturing Process
Figure 17. Process Flow Diagram for Formulation, Filling and Labelling
Figure 18. Pilot PRNP-CHARM-001 RT-qPCR Potency Assay
Figure 19. CSF PrP levels are stable in presymptomatic prion disease mutation carriers
Figure 20. Proposed dose escalation plan for the PRNP-CHARM-001 first-in-human study

List of Tables

Table 1: Evidence that PrP is central to prion disease pathophysiology. Reproduced from Vallabh 20204. 16
Table 2. Non-clinical in vivo potency and tolerability studies 30
Table 3. Summary of mouse lines used during the development of PRNP-CHARM-001 31
Table 4. Cohorts in the PRNP-CHARM-001 dose translation and titer bridging study 37
Table 5. Cohorts in the PRNP-CHARM-001 Safety and Biodistribution Study 38
Table 6. Planned cohorts in the GLP PRNP-CHARM-001 Safety and Biodistribution

Study 39
Table 7. Human cell lines chosen for PRNP-CHARM-001 off-target analysis. 41
Table 8. Planned cohorts in the PRNP-CHARM-001 Long-term Off-Target Accumulation Study 42
Table 9. Manufacturer information 44
Table 10. Draft drug substance testing plan 47
Table 11. Name, address, and responsibility for the drug product manufacture 48
Table 12. Draft drug product testing plan 50
Table 13. Stability testing plan for T-POLARIS-123 batch 51
Table 14: Utility of CSF PrP measurement in different prion disease patient populations. 55
Table 15. Profiles of symptomatic and presymptomatic prion disease patients. 57
Table 16: Key aspects of the proposed clinical trial design. 60
Table 17. Safety risks and response plans. 67
Table 18. Planned designation applications for the PRNP-CHARM-001 gene therapy program 72

1. Application Number

STN PS009720.

2. Product Name

PRNP-CHARM-001.

3. Product Description

PRNP-CHARM-001 is a recombinant adeno-associated virus (rAAV)-based gene therapy comprised of a single-stranded AAV2 genome encoding an epigenetic editor (dubbed CHARM) targeted to the *PRNP* promoter by a zinc finger (ZF) binding domain and an engineered AAV9-derived capsid TfR1 CapX, that has been designed to enter the human CNS via its ability to bind to human Transferrin receptor (hTfR1).

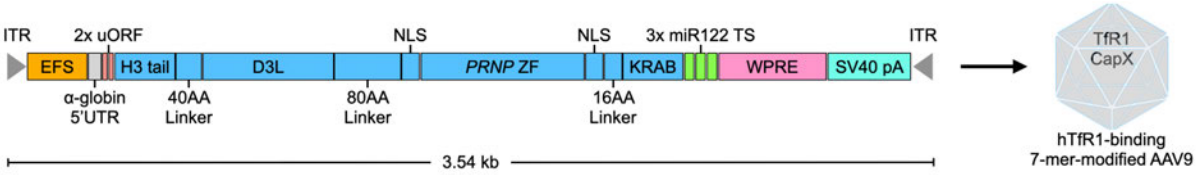


Figure 1. Schematic representation of PRNP-CHARM-001. Individual elements are described below.

The PRNP-CHARM-001 product consists of:
Payload: a single-stranded AAV2 genome containing AAV2 ITRs flanking the following payload (**5' to 3'**):

- Human Elongation Factor 1 alpha, short (EFS) promoter
- Two tandem upstream open reading frames (uORFs) embedded in a non-coding fragment of the alpha-globin 5'UTR sequence. Each uORF comprises a nine base pair ORF (Met-Gly-stop): (1) ATG-GGT-TGA or (2) ATG-GGA-TAA designed to reduce the overall levels of translation of the CHARM transgene protein
- A cDNA payload with the following features:
  - **CHARM effector domain**. CHARM stands for Coupled Histone tail for Autoinhibition Release of Methyltransferase[1]. This effector domain is a fusion protein comprised of a) a N-terminal unmethylated histone H3 tail (31AA), b) a 40 amino acid (AA) linker, c) a DNMT3L C-terminal domain (D3L), which recruits and stabilizes the endogenous DNMT3A and DNMT3A1 DNA methyltransferase enzymes, d) a 80AA linker
  - **Zinc finger (ZF) DNA-binding domain** flanked by two bipartite nuclear localization sequences**.** Targeting to the *PRNP* promoter is achieved using a zinc finger protein domain. *Note that the ZF domain is solely present for DNA targeting; it does not have an associated nuclease or catalytic activity and neither DNA cutting nor base editing are involved in the drug's mechanism.*
  - **KRAB domain.** The KRAB transcriptional repression domain from human ZFP10 with an N-terminal 16 AA linker.
- Three binding sites for miR-122-5p incorporated in the 3'UTR to reduce transgene expression in hepatocytes[2].
- A WPRE 3' UTR element (engineered to eliminate protein X expression)[3]
- An SV40 late polyadenylation sequence to terminate transcription

AAV Capsid: TfR1 CapX. An engineered capsid that crosses the blood-brain barrier (BBB) by binding human TfR1. The capsid is derived from AAV9 and contains a 7-mer insertion and several adjacent substitutions in variable loop VIII that facilitate the interaction with TfR1.

Formulation: 20mM Tris, 200mM NaCl, 1mM $MgCl_2$, 0.005% Pluronic F-68, pH 8.

4. Proposed Indications

Prion disease.

1. Patients symptomatic with prion disease
2. Presymptomatic carriers of high penetrance pathogenic *PRNP* variants

5. Purpose and Objectives of the Meeting

The goals of this meeting are to obtain the Agency's feedback on questions that are critical to the development of the PRNP-CHARM-001 product. The Sponsor seeks feedback on the set of questions listed in Section 14, which concern non-clinical, CMC, and clinical aspects of the PRNP-CHARM-001 gene therapy program.

6. Proposed Agenda

Topic	Sponsor Speaker	Time allotted
Introduce participants	Sonia Vallabh	5 minutes
Brief introduction to the PRNP-CHARM-001 gene therapy program and our patient community	Sonia Vallabh	5 minutes
Discussion of questions to the Agency and any other input from the Agency	Sonia Vallabh and Ben Deverman	40 minutes
Summary and action items	Sonia Vallabh	10 minutes

7. Sponsor Attendees

Name	Title
Sonia Vallabh, PhD	Director, Prion Therapeutic Science, Broad Institute
Benjamin Deverman, PhD	Senior Director, Vector Engineering, Broad Institute

Eric Minikel, PhD	Director, Prion Therapeutic Science, Broad Institute
Andrew Steinsapir, MBA	Nonclinical consultant; Director, Gene Therapy Program Lead, Deerfield Discovery and Development; Acting CTO, Apertura Gene Therapy
Christopher Davis, PhD	Nonclinical consultant; Director, Nonclinical and Translational Research, Apertura Gene Therapy
Stephen Sofen, PhD	CMC consultant
Monika Chabicovsky, PhD	Toxicology consultant
Tim LaVaute, PhD	NIH Program Officer
P.J. Brooks, PhD	NIH Program Officer
Chris Boshoff, PhD	NIH Program Officer
Steven E. Arnold, MD	Neurologist, Massachusetts General Hospital; Professor of Neurology, Harvard Medical School
Brian S. Appleby, MD	Director, National Prion Disease Pathology Surveillance Center
Daniel A. Sprague, PhD	Computational Scientist, Broad Institute
Alissa Coffey, PhD	Project Manager, Broad Institute
Fiona Serack, PhD	Research Scientist, Broad Institute

8. History of regulatory engagement

8.1 History of Development Program and Relevant Communication with FDA (Consolidated)

Sponsor is currently involved in various therapeutic development programs related to *PRNP.*

8.1.1. Relevant history with CDER: 2017 Critical Path Innovation Meeting (CPIM)

We have previously interacted with CDER under PIND 141250 about the strategy of lowering PrP in prion disease. Of particular note, on November 14, 2017, we met with twenty-five CDER scientists in the context of a Critical Path Innovation Meeting to discuss tools needed to ready the field for preventive trials of PrP-lowering therapeutics. The notes from this meeting are attached (Appendix II), and the white paper we submitted as prereading material has since been adapted for publication[4]. The need for preventive

treatment in prion disease was recognized in this meeting, and the discussion focused on enabling steps. Feedback received at this meeting and a subsequent Type C meeting (Oct 31, 2019 also under Pre-IND 14125) has motivated many of our efforts since, including:

- Validation that PrP lowering is protective against prion disease in additional in vivo paradigms: rats, hamsters, humanized mice infected with human prions, and a spontaneous mouse model of prion disease.
- Creation of an online patient registry (www.prionregistry.org)
- Expansion of natural history efforts to demonstrate stability of CSF PrP in individuals of various *PRNP* genotypes, over the timespan of years (Figure 19)
- Validation that treatment-mediated CNS reduction of PrP is dose-dependently reflected in CSF PrP levels (Figure 3D).

At both of these meetings, CDER scientists expressed high-level support for the concept of CSF PrP lowering as a surrogate biomarker suitable for Accelerated Approval, provided that the assay can be appropriately validated. These meetings included some discussion of the PrP-lowering antisense oligonucleotide (ASO) program on which we have collaborated with Ionis Pharmaceuticals, which is now in a Phase 1/2a trial (PrProfile; NCT06153966), but the discussion focused primarily on PrP lowering as a therapeutic hypothesis, regardless of the drug modality employed.

8.1.2. 2025 PrP-lowering divalent siRNA IND

We are the Sponsors of an academically developed PrP-lowering small interfering RNA (siRNA) termed 2439-exNA. Following a Type B meeting (pre-IND 167326) in June 2023 we submitted an IND containing two clinical protocols, one for symptomatic prion disease patients and one for pre-symptomatic at-risk individuals with high penetrance *PRNP* variants. As of December 2025, both protocols have been cleared by CDER for first in human dosing. The symptomatic arm of the study is currently funded through NIH, and began enrolling in April 2026 (PRiSM; NCT07444580); the first patient was dosed in June 2026. The pre-symptomatic arm of the study has received notice of NIH funding; receipt of funding is projected for August 2026 to support enrollment launch in Q3 2026. As noted in the clinical section (Section 12) of this document, the first-in-human clinical trial structure herein proposed for PRNP-CHARM-001 – including patient populations, enrollment criteria, schedule of assessments, and endpoints – closely mirrors that of the PRiSM trial.

8.2 Relevant Prior Agency Communications regarding PRNP-CHARM-001

INTERACT submission for PRNP-CHARM-001 (PTS #PS009720, Meeting ID #20961)**:** On Nov 26, 2024, the Sponsor submitted a new INTERACT meeting request and the information package. On Feb 5, 2025, the Sponsor received the FDA's preliminary responses and was granted an INTERACT meeting scheduled for Feb 10, 2025. The FDA's responses were sufficiently clear and complete, so the Sponsor chose to forgo the INTERACT meeting.

The INTERACT's five questions and associated feedback from the Agency established that:

1. Clinical trials for PRNP-CHARM-001 can be enrolled with both symptomatic prion disease patients and pre-symptomatic carriers of high penetrance pathogenic *PRNP* mutations.
2. Measurement of prion protein levels in cerebrospinal fluid (CSF) is suitable as a pharmacodynamic biomarker of PRNP-CHARM-001 activity in the brain. The Agency requested additional information about the specific assay used to measure CSF levels of prion protein, as well as the anticipated threshold of CSF prion protein level reduction considered to be reasonably predictive of clinical benefit.
3. Homozygous humanized TfR1 knock-in mice (*hTFRC* KI) are appropriate and sufficient as the single species for definitive biodistribution and toxicology studies. The Agency requested additional information on how reasonably safe and biologically active dose levels of PRNP-CHARM-001 will be selected based on relevant study endpoints, as well as the rationale for dose level extrapolation from animals to humans.
4. Humanized *PRNP* (*hPRNP*) mice are an acceptable model to evaluate the biological activity of PRNP-CHARM-001, which contains a human-specific zinc finger (ZF) targeting the *PRNP* locus.
5. RT-qPCR-based quantification of *PRNP* RNA in human HEK293T cells is appropriate as the potency assay for product release. Additionally, luminescence-based quantification of tagged endogenous PrP in human U251MG cells is suitable as an alternative potency assay for product release.

- **Pre-IND Submission for PRNP-CHARM-001 (Current Submission):** On May 11, 2026 the Sponsor contacted the Agency to submit the present Pre-IND Meeting Request. On May 21, 2026 the Sponsor received a response from the FDA granting the meeting for July 7, 2026 and confirming the pre-assigned Submission Tracking Number (STN) for the program: STN PS009720.

9. Therapeutic hypothesis, CHARM mechanism, and delivery strategy

9.1 Background on prion disease and the therapeutic strategy of PrP lowering

9.1.1 Prion disease is a fatal neurodegenerative disease with no existing cure caused by PrP

Prion disease is a fatal and incurable neurodegenerative disease caused by prion protein (PrP)[5] that kills an estimated 500-600 people in the United States every year. PrP is encoded by a chromosomal gene, *PRNP*, and is expressed in the brains of all mammals, including humans. In prion disease, PrP undergoes post-translational misfolding into a self-replicating conformer known as a prion. Prions spread exponentially throughout the brain by recruiting other molecules of PrP, eventually accumulating to a level where they kill neurons. Subtypes of prion disease known historically by various names including Creutzfeldt-Jakob disease

(CJD), fatal familial insomnia (FFI), and Gerstmann-Straussler-Scheinker (GSS) disease all share this same fundamental molecular mechanism.

First symptoms of prion disease can include a mix of cognitive, motor, and autonomic dysfunction but typically converge swiftly towards a rapidly progressive dementia[6]. Activities of daily living are swiftly lost as patients progress toward akinetic mutism. A typical patient will live only five months from first symptoms. 85% of cases are sporadic, apparently due to stochastic protein misfolding events, while the other 15% are genetic, due to gain-of-function mutations in *PRNP*[7]. Acquired prion disease caused by exposure to exogenous prions, for example through dietary routes or medical procedures, has thankfully become vanishingly rare, today accounting for <1% of cases[8]. There is no single point of origin for prions in the brain; as many regions can be first affected and prions readily spread across all regions, prion disease is best conceived of as a whole-brain disease. Near definitive pre-mortem diagnosis can be achieved through the combined deployment of RT-QuIC, a clinical assay that detects prion seeds in CSF[9], and targeted sequencing of *PRNP*.

To date there is no proven therapy for prion disease and no clinical standard of care. All available data from human genetics, biochemical approaches, laboratory animal models, and observations in wild and agricultural animals that are natural hosts of prion disease, are unanimous that PrP is the cause of prion disease. The therapeutic hypothesis of PrP lowering is backed by decades of genetic experiments in animal models and more recently by preclinical proof-of-concept studies in mice using PrP-lowering oligonucleotides (see below)[4,10–17]. A PrP-lowering ASO, ION717, has now progressed to a first-in-human trial under the sponsorship of Ionis Pharmaceuticals (first patient dosed early 2024, Phase I/II ongoing). A divalent siRNA, 2439-exNA, developed by our team has more recently progressed to an investigator-initiated first-in-human trial under our (the Broad Institute's) sponsorship. The PRiSM trial (NCT07444580) opened enrollment in April 2026 and the first patient was dosed in June 2026.

While these therapies may successfully lower PrP by some amount in the brain, intrathecal oligonucleotide therapies are intrinsically limited in terms of potency, biodistribution across the brain, peak/trough target engagement kinetics, and the clinical burden of repeat intrathecal dosing. Therefore, the development of additional PrP-lowering therapeutics is urgently needed. We have developed PRNP-CHARM-001 as a one-time therapy for prion disease, aimed at epigenetically silencing the *PRNP* gene, lowering the amount of PrP mRNA produced and thereby cutting off PrP production at the source. By targeting the DNA we reduce the number of targets per cell compared to an RNA-targeting therapy, with implications for potency. By delivering systemically with TfR1 CapX, we seek to leverage the capillary network to reach the whole adult brain. On the basis of these advantages, we are advancing PRNP-CHARM-001 as a one-time permanent therapy for the treatment and prevention of prion disease.

9.1.2 PrP lowering is a well-validated therapeutic hypothesis in prion disease

Decades of work from multiple orthogonal approaches including human genetics, histological and biochemical approaches, and animal models, all converge on PrP as *the* molecular cause of prion disease (Table 1). Briefly, infectious prions are composed of

misfolded PrP[5,18,19]; PrP deposition is pathognomonic for prion disease neuropathology[20]; prion strains are encoded in PrP conformation[21–23]; PrP genetic variants cause genetic prion disease, impact sporadic prion disease risk, affect prion disease duration and presentation, govern species barriers and affect transmission risk in humans and other animals[24]. Because all prions, regardless of strain or species, are comprised of PrP, it has long been expected that depleting PrP substrate should be a universal therapeutic for prion disease regardless of the patients' etiology (sporadic, genetic, acquired) or subtype; recent studies have provided corroborating evidence that both pharmacologic and genetic lowering of PrP increase survival across prion strains[16].

Table 1: Evidence that PrP is central to prion disease pathophysiology. *Reproduced from Vallabh 2020[4].*

Category	Evidence
Biochemical	Prions, the infectious agent in prion disease, are composed of PrP[19]. Prion "strains" are encoded in distinct conformations of PrP[21–23]. Prion infectivity can be generated *in vitro* from purified PrP[25–28].
Human genetics	All genetic prion disease families possess protein-altering variants in *PRNP*[29]. Certain *PRNP* missense variants confer protection against prion disease[30–32].
Animal genetics	PrP is required for prion propagation[10]. PrP is required for prion neurotoxicity[12]. PrP dosage and incubation time are inversely correlated[11]. PrP amino acid sequence governs the "species barrier"[33].

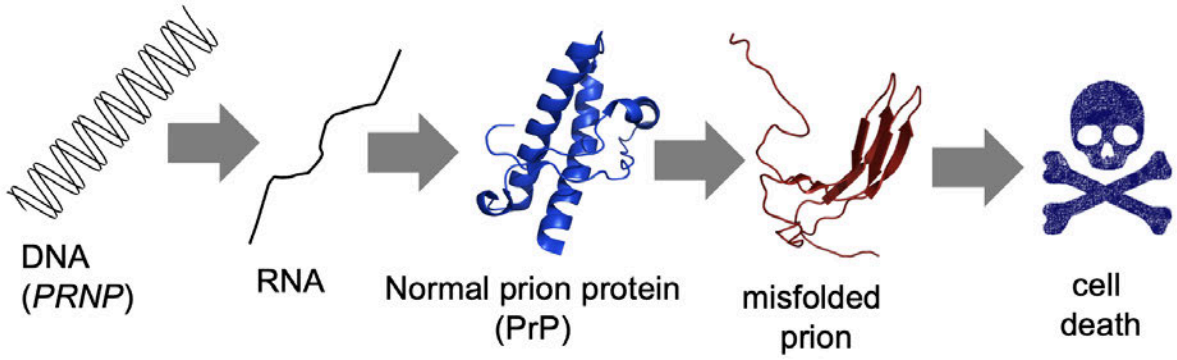


Figure 2. The pathway of prion disease. *Adapted from Vallabh 2020[4].*

The advent of transgenic and knockout mice 30 years ago provided the first genetic evidence that PrP dosage governs the tempo of prion disease[11] (Figure 3A). These models are relevant because prion disease naturally afflicts not only humans but also diverse mammals including deer, elk, sheep, goats, cattle, camels, and mink. It is exceptionally well modeled in mice, where intracerebral inoculation of prions into naive wild type (WT) animals leads to fatal disease within 5–6 months, with all of the same pathological hallmarks seen in the human disease[34]. Heterozygous knockout mice survive prion infection more than twice as long as

WT mice (Figure 3A), and homozygous knockouts are incapable of propagating prions or developing prion disease[10,17]. Conversely, disease is accelerated in transgenic mice overexpressing PrP[11]. This finding has been replicated in conditional genetic models, where lowering or abolishing PrP expression can respectively delay or prevent development of disease[13,14].

Benefit is directly responsive to PrP dosage. Chronic dosing with an ASO that reduces cortex *Prnp* RNA by 50%, beginning at an early treatment time point, triples survival of prion infected animals (Figure 3B). In a transient dosing paradigm, a low dose of ASO corresponding to 21% knockdown leads to a significant 15% increase in survival time, but progressively higher doses achieving deeper target engagement offer increasing survival benefit[16] (Figure 3C). These findings suggest that there is no fixed threshold for effect; a modest level of PrP reduction that is robustly quantifiable can be expected to convey protection against disease, and deeper knockdown will progressively convey greater benefit. ASO-mediated reduction of PrP in the brain is dose-dependently reflected in the lowering of CSF PrP in rats (Figure 3D), indicating that CSF is a relevant sampling compartment in which to monitor brain PrP levels[35].

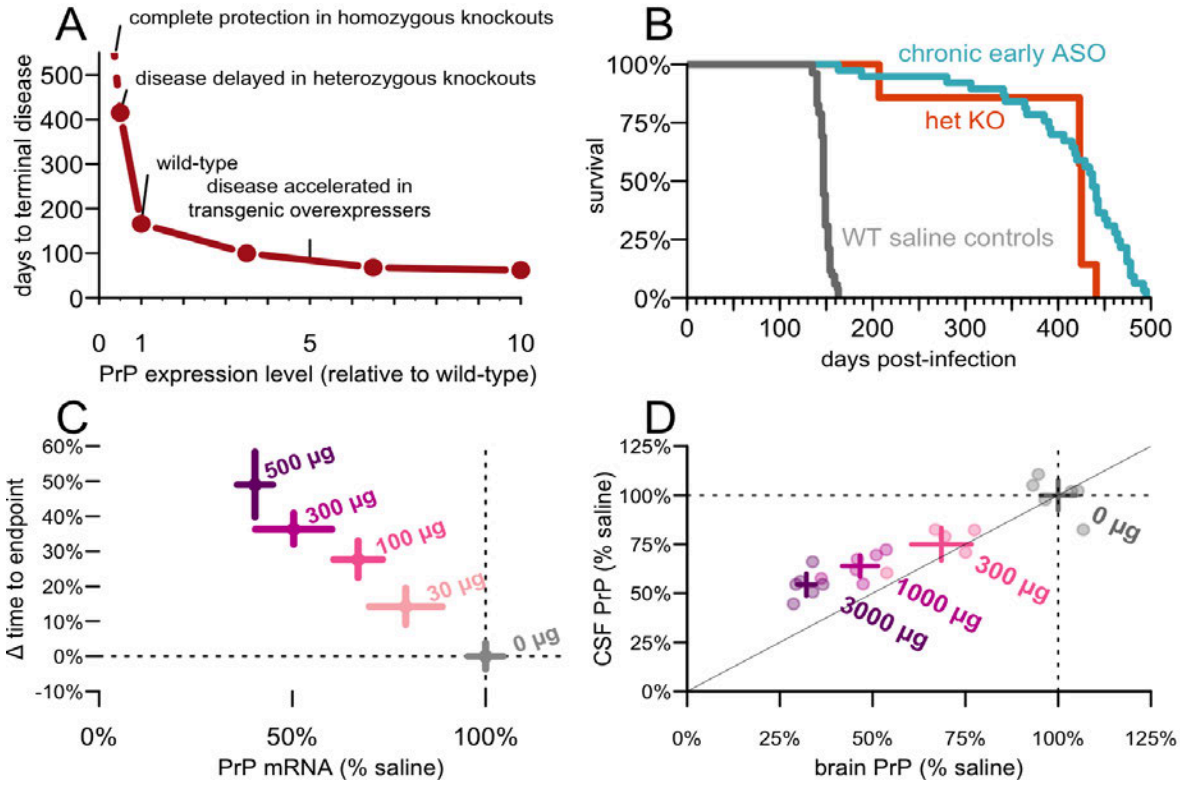


Figure 3. Published genetic and pharmacologic proofs-of-concept validating PrP lowering as a therapeutic hypothesis in prion disease. (**A**) PrP expression level determines survival time following intracerebral prion inoculation in mice across wild-type, transgenic, and knockout lines. (**B**) The benefit of chronic early ASO treatment on a symptomatic endpoint mirrors heterozygous knockout in mice. (**C**) Following treatment with varying doses of PrP-lowering ASO, a continuous dose-response relationship emerges between brain PrP level and time to symptomatic endpoint in mice. (**D**) CSF PrP mirrors brain PrP knockdown after ASO administration in rats. *Panel A adapted from Fischer 1996[11], B-C from Minikel 2020[16], D from Mortberg 2022[35]*.

9.1.3 PrP is dispensable for healthy life

In contrast to its undisputed central role in prion disease, PrP appears dispensable for healthy life. PrP knockout mice[10], cows[36], and goats[37] are all healthy and viable and initially defied efforts to identify any knockout phenotype other than resistance to prion infection. It was eventually discovered that homozygous (but not heterozygous) PrP KO mice exhibit a myelin maintenance deficiency in peripheral nerves (but not in the CNS) leading to a mild peripheral neuropathy[38]. This is apparently due to a lack of signaling of an N-terminal PrP peptide to Adgrg6, a receptor expressed on peripheral Schwann cells but not in the CNS[39]. While this myelin phenotype is histologically evident in aged animals, it is phenotypically subtle. Identifying a difference in grip strength, rotarod performance, or hot plate response is difficult, and some aged (60 week) PrP KO mice but not others exhibit a small but significant difference from wild-type animals, apparently as a function of mouse strain[38]. The histological phenotype has been further replicated in PrP KO goats, where no outward phenotypic difference was reported[40]. We have identified older humans with naturally occurring heterozygous loss-of-function mutations in *PRNP* who appear to be healthy[7,41]. Moreover, such mutations occur in the population at exactly the frequency expected based on mutation rates; unlike the vast majority of genes in the human genome, there appears to be no purifying selection against loss-of-function variants in *PRNP*[41]. While Phase I studies should be conducted with the usual high level of care and monitoring, we have no reason to expect on-target toxicity from lowering PrP to the minimum achievable level.

9.1.4 Neurons are the cell type of interest for PrP-lowering therapies

While prion disease affects all brain regions, prion toxicity is both specific to neurons and cell-autonomous, meaning that every neuron in which PrP is depleted is protected from disease. The progression of prion pathology halts following neuronally specific PrP depletion as shown in conditional knockout mice where *Prnp* is depleted in only neurons[13]. A comparison of mice selectively expressing PrP under neuronal versus astrocytic promoters found that neuronal PrP alone supported typical prion disease, while mice expressing only astrocytic PrP failed to experience clinical symptoms following prion inoculation[42]. Though PrP knockout mouse brains neither propagate prions nor experience prion neurotoxicity following prion inoculation, a PrP-expressing graft introduced into the same knockout mouse brain will both amplify prions following prion challenge, and degenerate. The restriction to PrP-expressing tissue is precise; even knockout tissue adjacent to the graft shows no pathology[12]. These findings highlight neurons as the cell type of interest in prion disease and suggest the potential of a therapy to cell-autonomously protect the neurons in which it effectively silences PrP. As described in detail below, the TfR1 CapX capsid used for

PRNP-CHARM-001 crosses the BBB by interacting with the human transferrin receptor, offering the prospect of efficient delivery of PRNP-CHARM-001 to neurons across the brain following intravenous administration.

9.1.5 PrP-lowering is beneficial at both presymptomatic and symptomatic timepoints – but the benefit differs in both degree and kind

The most striking finding of one decade of preclinical studies with PrP-lowering therapies has been the importance of treatment timepoint to outcome.

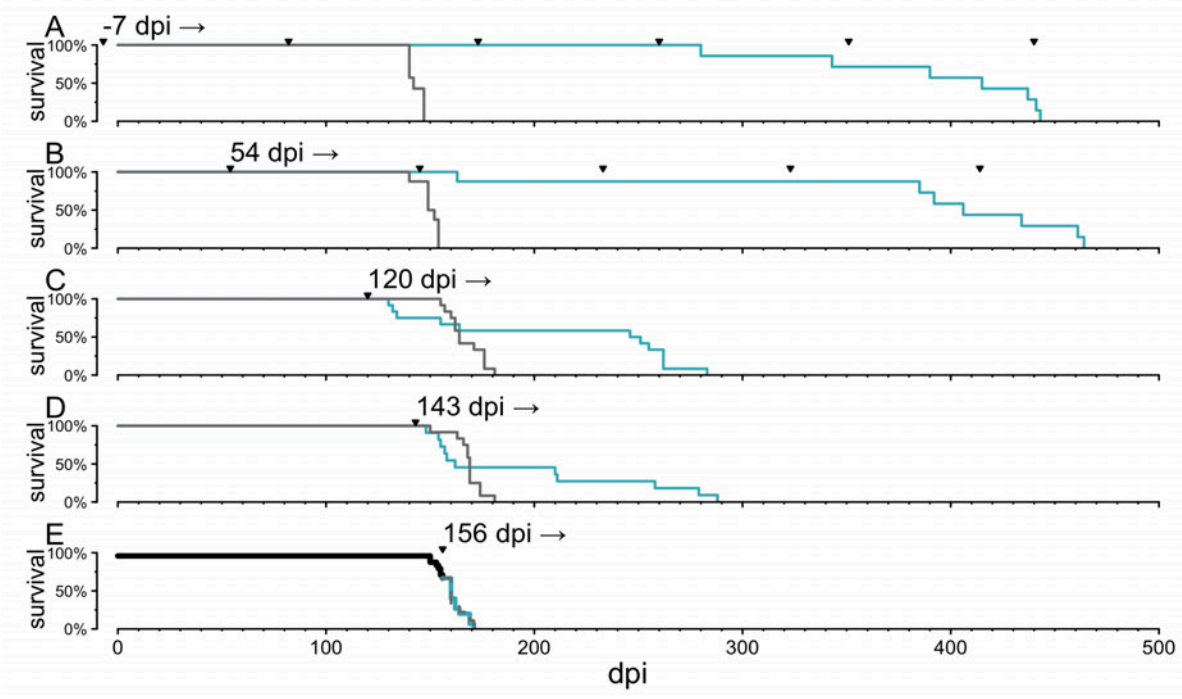


Figure 4: Treatment time point is a key determinant of efficacy of PrP-lowering therapy. dpi = days post inoculation. Adapted from Minikel 2020[16]. PrP-lowering ASO treatment conveys a different magnitude of survival benefit to prion-inoculated mice depending on the timepoint at which treatment is initiated, with earlier initiation corresponding to larger benefit and treatment at an advanced disease point failing to convey benefit. Mice received ASO treatment (cyan) or saline (gray) initiated prophylactically at 7 days before inoculation (**A**), just prior to detectable plasma neurofilament light chain (NfL) rise at 54 dpi (**B**), just prior to symptom onset in the presence of marked neuropathology at 120 dpi (**C**), after frank symptoms and weight loss in all animals at 143 dpi (**D**), or at 156 dpi, after some animals have already reached endpoint (**E**). In the early treatment experiments (**A, B**), ASO was administered chronically every 90 days, and the endpoint was the presence of five prion disease symptoms. In the late treatment experiments (**C–E**), a single dose was administered and the endpoint was 20% weight loss. In (**E**) the black portion of the curve indicates survival before the dose was administered.

Figure 4 (Panels A and B) shows that chronic administration of an ASO lowering PrP by ~50% can triple survival in prion-infected mice[16]. This result was obtained as long as therapy is initiated at any time from prior to infection up through 78 days post-inoculation (dpi),

when animals are presymptomatic but neuropathological sequelae including astrogliosis and increase in plasma neurofilament (NfL) are emerging[16]. Notably, survival benefit in early-treated animals consisted mostly of an extension of healthy life.

In animals treated at the cusp of frank symptom onset, a single dose of ASO was still able to increase survival by 70%[16] (Figure 4C), however this timepoint does not correspond to a systematic clinical intervention timepoint in humans patients, the majority of whom are identified based on onset of frank symptoms. For the 15% of cases caused by a protein-altering *PRNP* variant, risk can be identified ahead of symptoms; however, these individuals do not reliably show even molecular signs of disease before onset of clinical symptoms, making presymptomatic treatment of carriers more analogous to the pre-pathological treatment paradigms shown in Figures 3B, 4A, and 4B. Trial design considerations for this population are described in following sections.

If treatment was administered at a timepoint when all animals had measurably lost weight, the earliest consistent clinical sign of prion disease in mice, it was possible to extend survival in a subset of the animals (Figure 4D), though they did not regain weight that they had lost, nor did their number of symptoms decline. Rather than extending healthy life, treatment at this symptomatic timepoint extended symptomatic decline of the animals. If treatment was administered at a timepoint corresponding to advanced disease (Figure 4E), when the first animals in the cohort had begun to reach prion endpoint, no animals benefited from treatment. This time dependence likely reflects both the increasing difficulty of modifying disease course as it progresses, and the 5-day *in vivo* half-life of PrP, which dictates that even a therapy that acts on the PrP RNA within days will require one month post-dose to achieve full target engagement at the protein level[43]. These constraints are expected to apply to any therapy that lowers PrP by acting on the gene or RNA precursor, including PRNP-CHARM-001.

9.2 Background on targeted epigenetic silencing using CHARM

Compared to chronic oligonucleotide therapies, a gene therapy could potentially achieve one-time clamp suppression of PrP levels that would not be subject to peak-trough fluctuations in target engagement between doses. Epigenetic transcriptional silencing represents an attractive approach for permanently lowering PrP levels without the need to mutate the underlying DNA sequence[44–49]. CHARM achieves this durable silencing by recruiting the DNA methyltransferase enzyme DNMT3A[50–57] to the target locus and releasing its autoinhibition, resulting in methylation of the target locus. Alongside the DNMT3A recruitment domain (D3L) and DNMT3A autoinhibition release domain (H3L), CHARM can be designed to include a KRAB repressive domain which likewise leads to heterochromatin formation and repression of the locus through recruitment of the Kap1 co-repressor domain (Figure 5). Critically for a CNS therapy, CHARM can be directed to its target locus by a zinc finger protein (ZFP), a sufficiently compact DNA-binding domain to allow for efficient packaging within the 4.7kb capacity limit of a single AAV vector. ZF-CHARM designs, both with and without KRAB, work solely by recruiting endogenous enzymes and repression complexes; ZF-CHARM lacks direct enzyme activity and does not require any nicking or double stranded nuclease activity.

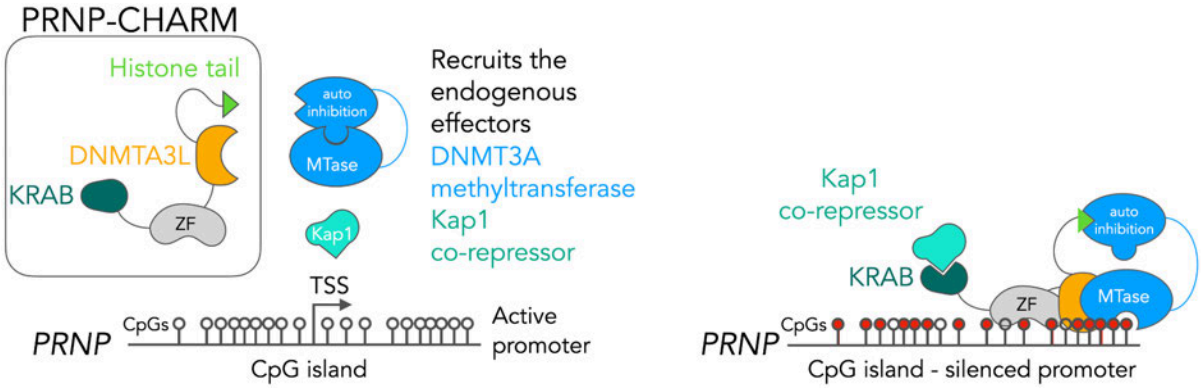


Figure 5. CHARM: Coupled Histone tail for Autoinhibition Release of Methyltransferase. A cartoon depiction of the PRNP-CHARM transgene (box). (Left) The D3L domain of DNMTA3L recruits the DNMT3A methyltransferase domain and the histone H3 tail binds DNMT3A and releases autoinhibition of the catalytic activity. The KRAB repressor from human ZFP10 recruits repressor complexes through interactions with Kap1. (Right) The CpG island is methylated and *PRNP* transcription is turned off by the targeted local recruitment of endogenous methyltransferases and repressor complexes by the *PRNP* promoter-specific ZF.

In early 2023, our team at the Broad Institute partnered with Dr. Jonathan Weissman's team at the Whitehead Institute and Dr. Ben Deverman's team at the Broad Institute to develop an AAV-mediated, *PRNP*-targeting CHARM therapeutic for prion disease. We applied for and were awarded an NIH grant (NINDS 5U19NS132315) to support preclinical proof-of-concept and IND-enabling studies.

Our proof-of-concept studies targeting CHARM to the mouse *Prnp* locus have been described[1]. Briefly, these studies demonstrated that CHARM can methylate the mouse *Prnp* promoter (Figure 6A) and potently lower PrP levels *in vivo*, across the mouse brain at both the RNA and protein level (Figure 6B). When a single dose of CHARM was delivered intravenously in the AAV-PHP.eB vector known to broadly transduce mouse neurons following IV dosing, *Prnp* RNA levels were reduced by 82.1%, the largest treatment-mediated reduction ever observed in our studies or reported by any group. CHARM reduced both *Prnp* RNA and PrP protein in a dose-dependent manner across a four-fold dose range (Figure 6B) and we observed no significant difference in target reduction at 6 weeks, 12 weeks, 6 months or 12 months after product administration, consistent with the long-term activity expected of the CHARM mechanism (Figure 6C). Finally, in the best-established model of prion disease, wild-type (C57BL/6) mice intracerebrally inoculated with the RML strain of mouse prions, animals treated with CHARM 120 days after prion inoculation, at a timepoint corresponding to robust pathology and the cusp of clinical symptoms, exhibited a mean extension of survival of roughly 80% relative to untreated controls (Figure 6D).

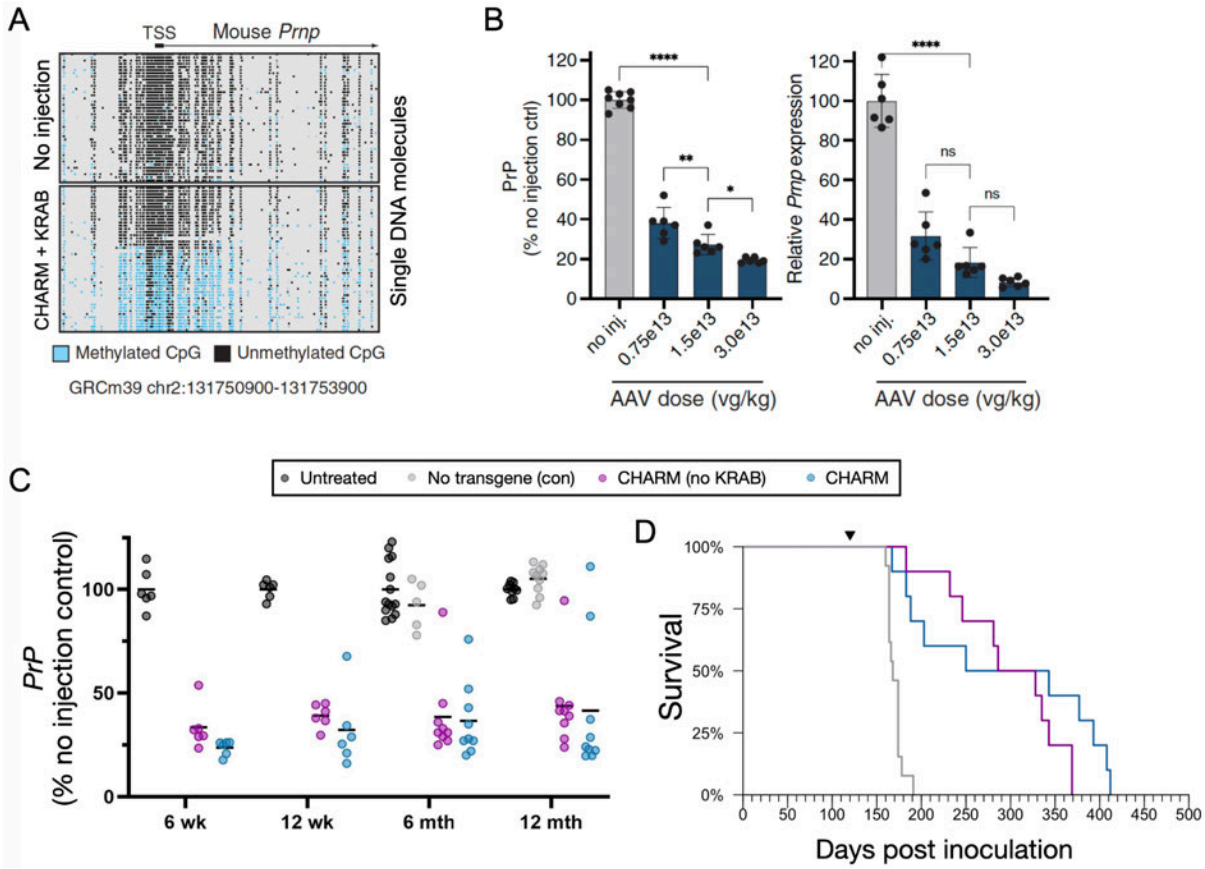


Figure 6. CHARM methylates the *Prnp* promoter, dose-responsively and durably lowers brain PrP, and extends survival of prion-infected mice. CHARM was delivered via a single retro-orbital AAV injection to mice between 5-8 weeks of age. Unless otherwise noted, the AAV dose was 1.5e13 vg/kg and whole brains hemispheres were collected and homogenized for analysis 6 weeks post dose. (**A**) DNA methylation was assessed by targeted nanopore long-read sequencing in genomic DNA extracted from whole brain hemispheres 6 weeks post dose. (**B**) CHARM was assessed in dose response by PrP ELISA and *Prnp* RT-qPCR performed on whole brain hemisphere homogenates. Data are mean ± SD of n = 5 to 6 replicates. (**C**) N=8-10 mice per group were injected with CHARM or CHARM without KRAB and whole brain hemispheres were assayed for PrP by ELISA at 6 weeks, 12 weeks, 6 months, or 12 months post-dose. (**D**) N=10-12 wild-type C57BL/6N mice per group were intracerebrally inoculated with RML prions (30 µL of 1% weight/vol brain homogenate) at 8-10 weeks of age on Day 0, then intravenously dosed via retro-orbital injection with 1.5e13 vg/kg AAV-PHP.eB containing CHARM, CHARM without KRAB, or a no transgene control at 120 days post inoculation. Humane endpoint was defined as 20% weight loss from baseline at 90 days, moribundity, or inability to reach food or water. Curves are plotted as all-cause mortality with the exception of 1) deaths due to surgical complications on the day of surgery, 2) deaths prior to treatment group, or 3) experimental error on the day of surgery.

Having established proof of concept by targeting mouse *Prnp*, we transitioned to identifying and characterizing a human CHARM candidate with a zinc finger targeting the human *PRNP*

promoter. Key non-clinical studies that led to the selection of our clinical lead candidate and ongoing characterization studies are described in Section 10.

9.3 Background on TfR1 CapX

An effective prion disease therapy requires efficient PrP lowering in neurons throughout the entire CNS, ideally via a systemically delivered, BBB-crossing vector that can reach all brain regions via the capillary network. PRNP-CHARM-001 uses TfR1 CapX, an engineered AAV9-derived capsid purpose-built for this task. Our confidence in TfR1 CapX for first-in-human use rests on four pillars: (1) the capsid exploits a well-validated, constitutively active CNS delivery pathway, receptor-mediated transcytosis (RMT) via the human transferrin receptor (TfR1), that has been validated in mice, non-human primates, and humans; (2) TfR1 CapX achieves efficient neuron transduction at doses well below clinical precedents for AAV9; (3) TfR1 CapX-delivered *Prnp* ZF-CHARM enabled a 50% reduction of whole-brain hemisphere PrP at less than 1 vg/cell in the brain indicating a high potency per particle delivered to the brain (see Section 10.1.2), (4) the capsid's strict human specificity for its enhanced tropism is mechanistically defined to a single amino acid in hTfR1 (Y247), providing an exceptionally clear biological basis for species selection in preclinical studies; and (5) the capsid is manufacturable at clinical scale under cGMP conditions using standard AAV9-compatible processes.

This section provides the scientific basis for these claims and supports the Sponsor's position, stated in Question 3, that *hTFRC* KI mice are the appropriate and sufficient single species for definitive biodistribution and toxicology studies, a position with which the FDA concurred in response to the Sponsor's 2025 INTERACT meeting (PTS#PS009720 Meeting ID#20961).

TfR1 CapX enables dramatically improved CNS delivery compared to AAV9

PRNP-CHARM-001 uses TfR1 CapX, an AAV9-derived capsid, specifically designed for systemically delivered human CNS gene therapy applications. TfR1 CapX is a second generation variant of the recently developed capsid BI-hTFR1[58] that crosses the BBB using receptor-mediated transcytosis via interactions with the human Transferrin receptor (TfR1) (Figure 7). The key strengths of TfR1 CapX are:

(1) TfR1 CapX is derived from AAV9, the best-studied natural AAV for systemic CNS delivery. AAV9 has been successfully administered to more than 3,000 infants with spinal muscular atrophy (SMA). The extensive safety and manufacturing experience with AAV9 provides a strong foundation for clinical development of TfR1 CapX.

(2) A clinically validated CNS delivery mechanism. TfR1 mediates rapid constitutive, ligand-independent RMT across the CNS vasculature[59-61] and has a well-established track record as a target to increase CNS delivery of biologics in mice[62-64], non-human primates[65-67], and humans, as investigational therapies (NCT07169578) and approved drugs[68-69], at doses predicted from *hTFRC* KI mouse studies[70-72]. Indeed, TfR1-mediated transport into the CNS is validated as the mechanism of action of two approved antibody-based therapeutics for Mucopolysaccharidosis type II[68-69]. Unlike delivery approaches that depend on novel or

poorly characterized receptors, TfR1-mediated transport across the BBB is a well-characterized pathway in brain capillary endothelium, with clinical proof of concept for CNS delivery in humans.

(3) Superior CNS tropism at low systemic doses. In adult *hTFRC* KI mice, TfR1 CapX can transduce more than 80% of $NeuN^+$ cortical and spinal cord neurons following intravenous administration at 2E12 vg/kg, a dose that is approximately 50-fold lower than clinical precedents with AAV9 (Figure 8). The enhanced tropism is CNS-selective: TfR1 CapX does not increase targeting to the liver or other assessed organs compared to AAV9 (Figure 9). Furthermore, when used to deliver a mouse *Prnp* ZF-CHARM cargo, TfR1 CapX enabled a 50% reduction of whole-brain hemisphere PrP as compared with untreated controls at less than 1 vg/cell on average in the brain indicating that TfR1 CapX requires few particles per cell to achieve efficient PrP lowering (see Section 10.1.2).

(4) Binding of the TfR1 apical domain in the presence of holo-transferrin. TfR1 CapX shares the identical TfR1-targeting 7-mer present in BI-hTFR1 and thereby engages human TfR1 at the same site within the apical domain as the first-generation capsid BI-hTFR1 (Figure 7, 10, and 11). Critically, TfR1 CapX binds TfR1 in the presence of its natural ligand, holo-transferrin.

(5) Extensive internal and external validation of TfR1 CapX manufacturing and potency. Consistent with our experience completing a 50L batch (batch no. T-POLARIS-123) of PRNP-CHARM-001 (see Sections 11.8 and 14, CMC Question 4), multiple independent groups have successfully manufactured TfR1 CapX-based AAVs[73-75].

The highly potent CNS transduction mediated by TfR1 CapX has been validated in *hTFRC* KI mice by our team at the Broad across multiple transgene designs, including reporters (Figures 8 and 9), CHARM studies (Appendices VI, VII, VIII), and base editor studies[76] representing over 200 *hTFRC* KI mice dosed. TfR1 CapX has also been independently validated non-clinically by several groups[77-80]. As of this writing TfR1 CapX has not been clinically dosed. Two sponsors, Finding Hope for FRSS1L and AviadoBio, have publicly stated their intention to take TfR1 CapX encapsidated gene therapies into first-in-human dosing for CNS diseases by the end of 2026. We will monitor clinical use of this capsid, public statements and data as they become available and assess for relevance to our program.

Human endothelial BBB models confirm TfR1 CapX mechanism and CNS crossing

To confirm that enhanced CNS tropism reflects active transcytosis rather than passive uptake, the first-generation capsid BI-hTFR1, which shares the identical 7-mer TfR1-targeting peptide with TfR1 CapX, was tested in human brain endothelial cell models. BI-hTFR1 efficiently transduced human brain microvascular endothelial cells (hBMVECs) and hCMEC/D3 cells compared with AAV9 (Figure 7A), and this transduction was blocked by TfR1 apical domain-blocking antibodies (Figure 7B). In a transwell BBB model, BI-hTFR1 was actively transported from the apical to basolateral chamber at 37°C but not 4°C, confirming temperature-dependent, active transcytosis (Figure 7C-D). Because TfR1 CapX carries the identical targeting peptide and engages the same apical domain binding site, these mechanistic findings apply directly to TfR1 CapX.

The CNS tropism of TfR1 CapX is strictly human-specific: humanized mice are the only physiologically relevant preclinical species

The enhanced transduction phenotype of TfR1 CapX is strictly dependent on human TfR1. TfR1 CapX does not exhibit enhanced tropism in cells expressing TfR1 from macaques, marmosets, or mice (Figure 10). We have precisely defined the mechanistic basis of this human specificity: a single human-specific amino acid in the apical domain of human TfR1, tyrosine at position 247 (Y247), is essential for TfR1 CapX binding (Figure 11). Substituting macaque residues D247 and S248 into human TfR1 fully abolished TfR1 CapX transduction, and substituting human Y247 into macaque TfR1 was sufficient to confer permissivity. Y247 is unique to human TfR1 and is absent from TfR1 in all common preclinical and domesticated species, including macaques, marmosets, mice, rats, dogs, and minipigs (Figure 11H).

Based on these data, we conclude that macaques are not a physiologically relevant species for assessing the biodistribution and toxicity of TfR1 CapX and PRNP-CHARM-001.

This strict human specificity has two critical implications. First, CNS biodistribution of TfR1 CapX would not be expected in non-human primates or any other non-humanized preclinical species following intravenous dosing. This prediction is confirmed by independent data from Apertura Gene Therapy (AGTX-1111 program, see Appendix III for a memorandum authorizing reference to the CBER INTERACT/PTS#PS009041 submission and associated correspondence). Pooled library studies in cynomolgus macaques showed no enrichment of TfR1 CapX (termed TfR1Cap02 in their filings) or BI-hTFR1 (TfR1Cap01) in the CNS following IV delivery, despite robust CNS transduction in *hTFRC* KI mice using the same capsids (Studies AS-1901 and AS-2803; CBER INTERACT submission INTERACT/PTS#PS009041, pp. 43-45 and 40-42). Apertura's data also confirm tolerability of a 1E14 vg/kg intravenous dose of BI-hTFR1 in cynomolgus macaques (see Appendix IV for a detailed study report). A summary of all relevant Apertura studies is provided in CBER INTERACT submission INTERACT/PTS#PS009041, pp. 13-16 (Appendix III). Biodistribution and toxicology studies in NHPs are not pharmacologically relevant as the distribution and transduction capabilities of TfR1 CapX would not be representative of dosing in humans.

Second, *hTFRC* KI mice, in which mouse *Tfrc* exons 4-19 encoding the extracellular domain have been replaced with the corresponding human *TFRC* sequence, are the only commonly available preclinical species in which TfR1 CapX achieves pharmacologically relevant CNS biodistribution and can be meaningfully assessed. The FDA concurred with this single-species justification in response to the Sponsor's INTERACT submission (PTS#PS009720, Question 3, February 2025).

We expect that IV dosing of TfR1 CapX in *hTFRC* KI mice will be quantitatively representative of IV dosing in humans based on the following lines of reasoning: (1) The mechanism of transport of the capsid across the BBB (via binding TfR1) is conserved across species. (2) TfR1 protein levels in mouse and human microvasculature are similar. (3) Receptor mediated transcytosis across the vasculature scales with brain size because brain

capillaries in both mice and humans are typically no more than 40 µm apart and most neurons are therefore within 20 µm of a capillary[81]. Together, these considerations support the use of vg/kg dosing in *hTFRC* KI mice as a direct basis for human dose selection.

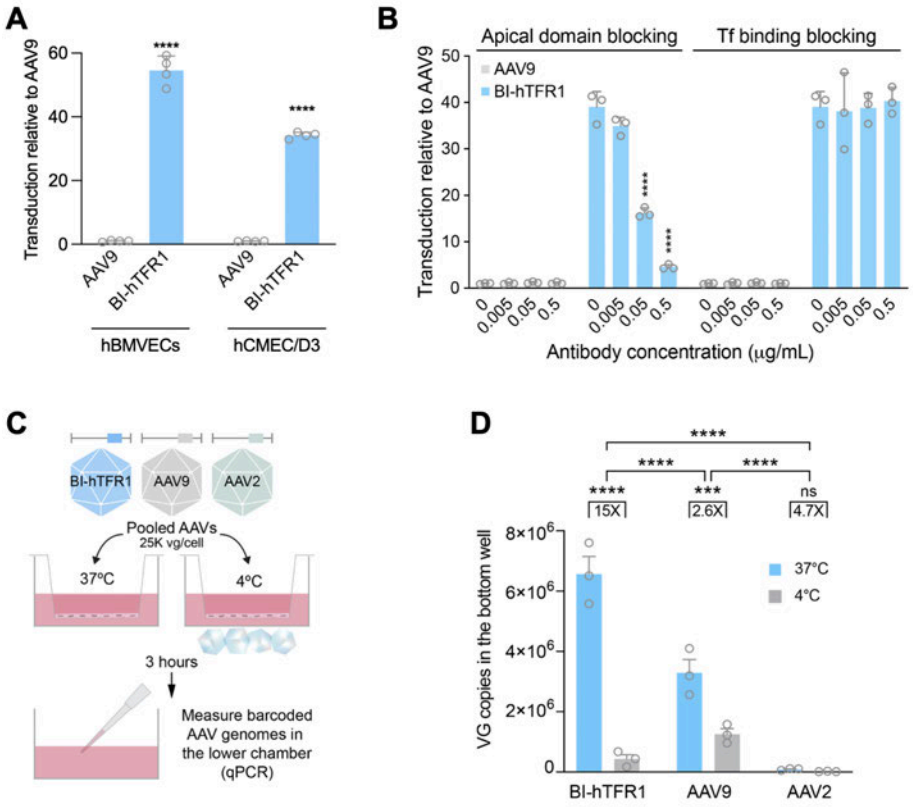


Figure 7. BI-hTFR1 more efficiently transduces and crosses human brain endothelial cell monolayers in vitro by binding the apical domain of human TfR1. *Figures adapted from Huang and Chan et al Science 2024*[58]. (**A**) BI-TFR1 more efficiently transduces hBMVEC and hCMEC/D3 cells as compared with AAV9 (luciferase reporter assay). (**B**) Transduction (luciferase activity) of hCMEC/D3 cells incubated with 3 x 10^8 vector genomes (vg)/mL of the indicated AAV and specified concentrations of OKT9 or AF2474 antibody is shown (two-way ANOVA using the no antibody control for each condition as the main comparison group with Bonferroni multiple comparison correction: **** indicates $p \leq 0.0001$; n = 3 replicates, error bars indicate ± SEM). In (**A**) and (**B**), values are normalized to AAV9 (reported as fold change) in each cell line. (**C**) The transwell BBB model experimental design is shown. (**D**) More BI-hTFR1 is actively (37C vs 4C) transported from the apical (top) to basolateral (bottom) chamber as compared to AAV9 and AAV2. Vector genomes in the bottom chamber were quantified by qPCR (two-way ANOVA with Bonferroni multiple comparison correction: **** and *** indicate $p \leq 0.0001$ and ≤ 0.001 respectively; n = 3 transwell replicates, error bars indicate ± SEM).

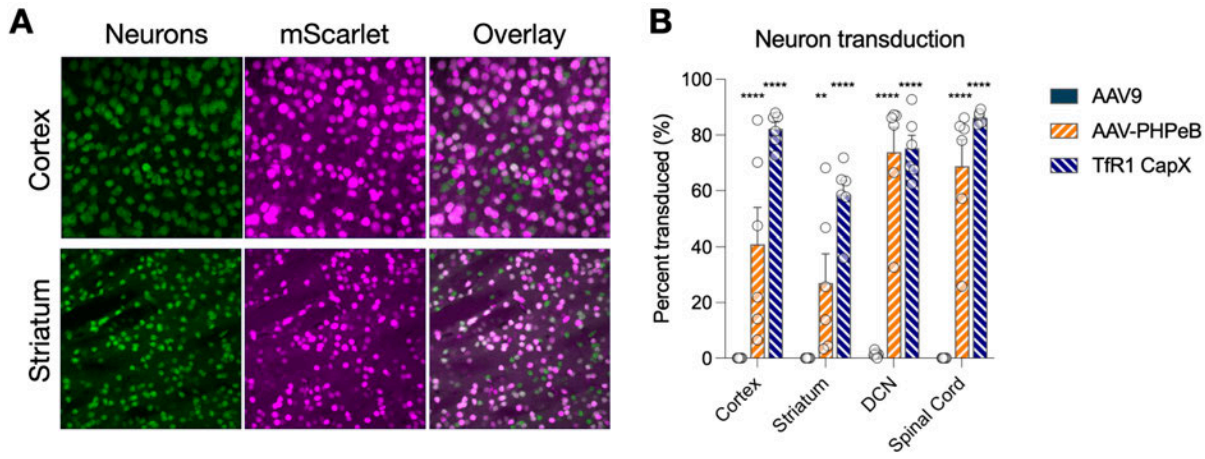


Figure 8. TfR1 CapX efficiently delivers genes to neurons in the CNS of *hTFRC* KI mice. AAV9, AAV-PHP.eB, BI-hTFR1, or TfR1 CapX encoding CAG-NLS-mScarlet-P2A-Luciferase-WPRE-SV40pA were intravenously injected into adult C57BL/6J or *hTFRC* KI mice at 2E12 vg/kg. Representative brain and spinal cord images from the indicated groups of mice are shown. (**A**) Images show the colocalization of mScarlet reporter expression with $NeuN^+$ neurons in the indicated brain regions. (**B**) The percentages of transduced $NeuN^+$ neurons in several brain regions is shown (two-way ANOVA with Bonferroni multiple comparison correction: **** indicates $p \leq 0.0001$ vs AAV9; each data point represents an individual mouse, error bars indicate ± SEM). DCN: deep cerebellar nuclei.

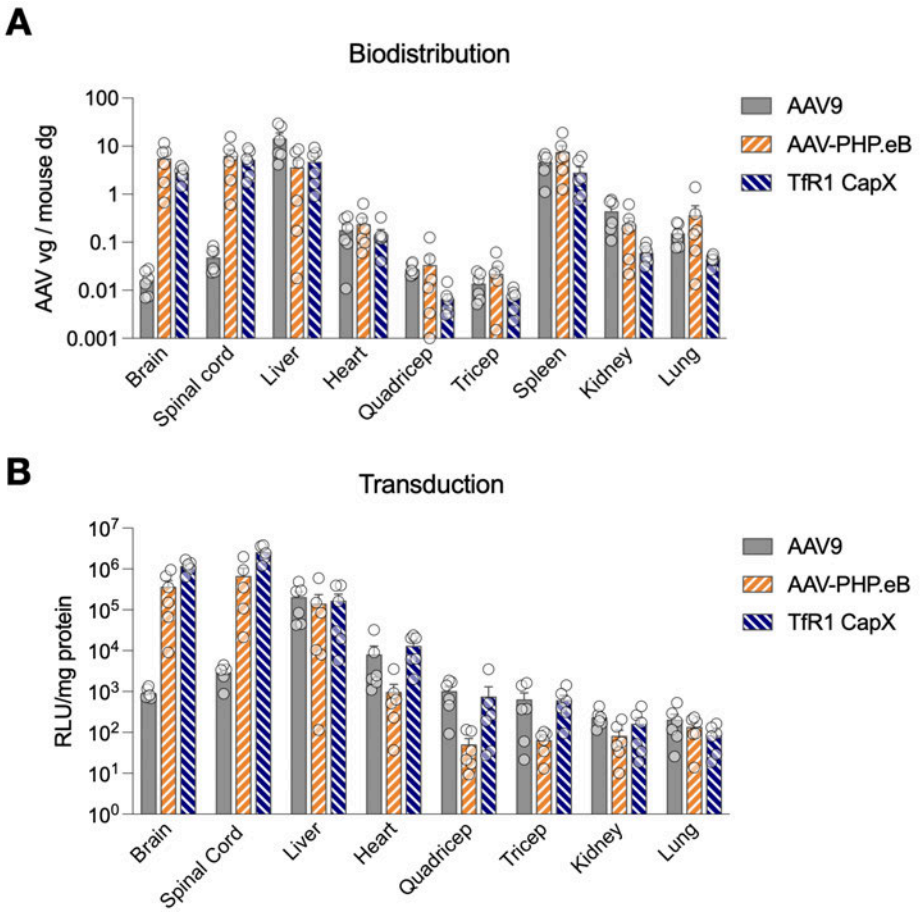


Figure 9. TfR1 CapX exhibits enhanced tropism to the CNS of *hTFRC* KI mice. AAV9, AAV-PHP.eB, BI-hTFR1, or TfR1 CapX encapsidating CAG-NLS-mScarlet-P2A-Luc-WPRE-SV40pA were intravenously injected into adult female C57BL/6J or *hTFRC* KI mice at 2E12 vg/kg. The biodistribution reported as AAV vector genomes (vg) per mouse diploid genome (dg) (**A**) and transduction based on luciferase relative light units (RLUs/ug protein) in lysates (**B**) are shown at three weeks post-injection.

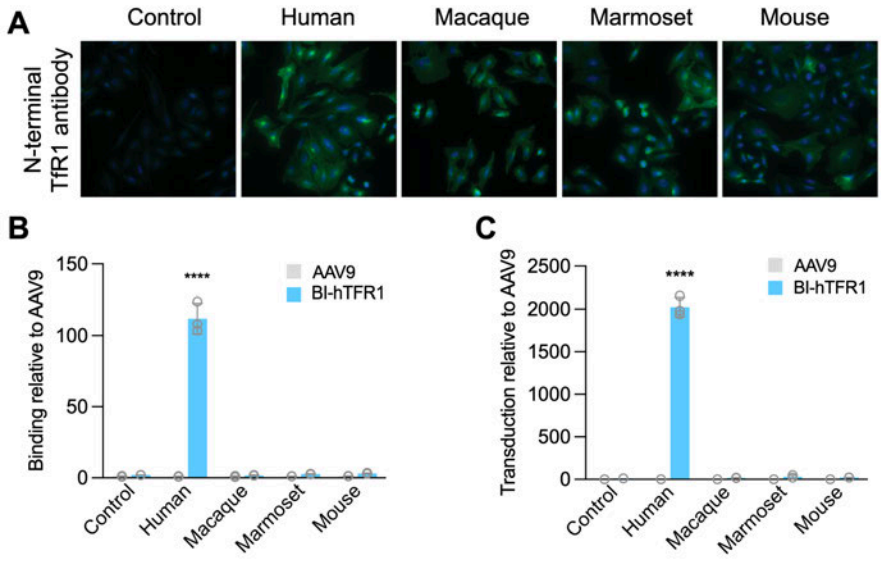
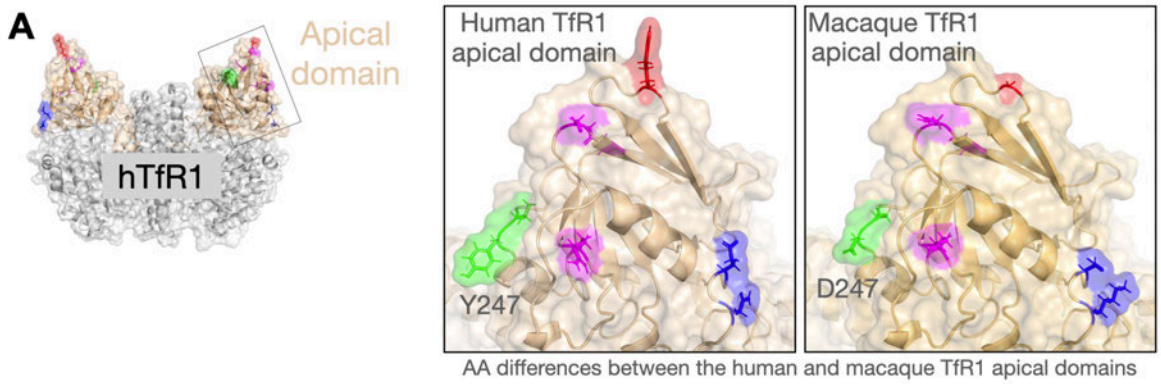


Figure 10. The enhanced binding and transduction phenotype of BI-hTFR1 is specific to human TfR1. (**A**) CHO cells were generated that stably express TFRC from the indicated species. Images show immunostaining using an anti-TfR1 antibody that recognizes a common N-terminal epitope. BI-hTFR1 associates with (**B**) and transduces (**C**) cells expressing human TfR1, but not cells expressing macaque, marmoset, or mouse TfR1.



AA differences between the human and macaque TfR1 apical domains

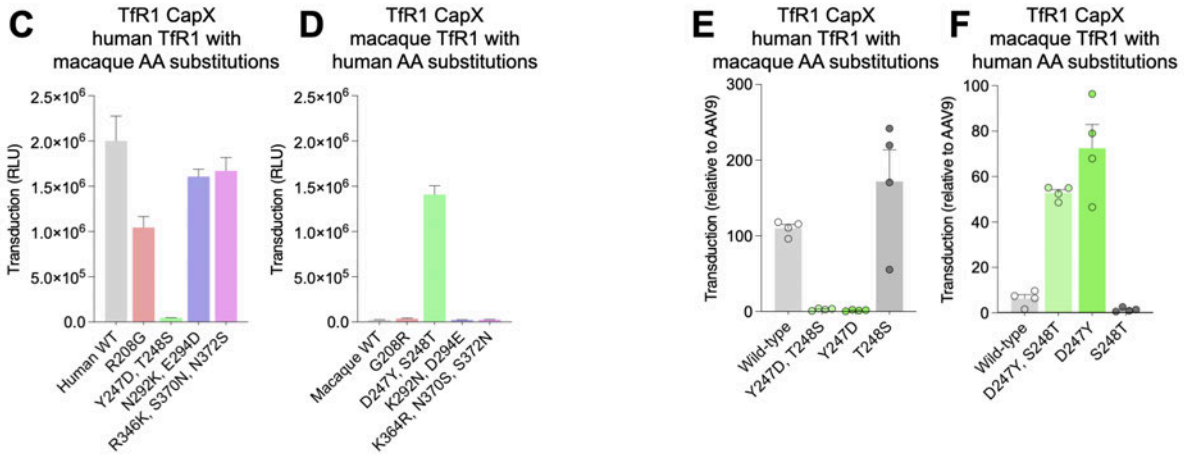


Figure 11. Binding of the TfR1 CapX capsid to the apical domain of human TfR1 depends on the Y247 residue that is unique to humans. (**A**) (Left) An AlphaFold 2 structural model of the hTfR1 dimer shows the apical domain highlighted (wheat). Amino acid differences between humans and macaque are highlighted with color corresponding to specific amino acids shown in the amino acid sequence alignment (**B**) and on AlphaFold2 models of the human and macaque TfR1 apical domain (**A**, right). (**C**) TfR1 CapX transduces CHO cells expressing human TfR1 but not CHO cells expressing human TfR1 where residues Y247 and T248 have been replaced with the corresponding macaque residues D247 and S248. (**D**) Consistent with this finding, replacement of macaque D247 and S248 with the corresponding human Y247 and T248 is sufficient to impart the enhanced transduction phenotype. (**E, F**) Y247 is the critical amino acid residue required for TfR1 CapX binding to human TfR1. (**G**) The tyrosine (Y) residue at human TfR1 position 247 is not present in any commonly used preclinical or domesticated species.

10. Non-clinical product development

PRNP-CHARM-001 has been developed in alignment with existing FDA guidance for gene therapy products which incorporate genome editing. Specifically FDA guidance:

- Preclinical Assessment of Investigational Cellular and Gene Therapy Products - Finalized November 2013
- Human Gene Therapy Products Incorporating Human Genome Editing - Finalized January 2024
- Safety Assessment of Genome Editing in Human Gene Therapy Products Using Next-Generation Sequencing - Draft April 2026

The PRNP-CHARM-001 non-clinical program encompasses AAV payload candidate selection, lead construct confirmation with the clinical capsid, surrogate capsid and manufacturer bridging studies, and safety/biodistribution evaluation. We have completed *in vivo* drug candidate selection and confirmation studies, capsid and manufacturer bridging studies, and a non-GLP pilot toxicology study. A definitive GLP toxicology study in *hTFRC* KI is planned and we seek the agency's feedback on our study design in this meeting. The studies completed and ongoing are summarized in Table 2; in brief:

- **Transgene candidate selection; V1SION study.** A head-to-head comparison of five human *PRNP* ZF-CHARM transgene designs (see Appendix V) in *hPRNP* mice using the AAV-PHP.eB capsid (a surrogate capsid capable of delivery in all mouse models used in this program) for CNS delivery. The lead construct was selected based on the most favorable combination of CNS potency and attenuation of liver expression. For details, see Appendix V.
- **Capsid bridging; CABS study.** A comparison of TfR1 CapX and PHP.eB delivery of a mouse-targeted *Prnp* ZF-CHARM construct in *hTFRC* KI mice, confirming that TfR1 CapX achieves comparable CNS efficacy to AAV-PHP.eB in *hTFRC* KI mice.

This study enables the bridging of transgene selection data generated with AAV-PHP.eB in V1SION to the clinical capsid. For details, see Appendix VI.

- **Lead construct confirmation with TfR1 CapX; H3X study.** Confirmation of PRNP-CHARM-001 potency in the most translationally complete preclinical model, mice humanized for both *TFRC* and *PRNP* (*hTFRC KI;hPRNP*), using the TfR1 CapX capsid. For details, see Appendix VII.
- **Manufacturer bridging; BRAV3 study.** A potency comparison of Broad Institute-manufactured versus Viralgen-manufactured material (lead candidate packaged in TfR1 CapX) in *hTFRC* KI;*hPRNP* mice. This study bridges the efficacy dataset to Viralgen-manufactured material. For details, see Appendix VIII.
- **Safety and biodistribution (non-GLP pilot, ongoing).** A non-GLP safety and biodistribution study of PRNP-CHARM-001 in *hTFRC* KI mice at Labcorp, evaluating organ-level vector genome distribution and preliminary safety endpoints at 1 and 3 months. Results from this study are informing the design of the GLP study. For details, see Appendices IX and X.
- **GLP Safety and Biodistribution Study (planned).** A definitive GLP study of PRNP-CHARM-001 in *hTFRC* KI mice at Labcorp using 50L scale toxicology material manufactured at Viralgen. This study will provide the primary nonclinical safety dataset to support IND filing. The draft study protocol is overviewed in Section 10.1.6 and provided in full in Appendix XI.

Table 2. Non-clinical *in vivo* potency and tolerability studies

Study (Appendix)	**Goal**	**hu /mo *PRNP***	**hu/mo *TFRC***	**hu/mo ZF**	**Capsid**	**Status** (test article production site)
V1SION (V)	Define human CHARM lead construct	Human	Mouse	Human	AAV-PHP.eB	Complete (Broad)
CABS (VI)	CapX - PHP.eB capsid bridging	Mouse	Human	Mouse	TfR1 CapX and AAV-PHP.eB	Complete (Broad)
H3X (VII)	Multi-dose potency in fully humanized mice	Human	Human	Human	TfR1 CapX	Complete (Broad)
BRAV3	Multi-dose	Human	Human	Human	TfR1 CapX	In-life

(VIII)	potency of Broad and CDMO lead candidate material in fully humanized mice. Primary study (with GLP tox) informing FIH dose selection					complete; analysis on-going (Broad and Viralgen)
Non-GLP toxicology (IX, X)	Non-GLP biodistribution and toxicology at Labcorp	Mouse	Human	Human	TfR1 CapX	In-life complete; analysis on-going (Viralgen)
GLP Toxicology (XI)	GLP biodistribution and toxicology at Labcorp	Mouse	Human	Human	TfR1 CapX	Planned (Viralgen)

Mouse models used in the development of PRNP-CHARM-001 are fully described in Appendix I. Briefly, in summary:

Table 3. Summary of mouse lines used during the development of PRNP-CHARM-001

Short name	**hu/mo PRNP**	**hu/mo TFRC**	**Compatible with TfR1 CapX**	**Compatible with CHARM targeting human *PRNP* locus**	**Notes**
WT	Mouse	Mouse	No	No	C57BL/6N and C57BL/6J
hTFRC KI	Human	Mouse	Yes	No	Required for TfR1 CapX tropism
hPRNP	Human	Mouse	No	Yes	ZH3/ZH3 mouse *Prnp* knockout allele ensures for protein quantification that all PrP expressed is huPrP
hTFRC KI/hPRNP	Human	Human	Yes	Yes	ZH3/ZH3 allele – see above

*All mouse lines are compatible with AAV-PHP.eB, leveraged as a surrogate capsid for TfR1 CapX

Viral Genomic Titer and Interpretation

Preparations of AAV test articles used across all listed non-clinical studies have been subjected to a fit-for-purpose testing panel prior to use to ensure that material is adequately and sufficiently characterized. The Sponsor has endeavored to collect sufficient retains of critical preparations.

- All AAV test articles prepared by the Broad Institute were titered by the Broad Institute. All genomic titers listed for these materials throughout this document and associated appendices report the genomic titer and calculated dose-level using this Broad Institute titer unless otherwise specified.
- All AAV test articles prepared by Viralgen were titered by Viralgen using their qualified method. All genomic titers listed for these materials throughout this document and associated appendices report the genomic titer and calculated dose-level using this Viralgen titer unless otherwise specified.
- As discussed in subsequent sections, Viralgen produced AAV preparations and associated Viralgen titers will be used for the determination of human dose-levels.

Reasonable agreement has been found between Viralgen produced AAV preparations and Viralgen titer results with Broad Institute titer results from the same material. Nonetheless, should assessment of Broad Institute prepared materials be required, they will be tested at Viralgen using their qualified method.

10.1 In vivo potency and safety studies

Streamlined description of each key study

Route of administration. For all studies in this program, animals were or will be dosed intravenously.

10.1.1 PRNP-CHARM-001 V1SION Study: selection of the human CHARM lead construct

The V1SION study was executed in *hPRNP* mice capable of demonstrating target engagement and potency of human-targeting ZF-CHARM. *hPRNP* mice are maintained on a mouse *Prnp* null background (see Table 3 and Appendix I). V1SION was conducted at the Broad Institute with material produced at the Broad Institute. A detailed study report for V1SION can be found in Appendix V. PrP ELISA data from V1SION (Figure 12) successfully demonstrated PrP lowering in whole brain hemisphere homogenates and led to the nomination of PRNP164 as the CHARM construct carried forward as our lead. The AAV-PHP.eB mouse surrogate capsid was used for all conditions in this study.

Key comparisons in this study include:

- Inclusion of upstream open reading frames (uORFs) to attenuate CHARM expression in transduced cells, to test whether expression can be reduced on a per-cell basis without impacting potency.
- Inclusion of miRNA-122 binding sites to attenuate CHARM expression in hepatocytes. *PRNP* silencing in liver would not be therapeutically beneficial and reduced transgene expression is expected to improve tolerability.

- A comparison of the Broad and Viralgen backbones, which differ in their ITR and backbone sequences.

Additional non critical conditions tested in this study are fully detailed in Appendix V.

The key finding of this study was that ZF-CHARM brain potency was consistent for all key conditions. Assessment of *PRNP* mRNA levels in liver tissue confirmed the ability of miR-122 binding sites to reduce transgene expression and activity in hepatocytes. The key outcome of this study is that PRNP164 was advanced as our payload.

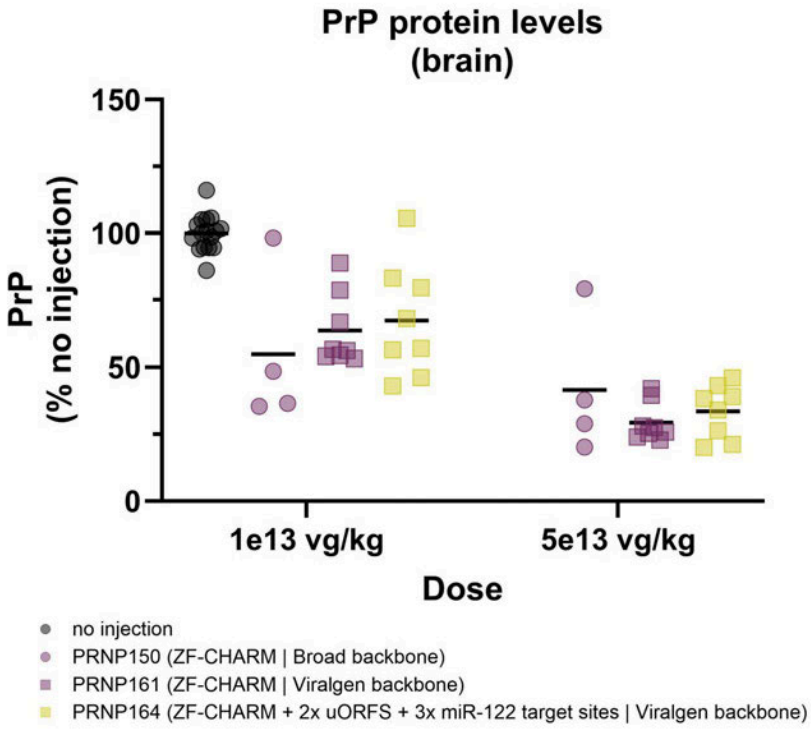


Figure 12. Lowering of PrP in the brain by human *PRNP* ZF-CHARM. *hPRNP* mice were injected with ZF-CHARM (Broad backbone), ZF-CHARM (Viralgen backbone), or ZF-CHARM 2x uORFs + 3x miR-122 target sites (Viralgen backbone) at one of two doses (1e13 vg/kg, or 5e13 vg/kg) at 9-17 weeks of age. Whole brain hemispheres were analyzed for PrP protein levels by ELISA at 6 or 8 weeks post-dose (6- and 8-week groups combined).

10.1.2 CABS Study: Capsid bridging between AAV-PHP.eB and TfR1 CapX using Prnp ZF-CHARM

The CABS study was executed in *hTFRC* KI mice responsive to both the mouse surrogate capsid AAV-PHPeB and the human-relevant capsid TfR1 CapX. This study used a mouse specific *Prnp* ZF-CHARM. The CABS study was conducted at the Broad Institute and carried out with material produced by the Broad Institute. A detailed study report for CABS can be found in Appendix VI. The CABS study demonstrated the relative biodistribution and

Prnp lowering potency of AAV-PHP.eB, the mouse surrogate capsid used in our proof of concept non-clinical studies, and TfR1 CapX, our clinical capsid.

Key readouts for the study included (1) measurement of *Prnp* mRNA and PrP protein in the brain, (2) *Prnp* mRNA in the liver, and (3) vector genome content in and PrP levels in the brain. Vector genomes were also quantified in other key tissues. Both the AAV-PHP.eB and TfR1 CapX-based test articles achieved robust and dose-dependent reduction of mouse *Prnp* mRNA and mouse PrP in the brain (Figure 13). At matched dose levels TfR1 CapX derived vector genomes were less abundant, but Prnp knockdown was more potent in the brain with TfR1 CapX compared to AAV-PHP.eB, suggesting greater potency per vg of TfR1 CapX.

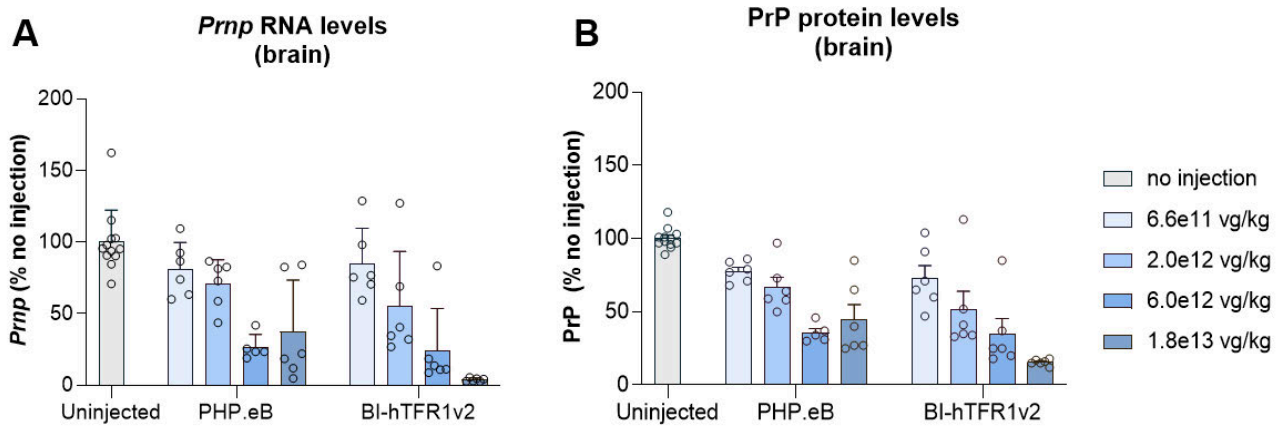


Figure 13. Comparison of CHARM-mediated *Prnp* silencing delivered with AAV-PHP.eB or TfR1 CapX. *hTFRC* KI mice were injected with 6.6e11 vg/kg, 2.0e12 vg/kg, 6.0e12 vg/kg, or 1.8e13 vg/kg AAV-PHP.eB or AAV-TfR1 CapX at 8-33.5 weeks of age. Whole brain hemispheres were analyzed for (**A**) *Prnp* RNA by RT-qPCR (housekeeping gene TBP) and (**B**) PrP protein levels by ELISA at 6 weeks post-dose.

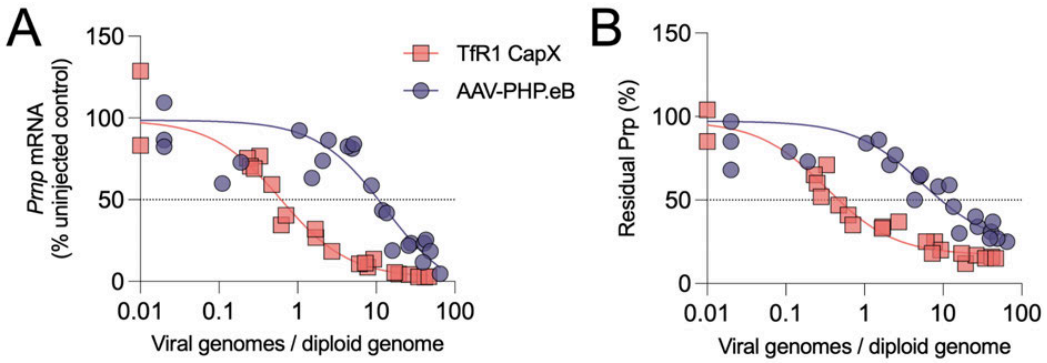


Figure 14. Brain *Prnp* silencing as a function of viral genomes/diploid genomes. *hTFRC* KI mice were injected with 6.6e11 vg/kg, 2.0e12 vg/kg, 6.0e12 vg/kg, or 1.8e13 vg/kg AAV-PHP.eB or TfR1 CapX at 8-33.5 weeks of age. Whole brain hemispheres were analyzed for (**A**) *Prnp* RNA by RT-qPCR (housekeeping gene TBP) or (**B**) PrP protein (ELISA) vs biodistribution (viral genomes/diploid genome) by qPCR (mouse genomes measured using mouse glucagon) at 6 weeks post-dose. Biodistribution (viral genomes/diploid genome) was measured by qPCR (WPRE for viral genomes, mouse glucagon for mouse genomes) at 6 weeks post-dose.

10.1.3 PRNP-CHARM-001 H3X Study: multi-dose potency and lead selection in fully humanized *TFRC* and *PRNP* mice (*hTFRC KI*;*hPRNP*)

The H3X study was executed in *hTFRC KI*;*hPRNP* mice in which relevant biodistribution and target engagement with our clinical candidate, PRNP-CHARM-001, are enabled by the presence of humanized TfR1 and *PRNP*. The H3X study was conducted at the Broad Institute and carried out with material produced by the Broad Institute. A detailed study report for H3X can be found in Appendix VII. PRNP-CHARM-001, our clinical candidate which combines our human targeting ZF-CHARM lead sequence payload with the TfR1 CapX capsid, was used for all conditions in this study.

The key readout for this study was PrP protein and *PRNP* RNA reduction in the brain across a three or four-point dose response curve of PRNP-CHARM-001, assessed at two timepoints. PRNP-CHARM-001 was compared to a ZF-CHARM construct lacking uORFs and miR122 binding sites, mirroring the payload comparison performed in the V1SION study. PRNP-CHARM-001 showed non-inferior potency across dose levels and timepoints, confirming this payload as our lead. Per our INTERACT feedback, kinetic analysis of CHARM transgene expression at both the 6 and 12 week timepoints is underway and the full results will be included in the IND. The 6 week timepoint represents anticipated peak expression; the 12 week timepoint will inform expression over time.

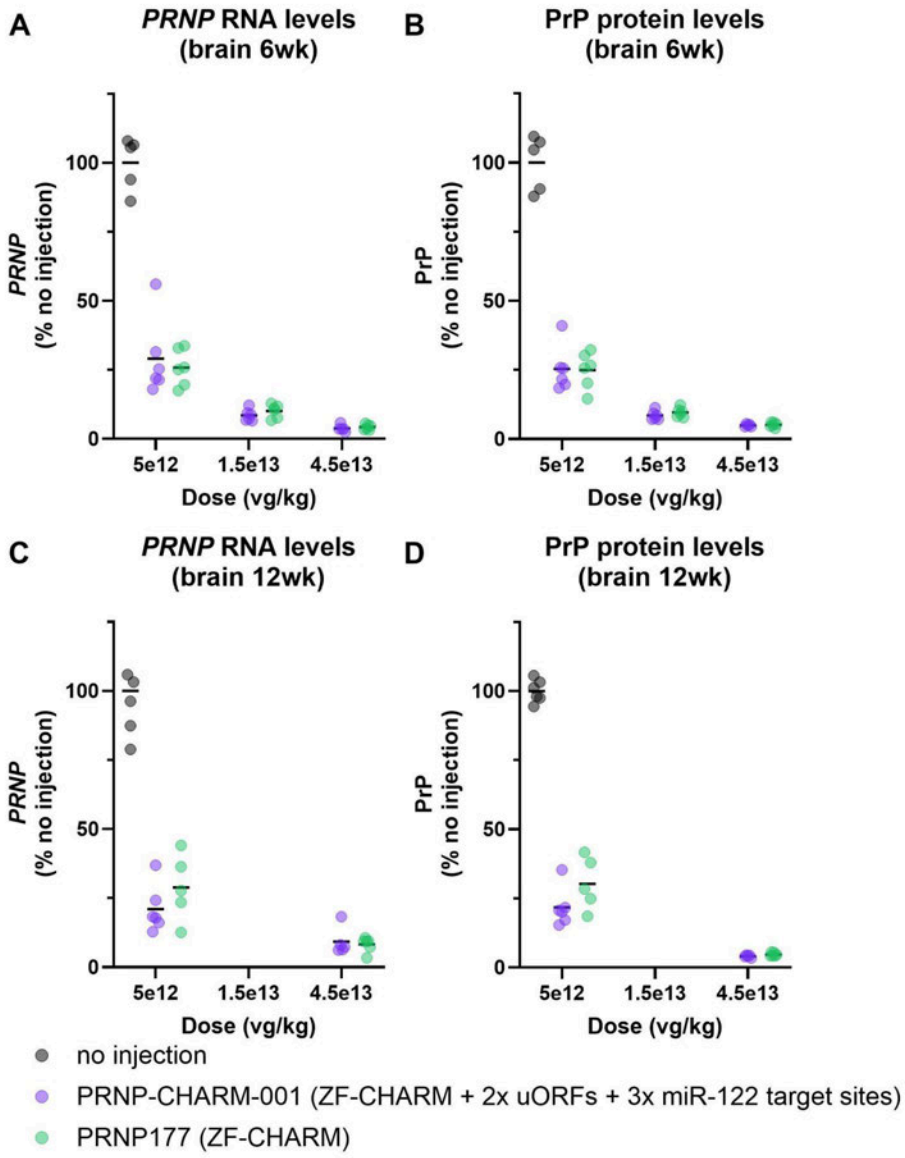


Figure 15. Comparison of candidate constructs in a biologically relevant mouse model. 9-17 week-old *hTFRC KI;hPRNP* mice were injected with 5E12 vg/kg, 1.5E13 vg/kg, or 4.5E13 vg/kg of PRNP-CHARM-001 (purple) or a comparator transgene (ZF-CHARM, green), also packaged in TfR1 CapX. Whole brain hemispheres were analyzed for (**A,C**) *PRNP* RNA by RT-qPCR (normalized to the housekeeping gene *Tbp*) and (**B,D**) PrP protein levels by ELISA at 6 weeks post-dose (**A,B**) or 12 weeks (**C,D**) post-dose. The study was designed to assess the 1.5E13 vg/kg dose at only the 6 week time point. Data points represent samples from individual animals.

10.1.4 PRNP-CHARM-001 BRAV3 study: dose translation and bridging of titers between Viralgen and Broad-produced material

The Pilot Tox and GLP Tox PRNP-CHARM-001 safety and biodistribution assessments were and are being conducted using PRNP-CHARM-001 in *hTFRC* KI mice. While relevant biodistribution is expected, PRNP-CHARM-001 will not achieve target engagement in these studies in the absence of a human *PRNP* allele. The Sponsor has therefore initiated the BRAV3 study to assess potency of PRNP-CHARM-001 in *hTFRC KI*;*hPRNP* mice across all dose levels used in Pilot Tox and GLP Tox at 7 weeks post-dosing. This study is being executed at the Broad Institute and uses PRNP-CHARM-001 test article produced both by the Broad Institute and Viralgen. A detailed study report for BRAV3 can be found in Appendix VIII.

Key readouts for the BRAV3 study will include measurement of *PRNP* mRNA in brain and liver and PrP levels in brain. A summary of the enrolled study groups, dose levels, and group sizes is presented in Table 4, and a more detailed description of this study is provided in Appendix VIII. This study will enable bridging of potency outcomes to biodistribution and safety readouts and will help to inform dose levels for clinical studies of PRNP-CHARM-001.

Table 4. Cohorts in the PRNP-CHARM-001 dose translation and titer bridging study

Group	Treatment	Manufacturer	Dose (vg/kg)	Total mice	Female mice	Male mice
1	N/A (untreated)	N/A	N/A	5	3	2
2	PRNP176	Broad Institute	5.0E12	5	4	1
3	PRNP176	Broad Institute	1.5E13	5	4	1
4	R-792	Viralgen	1.67E12	5	3	2
5	R-792	Viralgen	5.0E12	5	3	2
6	R-792	Viralgen	1.5E13	5	3	2
7	R-792	Viralgen	4.5E13	5	3	2

10.1.5 PRNP-CHARM-001 Safety and Biodistribution Study (Pilot tox): dose-range finding and risk mitigation for GLP toxicology study

The Pilot Tox study was executed in *hTFRC* KI mice to evaluate the biodistribution and safety of the PRNP-CHARM-001 product. The study is being executed at Labcorp (Madison, WI), a GLP-compliant preclinical contract research organization (CRO) with expertise in conducting *in vivo* nonclinical studies, including for AAV-based gene therapies. The study was performed using a research grade batch of PRNP-CHARM-001 (R-792) produced and titered at Viralgen. The in-life portion of this study is complete (March-May 2026) following scheduled animal takedown at 1- and 3-month timepoints.

As discussed in our INTERACT meeting, *hTFRC* KI mice were chosen for this study based on the relevant biodistribution of TfR1 CapX. This model benefits from characterization across multiple independent studies and sponsors, and commercial availability to support procurement of large, sex-balanced cohorts within a tight age range. The *hTFRC KI*;*hPRNP* model used in the H3X and BRAV3 studies is a novel model maintained at a small scale in an academic setting; it is appropriate in a research setting but has to date not been used or characterized beyond our laboratory. For these reasons we deemed the *hTFRC* KI mice most appropriate for our Pilot Tox and GLP Tox studies. This multi-dose study was structured as follows:

Table 5. Cohorts in the PRNP-CHARM-001 Safety and Biodistribution Study

Group	Treatment	Dose (vg/kg)	Dose Day 1	Necropsy Day 29-30	Necropsy Day 92-93
1 (Tox)	Vehicle	N/A	8M/8F	4M/4F	4M/4F
2 (Tox)	PRNP-CHARM-001 (R-792)	5.0E12	8M/8F	4M/4F	4M/4F
3 (Tox)	PRNP-CHARM-001 (R-792)	1.5E13	8M/8F	4M/4F	4M/4F
4 (Tox)	PRNP-CHARM-001 (R-792)	4.5E13	8M/8F	4M/4F	4M/4F
4 (Exploratory)	PRNP-CHARM-001 (R-792)	4.5E13	1M	1M	N/A

A detailed version of the executed study protocol, including all tissues collected and ongoing and planned analyses, is provided in Appendix IX. Histology and biodistribution analyses on key tissues are ongoing. For histology, brain, spinal cord (cervical, thoracic, lumbar), liver, heart, spleen, and skeletal muscle will be analyzed for vehicle, high, and mid dose animals at the 3-month necropsy with potential to also analyze low dose tissues if findings are identified in the high and mid dose. For biodistribution analysis, DNA will be extracted from brain (cerebrum, cerebellum, brainstem), spinal cord (cervical, lumbar, thoracic), lung, liver, heart, spleen, and blood and will be analyzed by using a qualified duplex quantitative polymerase chain reaction (qPCR) assay for the detection of the WPRE portion of the PRNP-CHARM-001 payload to measure viral genome abundance and mouse *Tert* to provide a measure of diploid genome content.

Cage-side clinical observations were performed twice daily. No PRNP-CHARM-001-related clinical observations were noted (Appendix X). During routine body weight measurements, two animals from Group 4 were observed to have minor body weight loss starting at Day 43, though the weight loss did not reach a defined humane endpoint. Diet gel was supplemented in all cages on Day 59 and for the remainder of the study to provide further nutritional support for animals with minor body weight loss and to control for the impact of diet gel supplementation on study readouts. Body weights recorded over the course of the study were analyzed to identify any test article-dependent effects on body weight. Accounting for initial baseline weight, treatment with 4.5E13 vg/kg PRNP-CHARM-001 led to reduced body weight gain relative to vehicle-treated animals. Treatment with 1.5E13 vg/kg and 5.0E12

vg/kg PRNP-CHARM-001 did not result in any reduction in body weight gain relative to vehicle-treated animals.

All animals survived to the end of the study and weights stabilized following provision of diet gel packs.

10.1.6 GLP PRNP-CHARM-001 Safety and Biodistribution Study (GLP toxicology study): definitive GLP toxicology study of PRNP-CHARM-001

The GLP Tox study will be executed in *hTFRC* KI mice to evaluate the biodistribution and safety of the PRNP-CHARM-001 product in a GLP setting. As above, this study is being executed at Labcorp (Madison, WI). This study will be performed using a 50L toxicology batch of PRNP-CHARM-001 produced and titered at Viralgen. Mirroring our Pilot Tox study design, takedowns are planned for 1 and 3 months post-dose.

We have incorporated learnings from the Pilot Tox non-GLP safety study into our GLP study design, including increasing the number of animals per group to obtain sufficient power for the planned panel of clinical chemistry and hematology assessments and improving randomization and assignment of animals to groups based on starting body weight. Additionally, we have shifted the range of dose levels evaluated downward to a range of 1.7E12 to 1.5E13 vg/kg based on observations of reduced weight gain in the 4.5E13 vg/kg cohort in the non-GLP study and to better bracket the anticipated clinical dose levels of PRNP-CHARM-001.

This multi-dose study is structured as follows:

Table 6. Planned cohorts in the GLP PRNP-CHARM-001 Safety and Biodistribution Study

Group	Treatment	Dose (vg/kg)	Dose Day 1	Necropsy Day 29-30	Necropsy Day 92-93
1	Vehicle	N/A	16M/16F	8M/8F	8M/8F
2	PRNP-CHARM-001	1.7E12	16M/16F	8M/8F	8M/8F
3	PRNP-CHARM-001	5.0E12	16M/16F	8M/8F	8M/8F
4	PRNP-CHARM-001	1.5E13	16M/16F	8M/8F	8M/8F

A detailed version of the draft study protocol, including plans for tissue collection and analysis, is provided in Appendix XI.

For histology, brain, spinal cord (cervical, thoracic, lumbar), DRGs, liver, heart, spleen, and skeletal muscle will be analyzed for vehicle, high, and mid dose animals at the 3-month necropsy with potential to also analyze low dose tissues if findings are identified in the high and mid dose groups. For biodistribution analysis, DNA will be extracted from brain (cerebrum, cerebellum, brainstem), spinal cord (cervical, thoracic, lumbar), DRGs, lung, liver, heart, spleen, and blood and will be analyzed by using a qualified duplex quantitative polymerase chain reaction (qPCR) assay for the detection of the WPRE portion of the

PRNP-CHARM-001 payload to measure viral genome abundance and mouse *Tert* to provide a measure of diploid genome content. Additionally, adrenal gland, kidney, lung, gonads (ovary or testis), and injection site will be collected and may be analyzed if needed.

Cage-side clinical observations will be performed twice daily. Detailed clinical observations will be performed weekly.

The sufficiency of this study design is the subject of Question and Sponsor Position 3.

10.2 Off-target analysis of PRNP-CHARM-001

In alignment with recent regulatory guidance for gene editing products, "Safety Assessment of Genome Editing in Human Gene Therapy Products Using Next-Generation Sequencing," we have executed multiple *in silico* and *in vitro* studies to assess potential off-target impacts of PRNP-CHARM-001 activity on gene expression.

PRNP-CHARM-001 demonstrated a high degree of target specificity in our off-target assessment. Across two orthogonal analyses, *PRNP* was the only gene identified as a candidate off-target. We applied (i) *in silico* identification of genes proximal to predicted CHARM zinc-finger binding sites and (ii) a dose-responsive RNA-seq screen across a panel of human cell lines representing the intended central nervous system target and potential off-target liver and muscle tissues, transduced over a range of MOIs sufficient to estimate dose-response curves. RNA-seq served as the primary functional readout because a reduction in gene expression is the direct, safety-relevant consequence of both the DNA methylation and KRAB-mediated repression through which CHARM silences its target. *PRNP* was the only gene that demonstrated consistent dose-responsive downregulation across the cell lines tested, and the only gene with a perfect, gene-proximal match to the CHARM zinc-finger target sequence.

On the basis of this risk assessment, we consider the off-target activity profile of PRNP-CHARM-001 reasonable to support the proposed clinical plan. Detailed methods, results, and supporting figures and tables are presented in Section 10.2 and in the *in vitro* off-target analyses draft study report (Appendix XII).

10.2.1 PRNP-CHARM-001 *In silico* Off-Target Analysis

To nominate candidate human off-target sites, we first conducted an *in silico* assessment of the human genome. The zinc finger (ZF) DNA-binding domain of PRNP-CHARM-001 recognizes a 19-bp target sequence (5′-CTCGGTCGTGAGNAGAGGA-3′) situated within a CpG island proximal to the *PRNP* transcription start site.

Genomic regions with the potential to recruit CHARM were identified by searching this sequence against the human reference genome (GRCh38) using two complementary bioinformatic tools: Bowtie 1, to enumerate exact and near-exact ungapped matches (≤3 mismatches), and BLASTN, to detect shorter or lower-stringency local matches.

Predicted binding sites were intersected with GENCODE gene annotations within a ±10 kb window to generate a sequence-similarity candidate gene list. *PRNP* was the only gene

identified with a perfect-match binding site proximal to a gene body, and was the sole gene nominated by both alignment tools.

The full list of predicted binding sites and proximal genes, along with specific bioinformatic tool versions and command-line parameters, is provided in Appendix XII.

10.2.2 PRNP-CHARM-001 Off-Target Analysis in Human Cells

Off-target analysis was also performed in multiple relevant human cell lines based on several criteria: 1) relevance for expected TfR1 CapX distribution, 2) transducibility by TfR1 CapX, and 3) confirmation of expression of *PRNP*, *DNMT3A*, and *TFRC*. The set of cell lines evaluated for analysis suitability is listed in Table 7. Detailed characterization of off-target analysis suitability for each cell line can be found in Appendix XII.

Table 7. Human cell lines chosen for PRNP-CHARM-001 off-target analysis.

Cell line	Tissue	Description	Source
hCMEC/D3	Brain	Immortalized human brain endothelial cells	Sigma
SVG p12	Brain	Immortalized human fetal glial cells	ATCC
LN-229	Brain	Human glioblastoma cells	ATCC
THLE-2	Liver	Human epithelial cells from the left lobe of a donor	ATCC
Immortalized skeletal muscle cells	Muscle	Immortalized human skeletal muscle cells	ABM
HEK293	Kidney	Human embryonic kidney cells	ATCC

PRNP-CHARM-001 exposure

Cells were treated with PRNP-CHARM-001 and differentially expressed transcripts were assessed by RNA-seq. PRNP-CHARM-001 was applied across a range of concentrations bracketing the IC50 for *PRNP* silencing. The timepoint was chosen to correspond to full target suppression. A full list of the RNA-seq samples sequenced and their associated read depth, mapping rate, and any failures in meeting acceptance criteria for inclusion in analyses is provided in Appendix XIV.

RNA-seq and off-target analysis

Potential off-target genes were identified based on dose-responsive downregulation mirroring the *PRNP* on-target effect, utilizing a three-component Bayesian mixture model.

Across the human cell lines evaluated, *PRNP* was the sole gene to exhibit consistent dose-dependent repression. To assess the functional relevance of predicted recruitment, *in*

silico candidate genes (Section 10.2.1) were cross-referenced with the RNA-seq dataset: among those with predicted zinc finger binding sites, only *PRNP* demonstrated a corresponding change in expression, whereas other sequence-similarity matches showed no detectable response. This indicates that sequence-based prediction alone over-estimates potential off-target activity, and underscores the necessity of functional readouts to differentiate hazard-relevant silencing from predicted binding without biological impact. The alignment of these orthogonal methodologies on a single gene—*PRNP*—confirms the high degree of target specificity of PRNP-CHARM-001. A complete description of the bioinformatic tools and parameters used in these analyses is detailed in Appendix XII.

10.2.3 PRNP-CHARM-001 LOTAS: Long-term *in vivo* Off-Target Accumulation Study

It is the Sponsor's understanding that an off-target accumulation assessment is an important component of the off-target assessments for an epigenetic editor. This understanding is based on Section VI-G of draft guidance 4-2026 *Safety Assessment of Genome Editing in Human Gene Therapy Products Using Next-Generation Sequencing*. Based on verbal communications with Agency representatives it is our understanding that this assessment should be made *in vivo*.

To this end, the Sponsor has designed a study to examine *in vivo* off-target activity of PRNP-CHARM-001 over an extended in-life duration after dosing in *hTFRC KI*;*hPRNP* mice capable of demonstrating target engagement and potency of PRNP-CHARM-001. The study will be executed by the Broad Institute and carried out with PRNP-CHARM-001 material produced and titered at Viralgen. This multi-dose study is structured as follows:

Table 8. Planned cohorts in the PRNP-CHARM-001 Long-term Off-Target Accumulation Study

Group	Treatment	Dose (vg/kg)	Dose Day 1	Necropsy Day 30	Necropsy Day 180
1	Vehicle	N/A	6M/6F	3M/3F	3M/3F
2	PRNP-CHARM-001	5.0E12	6M/6F	3M/3F	3M/3F
3	PRNP-CHARM-001	1.5E13	6M/6F	3M/3F	3M/3F

Below is a high-level summary of the study.

- **Animal model:** *hTFRC KI*;*hPRNP* mice, 5-12 weeks of age; 36 total mice will be dosed.
- **Test article:** PRNP-CHARM-001 or vehicle (AAV formulation buffer).
- **Formulation***:* Test article will be thawed on the day of dosing and diluted as needed using AAV formulation buffer.
- **Dose route/frequency:** Test articles will be diluted in formulation buffer at room temperature and will be administered via intravenous retro-orbital injection on day 1.
- **Cage-side clinical observation:** Animals will be observed daily for morbidity, mortality, and injury. Any animals in poor health will be identified for further monitoring.
- **Body weight measurements:** Prior to dosing on Day 1, and weekly.

- **Blood collection:** Blood will be collected via facial vein bleed 1 week (+/- 2d) prior to necropsy.
- **Necropsy:** Mice will be euthanized on Day 30 and 180 according to their cohort enrollment. Necropsy will be performed on scheduled and prematurely terminated animals as feasible. Terminal body weight will be recorded. Brain, spinal cord, liver, heart, and spleen will be collected.
- **Off-target analysis by RNA-seq:** RNA-seq will be used to measure gene expression in animals treated with PRNP-CHARM-001 relative to vehicle-treated animals. Potential off-target expression effects of PRNP-CHARM-001 treatment will be identified using an approach similar to that performed on cell lines (Appendix XII)
- **Transgene expression:** Transgene expression will be measured by RT-qPCR for WPRE.

The safety profile for a therapy that modulates gene expression depends on the number of genes differentially expressed, the functional importance of those genes (tumor suppressors, high dependency for viability, etc.), and the magnitude of their differential expression. We will consider the totality of the evidence across these dimensions to provide a holistic assessment of the severity of the transcriptomic effect.

We propose that results from the Day 30 necropsy be considered sufficient to proceed with the symptomatic arm of our clinical protocol considering the limited life expectancy of this population, and that results from both the Day 30 and Day 180 necropsy be fully analyzed before initiating the presymptomatic arm of our clinical protocol.

11. Chemistry, manufacturing and controls

A full description of all contract manufacturers and associated analytical testing organizations will be incorporated in the IND. Table 9 outlines the currently contemplated list of contract manufacturing partners. The Sponsor will establish a Quality Agreement with all such parties as per the recommendations in the November 2016 FDA guidance “Contract Manufacturing Arrangements for Drugs: Quality Agreements.”

11.1 Manufacturing plan and milestones for PRNP-CHARM-001

The Sponsor has selected Viralgen as the manufacturer of PRNP-CHARM-001 drug product. Viralgen is a reputable contract manufacturer with years of experience manufacturing AAV clinical material.

Viralgen, using their platform manufacturing process, has produced representative PRNP-CHARM-001 for critical non-clinical studies at a research scale and at a 50L-scale. Viralgen produced PRNP-CHARM-001 material will be administered in the upcoming PRNP-CHARM-001 GLP Tox safety and biodistribution study. Viralgen will manufacture a 500L-scale batch of clinical material for the PRNP-CHARM-001 program’s Phase I/II Clinical Trial.

Table 9. Manufacturer information

Site name	Site address	Responsibility
Viralgen Vector Core, S.L.U	Paseo Mikeletegi 83, 2ºP, 20009 San Sebastián (Spain)	Drug Substance Manufacturing
Wuxi AppTec	4751 League Island Blvd, Philadelphia, PA 19112 USA	Pro10™ Master Cell Bank and associated QC testing
Aldevron, Fargo	4055 41st Avenue South Fargo, ND 58104, USA	Helper plasmid manufacturing and testing
TAAV Biomanufacturing Solutions S.L.U	Paseo Mikeletegi 73B, 20009 San Sebastián (Spain)	GOI and RepCap plasmid manufacturing and testing

11.2 Overview of Selected Manufacturer (Provided by Viralgen)

Viralgen Vector Core S.L.U is at the forefront of gene therapy manufacturing, specializing in the production of recombinant adeno-associated virus (rAAV) vectors. Founded in 2017 as a joint venture between AskBio and Columbus Venture Partners, Viralgen aims to meet the increasing demand for large-scale cGMP (current Good Manufacturing Practice) production of gene therapies. The company operates an advanced manufacturing facility in San Sebastián, Spain, which is optimized for efficiency and scalability. By utilizing its proprietary Pro10™ suspension manufacturing platform, Viralgen is able to deliver industry-leading titers and maintain cGMP-certified quality across all AAV serotypes, significantly accelerating the clinical development and commercialization of life-saving genetic medicines.

The facility, authorized under number 6711-E (MIA reference 6711), has received GMP-compliance approval from the Spanish Medicines Agency (AEMPS). This recognition underscores Viralgen's commitment to adhering to the highest standards of quality and safety in the gene therapy sector. Viralgen operates two state-of-the-art facilities: the Clinical Facility (B1), which spans 4,000 m² and includes 1,250 m² of cGMP space with four independent manufacturing suites to produce 250/500L batches, and the Commercial Facility (B2), which covers approximately 28,000 m², featuring nine suites and the capability to produce up to 2000L batches. With a focus on innovative manufacturing processes, Viralgen is equipped to handle production scales from 50L to 2000L, ensuring that it can meet the diverse needs of its clients.

Viralgen's strategic growth has led to the approval of 40 clinical trials utilizing material manufactured by the company, primarily in key regions such as the United States, European Union, United Kingdom, and Canada. This robust pipeline reflects Viralgen's dedication to enhancing access to essential therapies and contributing positively to global health and human welfare. The company’s commitment to innovation, quality, and patient access

positions it as a leader in the gene therapy landscape, dedicated to making a meaningful impact in the lives of those in need.

11.3 Critical Starting Materials

The Sponsor recognizes that the plasmids (Rep/Cap, Helper, and Payload) used to manufacture PRNP-CHARM-001 are considered to be critical starting materials. Plasmids used for the manufacture of clinical lots of PRNP-CHARM-001 will be produced as "GMP-like" plasmid grade and not make use of research grade material. Plasmid production in support of clinical PRNP-CHARM-001 manufacturing will be managed by Viralgen.

In the IND, the Sponsor will incorporate the following information regarding the plasmids:

- Specifications for the plasmids and their associated cell banks
- Certificates of Analysis for plasmid lots used in the clinical manufacture of PRNP-CHARM-001 drug product
- Description of the manufacturing process and procedures (including those employed to avoid cross-contamination from other plasmid lots)
- A description of raw materials and equipment

The Sponsor recognizes that the HEK293 cells employed by Viralgen (Pro10™) to manufacture PRNP-CHARM-001 are considered to be a critical starting material. In the IND, the Sponsor will provide details on the applicable cell banks.

11.4 General description of Viralgen drug substance (DS) manufacturing process and process controls

Recombinant AAV vectors are manufactured at Viralgen Vector Core S.L.U, located in San Sebastian, Spain with authorization number 6711-E (MIA reference 6711). The Viralgen facility has been approved for GMP-compliance by the Spanish Medicines Agency (AEMPS, Agencia Española de Medicamentos y Productos Sanitarios).

The name, address, and responsibility of the manufacturers of PRNP-CHARM-001 Drug Substance is provided in Table 9. Contract laboratories involved in testing of the DS will be provided in the IND.

The manufacture of Drug Substance uses a process based on the triple plasmid transient transfection of a suspension HEK293 cell line, Pro10™ cell line. The upstream process is initiated with the thaw of a single vial of the Pro10™ master cell bank (MCB), and expansion in a series of single use shake flasks to produce sufficient cells to seed a wave bioreactor at the 50L scale, and sequentially to a 250L or 500L single-use production bioreactor (SUB).

After expansion of the cells to the target production volume, cells are transfected with a cocktail consisting of i) helper plasmid that provides Adenovirus helper proteins for efficient replication and packaging of the vector, but lacks essential Adenoviral structural and

replication genes to generate an Adenovirus, ii) AAV rep-cap plasmid (packaging construct) containing the AAV2 rep gene and TfR1 CapX capsid (cap) protein gene and iii) the therapeutic transgene encoding plasmid containing the PRNP-CHARM-001 expression cassette mixed with PEI Max transfection reagent. Cells are harvested approximately 72 hours post-transfection. Products used for cell culture are chemically defined, and do not utilize materials of animal-origin in either the production or purification processes.

The downstream purification process involves chemical lysis to release the viral vector from the transfected cells. Cellular debris is clarified by filtration and recombinant Adeno-Associated vectors (rAAV) particles are further captured by affinity chromatography.

Full capsids are selected from empty capsids by iodixanol gradient centrifugation. rAAV separates from other cellular components based on its specific coefficient of sedimentation, between the 40% and 60% iodixanol fractions. Purified Bulk Virus from the Iodixanol centrifugation step is further polished and concentrated using a quaternary amine coated chromatography resin (anion exchange column). Contaminant proteins that do not bind to the column are removed in the flow through and from column wash steps.

The column eluate containing purified rAAV vector is concentrated or diluted to the target concentration (if required) and diafiltered against Formulation Buffer. After the Tangential Flow Filtration (TFF) step, the product obtained, called TFF eluate, is filtered through a 0.2 micrometer filter. After obtaining the titer, the following step is the dilution of the TFF eluate to the final desired concentration leading to the obtention of Drug Substance (DS). A general flow diagram of the process is represented in Figure 16.

The Drug Substance is sampled for QC testing before the final filtration and filling. The Drug Substance is not stored prior to final sterile filtration. Following sampling, the Drug Substance is immediately filtered through a double sterilizing grade filter and filled in the final container closure as Drug Product (DP) as a continuous process described in Section 11.6.

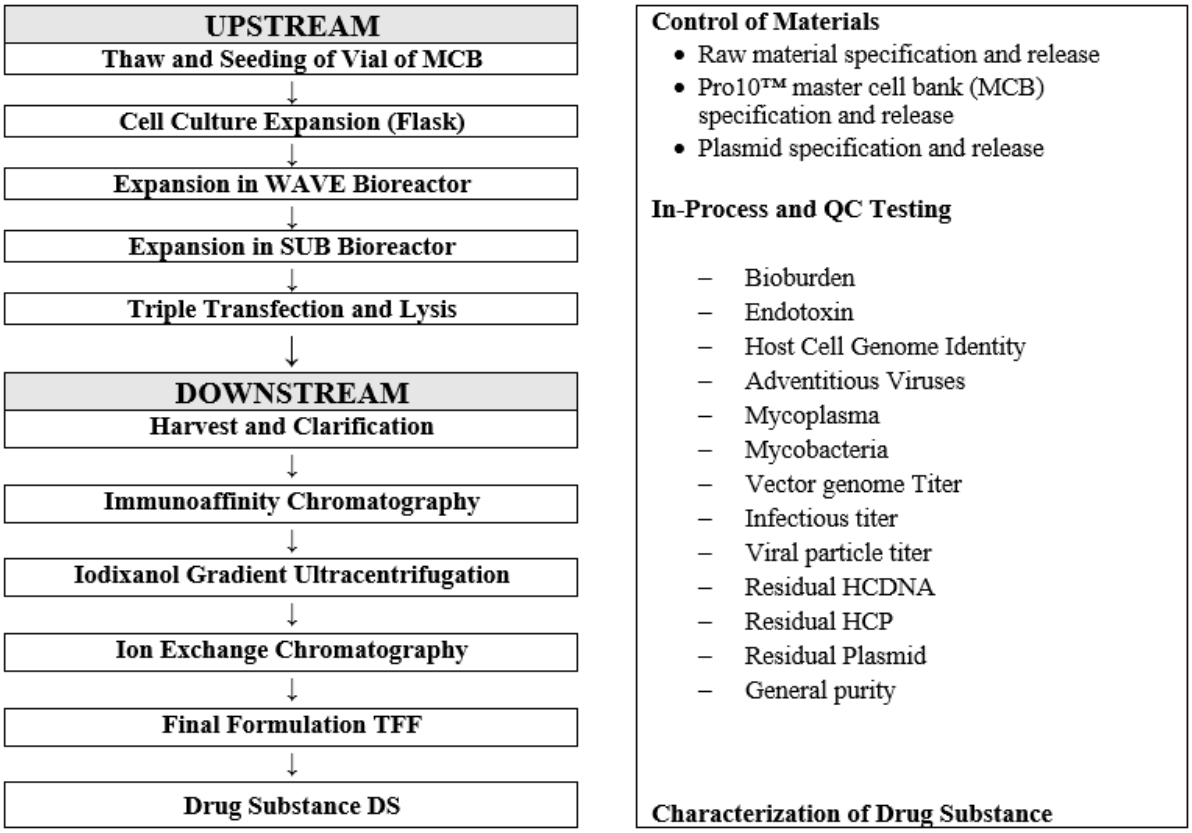


Figure 16. General Flow Diagram of the Viralgen Manufacturing Process

11.5 Draft drug substance (DS) testing plan

The draft quality attributes and the analytical methods to be assessed for GMP DS are provided in Table 10. Specifications for the prospective GMP batch of PRNP-CHARM-001 Drug Substance to be used in the clinical trial will be provided when available. In the IND, the Sponsor intends to propose preliminary acceptance criteria based on our collective experience manufacturing experience with PRNP-CHARM-001 and Viralgen's experience with their platform manufacturing process and TfR1 CapX.

Table 10. Draft drug substance testing plan

Quality attribute	Assay method	Expected values
SAFETY ASSAY		
Replication-competent AAV	Infection of permissive cell line/ qPCR	Report result
Mycoplasma*	Cell culture and indicator cells Ph. Eur. 2.6.7; USP<63>	Not detected
Mycobacteria*	Mycobacterium DNA qPCR	Not detected
Adventitious virus*	In vitro cell assay 3 cell lines	No cytopathic effect No haemagglutination No haemadsorption
Bioburden (CFU/mL)*	Surface spread	<10 TAMC <10 TYMC

Quality attribute	Assay method	Expected values
Bioburden (CFU/10mL)	Membrane filtration Ph. Eur. 2.6.12; USP<61>	≤1 TAMC
STRENGTH ASSAYS		
Vector genome titer (vg/mL)	ITR ddPCR	1.7E13-3.8E13
PURITY ASSAYS		
General purity	SDS-PAGE / Silver Stain	VP1, VP2 and VP3 are predominant bands
Residual Host Cell Protein (ng/mL)	Immunoassay method	Report result
Residual Host Cell DNA-18S (pg/mL)	Quantitative PCR of 18S. 2 Amplicons: 123 & 254bp	Report result
Residual Host Cell DNA E1A (pg/mL)	E1A ddPCR	Report result
Residual Plasmid DNA (copies/mL)	kanR ddPCR	Report result
Full/Empty Vector Particles %	CryoTEM	Report result
Residual chemicals (transfection reagent) (ppm, µg/mL)	HPLC	Report result
Residual chemicals (cell lysis reagent) (ppm, µg/mL)	HPLC	Report result
Residual chemicals (clarifying reagent) (ppm, µg/mL)	HPLC	Report result
Residual chemicals (Iodixanol) (ppm, µg/mL)	HPLC	Report result
Residual chemicals (Antifoam) (ppm, µg/mL)	ICP-OES	Report result
Residual immunoaffinity ligand (ng/mL)	ELISA	Report result
IDENTITY ASSAYS		
Genome identity	Sanger Sequencing	100% conform to sequence of reference
Capsid Protein Identity	SDS-PAGE/Western Blot	Detection of VP1, VP2 and VP3

* Mycoplasma, mycobacteria, adventitious virus and bioburden tests are performed on Transfection Pool step (harvest before lysis).

11.6 General Description of Viralgen Drug Product (DP) Manufacturing Process and Process Controls

The name, address, and responsibility of PRNP-CHARM-001 Drug Product (DP) manufacturer is provided in Table 11. Contract laboratories involved in testing of the DP will be provided in the IND.

Table 11. Name, address, and responsibility for the drug product manufacture

Site name	Site address	Establishment Function
Viralgen Vector Core, S.L.U	Building 1 Paseo Mikeletegi 83, 20009 San Sebastián (Spain) Or Building 2 Paseo Mikeletegi 8, 20009 San Sebastián (Spain)	GMP Drug Product fill & Finish, testing and release activities.

The formulated Drug Substance is 0.2µm sterile filtered and filled as PRNP-CHARM-001 Drug Product vials. One lot of Drug Substance is processed as one lot of Drug Product. The process is monitored through in-process testing and the quality of the Drug Product is verified at release testing. The flow chart of the drug product manufacturing process is presented in Figure 17.

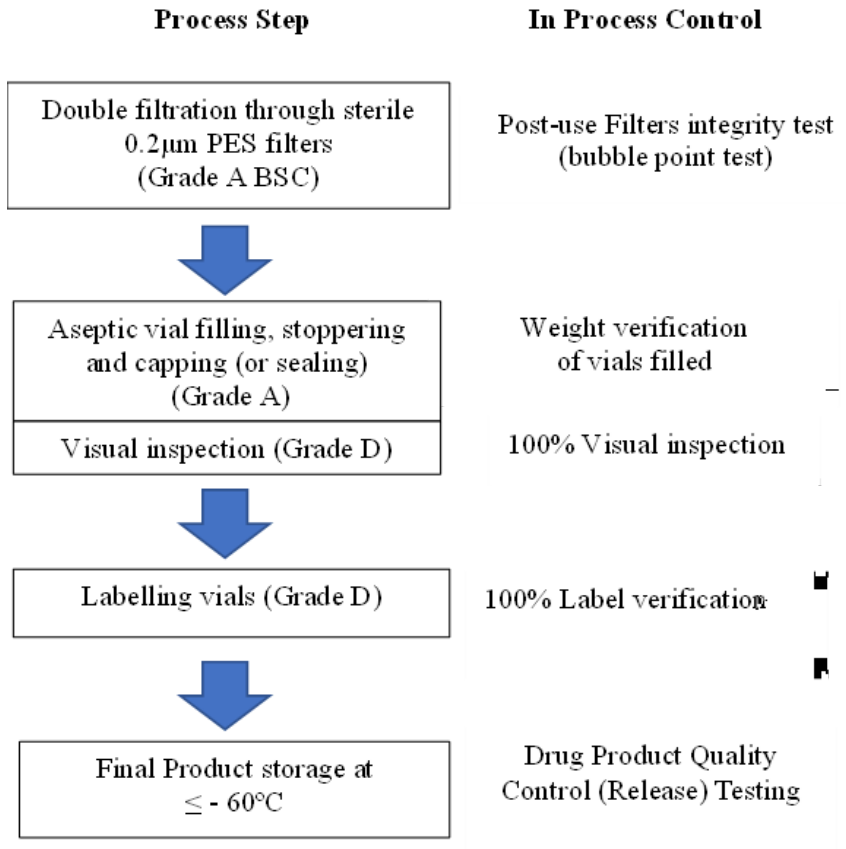


Figure 17. Process Flow Diagram for Formulation, Filling and Labelling.

11.7 Draft Viralgen Drug Product (DP) Testing Plan

The draft quality attributes and the analytical methods to be assessed for GMP PRNP-CHARM-001 DP are provided in Table 12. Specifications for the prospective GMP batch of PRNP-CHARM-001 DP to be used in the clinical trial will be provided when available. In the IND, the Sponsor intends to propose preliminary acceptance criteria based on our collective experience manufacturing experience with PRNP-CHARM-001 and Viralgen's experience with their platform manufacturing process and TfR1 CapX.

Table 12. Draft drug product testing plan

Quality attribute	Assay method	Expected values
SAFETY ASSAYS		
Sterility	Direct Inoculation	Absence of microbial growth
Endotoxin (EU/mL)	Kinetic Chromogenic	≤ 0.500
Appearance (Coloration and Clarity)	Visual Inspection	Colorless, clear to slightly opalescent
Appearance (Visible Particles)	Visual Inspection	Practically/essentially free of visible particles
Container Closure Integrity Test	Dye Ingress	No dye ingress
Subvisible Particles	Light Obscuration Particle Count Test. Test 1B USP<787>	≤6000 particles ≥10µm ≤600 particles ≥25µm
STRENGTH ASSAYS		
Vector genome titer (vg/mL)	ITR ddPCR	1.7E13-3.8E13
Vector genome titer (vg/mL)	GOI ddPCR	Report result
Infectious particles titer (TCID50/mL)	TCID50	Report result
Vector Genome and Infectious Particles Ratio	TCID50 + ddPCR	Report result
Biological Activity Assay / Potency Assay	RT-qPCR Quantification *PRNP* mRNA (Section 11.9)	To be determined
GENERAL QUALITY ASSAYS		
Osmolality (mOsm/KgH_2O)	Freezing point Depression Ph. Eur. 2.2.35; USP<785>	423 ± 30
pH	Potentiometry Ph. Eur. 2.2.3; USP<791>	8.0 ± 0.5
Particle size distribution	Dynamic Light Scattering	Report result
Packaging (Primary Container)	Visual Inspection	Clear and readable labels. Container without breakage or leakage
Extractable Volume	Extractable Volume Ph. Eur. 2.9.17; USP<697>	Not less than the nominal volume
Poloxamer 188 (ppm, µg/mL)	HPLC	20.0 – 100.0

11.8 Stability Plan

The PRNP-CHARM-001 product manufactured in the 50L batch (batch no. T-POLARIS-123), which was vialed on May 21, 2026, is currently being evaluated in a stability study. Stability timepoints are 3, 6, 12, 18, and 24 months post-vialing. Table 13 describes the quality attributes to be tested at each time point:

Table 13. Stability testing plan for T-POLARIS-123 batch

Quality Attribute	3M	6M	12M	18M	24M
Container Closure Integrity Testing	N/A	N/A	X	N/A	X
Vector Genome Titer by ITR ddPCR (vg/mL)	X	X	X	X	X
Infectious Particles Titer (TCID50/mL)	X	X	X	X	X
Vector Genome and Infectious Particles Ratio	X	X	X	X	X
Capsid Protein Identity (SDS-PAGE/Western blot)	X	X	X	X	X
pH	N/A	X	X	N/A	X
Particles Size Distribution	N/A	X	X	N/A	X
Osmolality (mOsm/kg H_2O)	N/A	X	X	N/A	X
PRNP mRNA RT-qPCR Assay Biological Activity Assay*	X	X	X	X	X

*Intended biological activity assay described in Section 11.9

A stability plan for clinical PRNP-CHARM-001drug product will be included in the future IND submission and follows an analogous format.

11.9 Potency Assay

The Sponsor confirms that the intended PRNP-CHARM-001 potency assay will be the RT-qPCR-based quantification of *PRNP* RNA in human HEK293T cells described in the INTERACT. This assay directly measures the biological endpoint most closely tied to PRNP-CHARM-001's mechanism of action: epigenetic and transcriptional silencing of *PRNP*, the molecular target of the therapy. Per our INTERACT feedback, product potency will be quantified as a percentage of the potency of a reference lot that will be assessed in parallel.

Briefly, HEK293T cells were selected as the assay cell line because they robustly express *PRNP* mRNA, express *TFRC* enabling efficient transduction by TfR1 CapX, and express the endogenous *DNMT3A* methyltransferase required for the CHARM silencing mechanism.

Cells were seeded in a 96-well plate by reverse transduction with serial two-fold dilutions of PRNP-CHARM-001 across a six-point MOI range (500 to 128,000 vg/cell), together with a no-CHARM control AAV (an AAV carrying a non-coding stuffer sequence packaged in TfR1 CapX) to control for non-specific effects of AAV transduction. After 72 hours of incubation at 37°C, cells are lysed and total RNA is analyzed by one-step RT-qPCR using TaqMan assays targeting *PRNP* mRNA (FAM) and the housekeeping gene TBP (VIC). *PRNP* mRNA levels are expressed as residual *PRNP* RNA normalized to *TBP* RNA.

Pilot studies conducted internally at the Broad Institute across three independent assay runs demonstrate consistent, dose-dependent *PRNP* silencing by PRNP-CHARM-001 (Figure 18). At the highest MOI tested (128,000 vg/cell), residual *PRNP* mRNA was reduced to approximately 1–2% of baseline (>98% knockdown). *PRNP* silencing was dose-dependent across all tested MOI levels. The no-CHARM control produced no reduction in *PRNP* mRNA at any dose, confirming the specificity of the observed silencing for ZF-CHARM activity. In the third assay run, material manufactured by the Broad Institute and by Viralgen produced comparable *PRNP* silencing curves, providing initial evidence supporting the potency bridging of Broad-manufactured and Viralgen-manufactured PRNP-CHARM-001.

The Sponsor will transfer the assay to a qualified contract research organization (CRO) for formal development and qualification as a GMP-compliant potency release assay. Given the rare nature of prion disease, the sponsor intends to utilize this assay to leverage efficacy data from the Phase 1/2 clinical studies to support a future license application. As recommended by the FDA in response to the Sponsor's 2025 INTERACT submission (PTS#PS009720), the qualified assay will report potency as percentage of the activity of a well-characterized reference material assessed in parallel, to improve assay precision. The assay will subsequently be validated to meet the requirements for a biologics license application (BLA) in support of licensure.

In the IND, the Sponsor will provide a detailed description of this functional potency assay describing how the assay is performed and other relevant information such as assay controls and reference materials.

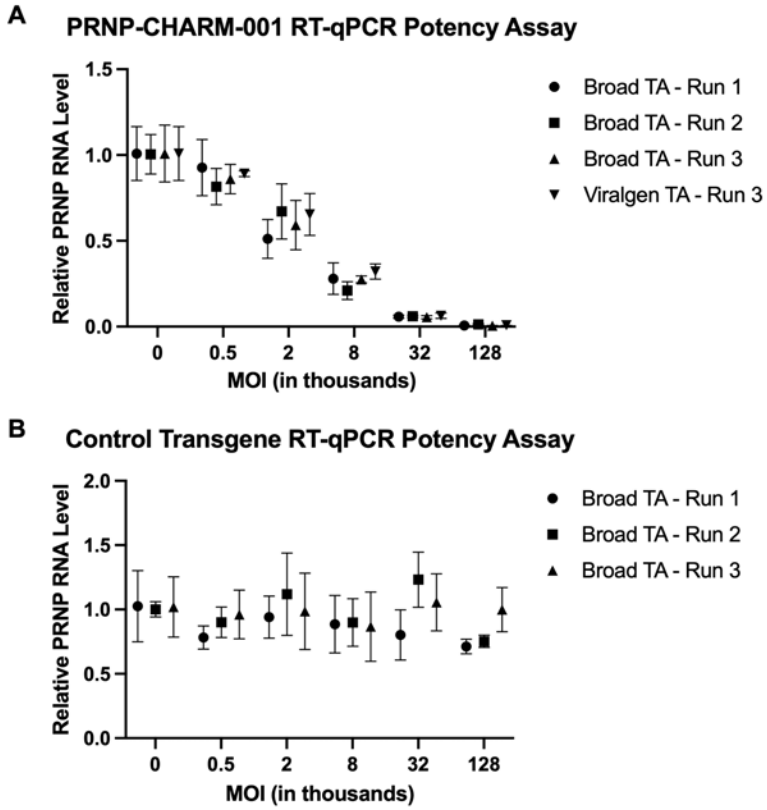


Figure 18. Pilot PRNP-CHARM-001 RT-qPCR Potency Assay. *PRNP* silencing in HEK293T cells transduced with PRNP-CHARM-001 as measured by RT-qPCR. (**A**) or a control AAV lacking CHARM activity (**B**) over a range of multiplicities of infection (MOI). Data points indicate mean relative *PRNP* RNA levels obtained in each assay run and error bars indicate standard deviation.

11.10 Device compatibility

The Sponsor recognizes that delivery-device compatibility must be demonstrated prior to the intended clinical trial. A draft delivery-device compatibility and in-use stability study design for the PRNP-CHARM-001 clinical product is presented in Appendix XIII. As this study has not yet been executed, this pre-IND submission will not include associated data and results from this study. The Sponsor intends to present the completed delivery-device compatibility and in-use stability study protocol, methods, and results in the forthcoming IND submission.

12. Clinical

12.1 Symptomatic and pre-symptomatic patients present opposing clinical opportunities and challenges.

Prion disease is uniformly fatal. There are two distinct patient populations of interest: symptomatic patients and pre-symptomatic individuals at known high risk of fatal genetic prion disease.

Symptomatic prion disease. While all cases of prion disease trace to the misfolding of PrP, most cases appear to occur randomly, and are not presently identifiable before symptoms. Symptomatic patients, whether sporadic or genetic in etiology, typically follow a rapid progression. For the most common prion disease subtype, sporadic CJD, median survival is 5 months from first symptom[82]. During this time, cognitive and functional decline is extremely rapid: most patients lose the ability to complete all basic activities of living such as speaking, walking, eating, and using the toilet, within weeks[83]. Moreover, most patients lose considerable time just seeking a diagnosis — historically prion-specific diagnostic tests were not even ordered until ~3 months into the disease course[84] — though there is some evidence that diagnosis is improving in recent years[85]. In previous randomized trials, patients died a median of ~2 months from randomization[86,87], and many were profoundly impaired well before this time[86].

Given rapid progression, achieving clinical benefit in this population may be challenging. This is particularly true because PrP's *in vivo* half-life of 5 days[43,88,89] means that a PrP-lowering drug will require weeks to achieve full effect, during which time patients will continue to progress. As described in Section 9.1.5, we have observed in mouse studies that treatment at a symptomatic stage is less effective than treating before symptom onset (Figure 4). While factors such as Met/Val heterozygosity at *PRNP* codon 129 in sporadic disease or the pathogenic P102L *PRNP* that causes genetic prion disease can impact rate of progression, trial design in the symptomatic population must anticipate that participants are likely to decline quickly [83].

Pre-symptomatic at-risk carriers. Roughly 15% of cases of prion disease trace to protein-coding variants in *PRNP*. Carriers of such variants can be identified years or decades in advance of symptoms, offering an opportunity to treat before the rapid symptomatic course begins. In recent years, case-control datasets have enabled penetrance estimates for *PRNP* variants to be refined, and individuals with high (often >90%) lifetime risk of fatal prion disease can now be reliably identified. Three pathogenic variants – E200K, D178N and P102L – comprise 85% of high-penetrance genetic prion disease cases[7].

Clinical trials in presymptomatic at-risk individuals offer the opportunity to test PRNP-CHARM-001 not just for its ability to intercept and prolong an ongoing disease process, but for its ability to preserve healthy life. Unlike symptomatic patients, presymptomatic carriers will reliably live to see the drug to reach full target engagement, increasing the potential for benefit. Natural history data show that cross-sectionally, in most carriers available for recruitment there is no sign of a disease process underway nor that initial prion formation has occurred[90]. Only in the presymptomatic population does a

PrP-lowering drug have the opportunity not only to slow prion propagation and neurotoxicity, but to slow or prevent the initial prion formation event, again enhancing the prospect for benefit.

For the purposes of initial study endpoints, pre-symptomatic at-risk individuals provide an unconfounded background against which to assess both safety and target engagement of PRNP-CHARM-001. Characterization of presymptomatic at-risk mutation carriers shows their health status to be normal[90], allowing for more straightforward assessment of safety than in symptomatic patients, where drug-related events must be differentiated from rapidly evolving and multi-faceted symptom progression.

CSF PrP can serve as a pharmacokinetic biomarker in the presymptomatic population

PrP is abundant in CSF and readily quantifiable by ELISA. Longitudinal CSF sampling has shown that CSF PrP levels are stable in presymptomatic genetic prion disease carriers across genotypes (Figure 19), just as they are in healthy controls. This known stability contrasts with symptomatic patients, in whom the kinetics of PrP accumulation into plaques and impact on soluble PrP levels in CSF over time are not clear[91] (Table 14). Meanwhile rat ASO treatment studies show that reduction of prion protein in the brain is dose-dependently mirrored in CSF[35] (Figure 3D), demonstrating the relevance of CSF as a sampling compartment.

Table 14: Utility of CSF PrP measurement in different prion disease patient populations.

Disease stage	Etiology	PrP ELISA useful for target engagement?
Pre-symptomatic	Genetic	Yes, established 10% intra-individual test-retest CV
Symptomatic	Genetic	Likely, but possible confounding from disease-related change. Natural history needs more characterization.
Symptomatic	Sporadic	Likely, but possible confounding from disease-related change. Natural history needs more characterization.

In addition to providing a readout of brain target engagement, CSF PrP may have additional significance in the presymptomatic population. Given that PrP is well established as the single causal molecule in prion disease and as a dose-dependent driver of the disease process, reduction in CSF PrP levels has also been proposed to be predictive of potential clinical benefit analogous to viral load in HIV/AIDS[4]. Indeed, as discussed above under Relevant History Section 8.1.1, we have previously discussed with CDER the potential for CSF PrP lowering to serve as a surrogate endpoint in preventive genetic prion disease trials of PrP-lowering oligonucleotide drugs[4]. In principle such an approach holds equal validity for any therapy that lowers PrP levels in the brain, including PRNP-CHARM-001.

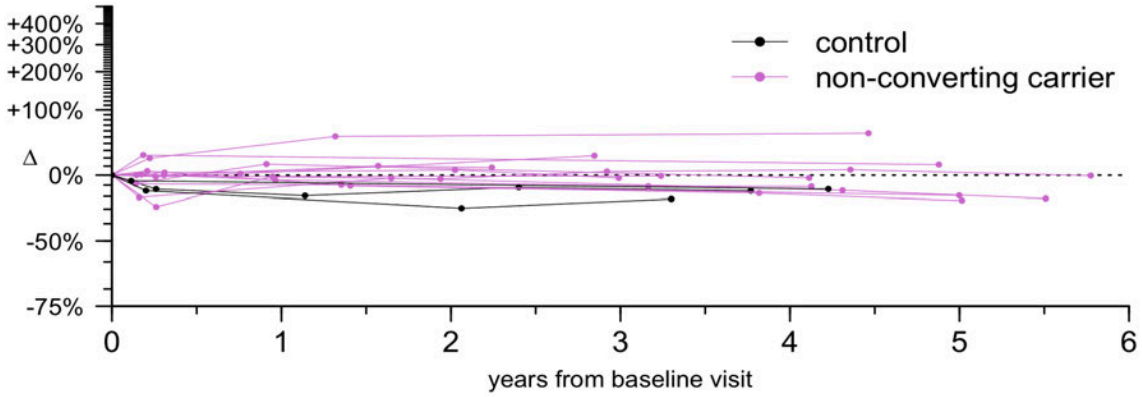


Figure 19. CSF PrP levels are stable in presymptomatic prion disease mutation carriers. Longitudinal CSF PrP levels in presymptomatic genetic prion disease mutation carriers across a range of genotypes, as well as non-mutation controls, show a mean test-retest CV of 10%, demonstrating assay reliability and stable PrP levels in presymptomatic genetic carriers. CSF was drawn roughly annually over up to six years. *Adapted from Vallabh 2024*[92].

12.2 Clinical plan for PRNP-CHARM-001

Proposed clinical study protocols for both the symptomatic prion disease population and presymptomatic high-risk population are overviewed below.

12.2.1 Overview of proposed clinical plan for first-in-human evaluation of PRNP-CHARM-001

We propose to evaluate AAV-PRNP-CHARM-001 in two patient populations. The summary below provides a high-level structural overview, with further detail provided in sections below.

- **Symptomatic patients** diagnosed with probable prion disease.
  - **a. Treatment arm:**
    Objective: To assess safety, target engagement (PrP lowering) and potential to slow disease progression in patients already experiencing prion disease symptoms.
    Eligibility: Probable prion disease by CDC criteria and an MRC Prion Disease Rating Scale score ≥15. (See full description of criteria below.)
    Design: A single ascending dose (SAD) escalation (3 patients per low/mid/high dose cohorts, an additional 6 in the highest tolerated cohort).
  - **b. Observational arm:**
    Objective: To gather comparator data on CSF PrP trajectory and clinical progression of symptomatic prion disease in the absence of treatment. See full justification below.

Eligibility: Probable prion disease by CDC criteria and an MRC Prion Disease Rating Scale score ≥15.
Design: N=15 patients total. Patients will be enrolled in the observational arm when it is not possible to enroll in the symptomatic treatment arm (for example, in pauses for analysis between dose cohorts, or if the patient is excluded from treatment by AAV seropositivity.) When spots become available in the symptomatic treatment arm, they may cross over if eligible.

- **Presymptomatic carriers** of high-penetrance pathogenic *PRNP* variants.
  Objective: To assess safety and target engagement (PrP lowering) in healthy individuals at high risk of genetic prion disease, in a paradigm that precedes irreversible neuronal loss.
  Eligibility: Positive test for a high-penetrance *PRNP* variant (e.g., E200K, D178N, P102L) who have established high lifetime risk of developing the disease. Age ≥40.
  Design: Parallel SAD escalation matching the symptomatic trial design. A single ascending dose (SAD) escalation (3 patients per low/mid/high dose cohorts, an additional 6 in the highest tolerated cohort). For each dose level, N=3 symptomatic patients will be dosed and monitored for a minimum of 10 weeks prior to dosing the first presymptomatic at-risk individual.

The proposed trial design is highly parallel to the design of the ongoing academically led PRiSM trial of PrP-lowering siRNA 2439-exNA (NCT07444580), of which we are also the Sponsor. Key structural similarities are the inclusion of both symptomatic and at-risk patient populations, the listed endpoints for both populations, and the inclusion of a symptomatic observational arm. The eligibility criteria and schedule of assessments also overlap except where gene therapy specific considerations arise.

12.2.2 Choice of study populations

We propose an investigator-initiated trial that encompasses the distinct clinical needs and data opportunities presented by symptomatic prion disease patients and presymptomatic at-risk carriers.

Symptomatic patients present the most urgent medical need, but their rapid decline (median survival of 5 months from symptom onset) makes safety and target engagement data difficult to interpret and limits the benefit that even an effective therapy can achieve. Presymptomatic carriers provide a stable baseline to reliably measure PrP lowering and safety, and offer the opportunity for an effective therapeutic to delay onset and extend healthy life. The complementary profiles of these two populations are outlined below (Table 15).

Table 15. Profiles of symptomatic and presymptomatic prion disease patients.

	Symptomatic patients	**Presymptomatic at-risk individuals**
Safety	Safety assessments complicated by rapid disease progression and limited to overt toxicity and immunogenicity.	As *PRNP* mutation carriers are healthy prior to symptom onset, safety **can be reliably assessed** in detail.
Target engagement	Measurement of drug-associated changes in CSF PrP levels may be complicated by disease-associated changes in CSF PrP levels[93,94], necessitating an untreated comparator arm.	As CSF PrP levels are longitudinally stable in presymptomatic individuals (mean CV = 10% across genotypes over up to six years, Figure 19), drug-associated changes in CSF PrP levels **can be reliably measured**.
Prospect for benefit	Potential benefit is possible, but **limited** by 1) rapid disease progression, 2) the 4-week time to full effect of the drug on the protein level, based on PrP's 5-day *in vivo* half-life[43,88,89], and 3) lack of basis on which to expect reversal of accumulated symptoms caused by neuronal loss.	In presymptomatic carriers the drug will have full time to reach maximal target engagement and the **prospect to extend healthy life.**
Risk vs. benefit	Risks of drug are **reasonable** given this population's imminently fatal diagnosis and the lack of standard of care for prion disease.	Risks of drug are **reasonable** in the context of highly penetrant *PRNP* variants, given the rapid lethality of disease once it strikes. Risks are mitigated by first testing each dose level in the symptomatic population, and by proceeding to pre-symptomatic individuals only upon acceptable safety and target engagement data. Expected permanence of therapy will be offset by specificity of the drug and the non-essentiality of the *PRNP* gene. Eligibility will be restricted to older individuals closer to expected age of disease onset.

Given their distinct opportunities and challenges, symptomatic and presymptomatic treatment are best conceived as two trial arms with coordinated dose timing. We propose to dose symptomatic patients first at each dose level, and monitor for safety prior to dosing the first presymptomatic individual at that dose level (see detailed dose escalation schedule below.) Each arm will enroll N=15 patients as outlined below.

12.3 Study design and rationale

The first clinical study will be a Phase 1/2 open-label, single ascending dose-escalation study designed to evaluate the safety, tolerability, and pharmacodynamic (PD) properties of a single infusion of PRNP-CHARM-001, as well as exploratory clinical and biomarker endpoints in the symptomatic arm of the study.

We propose 3 arms:

- Symptomatic treated
- Symptomatic observational
- Presymptomatic treated

Relationship between symptomatic treatment arm and symptomatic observational arm. In lieu of a randomized, placebo-controlled trial, we will enroll participants in the observational study arm whenever our dose escalation and sentinel criteria do not permit us to enroll patients in the symptomatic treated arm. If the treatment arm re-opens, observational participants who still meet inclusion criteria may have the opportunity to transition to the treatment arm (provided they have completed at least a 4-week follow-up visit in the observational arm). Scientifically, the observational arm will provide a comparator group for longitudinal changes in biomarkers, including CSF PrP, as well as provide useful information on AEs and clinical progression. In particular, this trial relies on lowering of CSF PrP in symptomatic patients as a measurement of PD target engagement. The observational arm will help to control for any changes in CSF PrP that occur as part of the natural history of prion disease.

Rationale for observational study in symptomatic patients. CSF PrP is lower in symptomatic prion disease patients than in patients with non-prion neurological diagnoses, likely due to a disease sink (PrP being incorporated into brain plaques instead of shedding into CSF)[91,93-96]. At present, the kinetics of CSF PrP decline within the natural history of prion disease is not well understood. There is minimal evidence for longitudinal decline in CSF PrP in symptomatic patients[93,94], and most historical samples are uninterpretable due to poor control of pre-analytical variables[91]. The observational arm will therefore allow us to determine whether, and at what rate, CSF PrP declines after prion disease diagnosis, as well as establishing a trajectory of disease-related biomarkers and clinical progression in untreated patients.

Rationale for presymptomatic study arm. See 12.2.2 and Table 15 above for the rationale for including this patient population in our first-in-human study; see 12.2.4.2 and Figure 20 for proposed staging of trial arms to balance risk and benefit for presymptomatic individuals.

12.4 Clinical study draft protocols

12.4.1 Schedule of assessments overview

The study duration for the observational arm will be limited to 8 weeks, making participation in this arm less burdensome for patients for whom no experimental drug is being administered. This arm will include CSF sampling (0, 4 and 8 weeks) and neurological and clinical monitoring, but will not include immunosuppressive therapy or an inpatient period. Gene therapy specific safety labs including seropositivity screening may be performed to determine eligibility to crossover to the treatment arm.

The study duration for all treated participants starting on the day of consent will be up to 51 weeks and will include:

1. Screening period (Day -21 to Day 0)
2. Initiation of immunosuppressive therapy (Day -14)
3. Baseline/Infusion Day (Day 0)
4. Inpatient period: Day 0 - Day 4 with option to extend to one week
5. Blood sampling for safety labs, particularly liver enzymes: weekly at weeks 1-12.
6. CSF sampling for PD biomarker at day 0, week 4, week 8, week 24, and week 48
7. Follow up phone calls or safety visits at week 16, week 20, week 28, week 32, week 36, and week 40.
8. Enrollment to a Long-Term Follow Up (LTFU) study to evaluate long-term safety for an additional 5 years. The LTFU study will enroll participants under a separate protocol.

Table 16: Key aspects of the proposed clinical trial design.

Study population	**Symptomatic prion disease patients**	**Presymptomatic adult carriers of high risk *PRNP* variants**
Eligibility criteria	Probable prion disease by CDC diagnostic criteria[97]	Positive test for high penetrance pathogenic *PRNP* variant: E200K, D178N, P102L, P105L, 5-OPRI, 6-OPRI, A117V, F198S Age ≥40 a) Note: As pathogenic biomarkers do not reliably predict onset of genetic prion disease, they will not determine enrollment.
Dose schedule escalation/ study design	a) Low single dose - 3 patients b) Mid single dose - 3 patients c) High single dose - 3 patients d) Expansion* - 6 patients	a) Low single dose - 3 patients b) Mid single dose - 3 patients c) High single dose - 3 patients d) Expansion* - 6 patients

	Each escalating dose cohort will be gated by an interim safety and PD determination. **Highest dose deemed tolerated based on interim analyses.*	Each escalating dose cohort will be gated by an interim safety and PD determination. **Highest dose deemed tolerated based on interim analyses.*
CSF collection	Goal: target engagement Timepoints relative to dose: at enrollment, pre-dose, 4 weeks, 8 weeks, 24 weeks, and 48 weeks.	Goal: target engagement Timepoints relative to dose: at enrollment, pre-dose, 4 weeks, 8 weeks, 24 weeks, and 48 weeks.
Primary endpoint	Safety/ adverse events	Safety/ adverse events
Secondary endpoints	CSF PrP concentration	CSF PrP concentration
Exploratory endpoints	a) Time to death or initiation of life-extending measures (permanent intubation or ventilation) b) Time to drop 10 points on MRC Prion Disease Rating Scale Pathological biomarkers: ● CSF prion titer ● CSF total tau levels ● Plasma and CSF neurofilament light chain levels	Pathological biomarkers: a) CSF prion titer b) CSF total tau levels c) Plasma and CSF neurofilament light chain levels
Long term follow-up	Safety	a) Safety b) Age of onset of genetic prion disease
Data analysis method	Intention to treat	Intention to treat

Target Engagement endpoint: CSF PrP lowering will be measured as a percentage change relative to individual baseline to control for inter-individual differences in CSF PrP concentration[35]. Animal models show that brain PrP reduction is dose-dependently reflected in the CSF. In presymptomatic humans, CSF PrP is longitudinally stable (mean CV = 10%), providing a reliable readout for drug effect (Figure 19). The natural history of CSF PrP is less well characterized in symptomatic prion disease patients with data indicating that CSF PrP levels may drop as a function of disease state, as disease progresses[93,94]. Data collected from the observational arm of this study will help to distinguish disease and drug related decline in this population.

Survival endpoint. Survival is an exploratory endpoint that will be measured as time to either death *or* life-extending measures, since a substantial amount of variance in time to death in prion disease is due to differences in family decisions around end-of-life care[98,99].

12.4.2 Dose Selection and Administration

a. One-Time Dosing: Because the PRNP-CHARM-001 mechanism acts through stable DNA methylation, a single treatment is expected to result in long-term suppression of the *PRNP* gene. Preclinical data indicate stability of PrP suppression in mice out to one year; continued suppression is expected based both on CHARM's long-term mechanism and on continued expression of CHARM from the AAV genomes that persist in treated cells.

b. Route of administration and dose scaling: PRNP-CHARM-001 will be administered via a one-time **intravenous (IV)** infusion.

We expect that IV dosing of TfR1 CapX in *hTFRC* KI mice will be representative of IV dosing in humans based on the following: (1) The mechanism of transport of the capsid across the BBB (via binding TfR1) is conserved across species. (2) TfR1 protein levels in mouse and human microvasculature are similar. (3) Receptor mediated transcytosis across the vasculature scales with brain size because brain capillaries in both mice and humans are typically no more than 40 μm apart and most neurons are therefore within 20 μm of a capillary[102]. Together, these findings support the expectation that AAV vg/kg dosing in *hTFRC* KI animals will be consistent with the human vg/kg dose. See Sponsor Position 2 for further discussion.

c. Dose finding, starting dose rationale and dose escalation.
We will select proposed low, mid and high doses based on animal studies, with even the low dose at a level predicted to measurably engage target and potentially modify disease. Initial human doses will be selected based on studies performed in humanized mice (Section 10), including: 1) multi-dose potency studies performed in *hTFRC KI;hPRNP* mice performed at Broad (H3X, BRAV3), and 2) biodistribution and toxicology studies in *hTFRC* KI mice performed at LabCorp (Pilot Tox, GLP Tox). Of studies performed at Broad, BRAV3, which contains all dose levels used in the Pilot Tox and GLP Tox studies administered with Viralgen produced and titered material as well as subset of these dose levels administered with Broad produced and titered material, is likely to provide key dose-finding data while also providing the information needed to bridge Viralgen and Broad titers in order to leverage the data from H3X.

Preclinical data show that PrP reduction in the brain is dose-dependently protective against disease, with greater reduction resulting in greater protection and full elimination of PrP resulting in full protection. As described in section 9.1.2, transient ASO-mediated 21% reduction of brain PrP has been shown to correspond to a 15% extension of survival in mice with greater brain PrP reduction corresponding to greater survival benefit (Figure 3). These data suggest that even modest levels of PrP reduction can confer a protective effect, and that

considerations of minimal efficacious dose must take into consideration quantifiability of target engagement as well as potential for efficacy and safety.

Balancing measurability and potential for efficacy with the safety considerations that attend a newly developed engineered AAV capsid, we propose to set our initial dose to correspond to 30% PrP knockdown across the whole brain based on the mouse studies listed above, with planned escalations to target 50% reduction at the mid dose level and 80% reduction at the high dose level pending clinical safety and target engagement data from prior doses. At each dose we will observe a sentinel patient for 8 weeks prior to dosing additional patients.

d. Presymptomatic dose staging. Recognizing the distinct risk-benefit profiles of our two patient populations, at each dose symptomatic patients will be dosed first. Presymptomatic patients will be dosed only beginning 10 weeks after the final symptomatic patient in that same dose cohort has been dosed (18 weeks after the sentinel symptomatic patient; see Figure 20), gated upon analysis of available safety and target engagement data. Unlike symptomatic patients, who are unlikely to survive long enough to be offered a different AAV gene therapy, presymptomatic patients on expectation have years to live, and therefore the opportunity cost of becoming seropositive upon receiving a subclinical dose is also a risk of this trial. To mitigate this risk, we propose to proceed to presymptomatic subjects only with dose level(s) that are supported by acceptable safety data in the symptomatic population *and* appear to confer at least 50% CSF PrP lowering based on available target engagement data from nonclinical studies and available data from the symptomatic population. In making this determination, autopsy data, such as brain parenchyma DNA methylation data, may be used to supplement CSF PrP target engagement data for deceased symptomatic subjects.

12.4.3 Proposed dose schedule

All patients in treatment arms will receive investigational drug. Since there will not be a placebo group, there will be no blinding. The Medical Monitor will review the safety data after each sentinel patient in collaboration with the clinical site PI and overall PI and selected on-call specialists as the data require (see list below) after each dose level is completed. We will analyze available pre-dose, day 0, 4-week and 8-week post-dose CSF samples to determine target engagement. Note that because this is a rapidly progressive disease, some patients will pass away before these follow-up visits and it is not expected that target engagement will be determined in all participants. Participants will be asked to consent to donate their brains for postmortem analysis, for which DNA methylation analysis can also quantify target engagement in deceased individuals. The Medical Monitor will make a determination as to whether to proceed to the next dose and/or to dose presymptomatic patients at the same dose level based on available data including safety and target engagement.

- Criteria for expanding from sentinel to other patients at same dose: safety and 4-week target engagement where available
- Criteria for expanding from symptomatic cohort to presymptomatic cohort at same dose: safety and 4- and 8-week target engagement where available

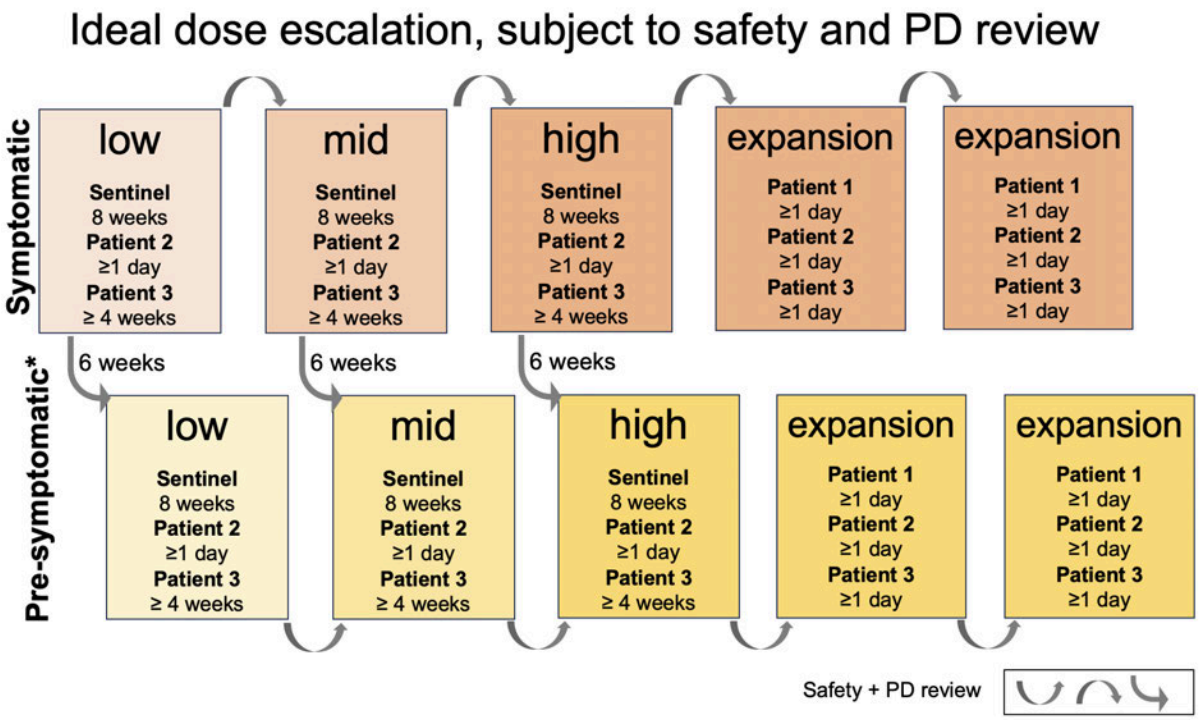


Figure 20. Proposed dose escalation plan for the PRNP-CHARM-001 first-in-human study. *While the presymptomatic trial arm is depicted as escalating through all three dose levels, initiation of this arm will be gated on safety and target engagement data as described above, with the result that in practice this arm may not encompass all dose levels.

12.4.4 Study populations

Inclusion criteria (symptomatic patients; both treatment and observational arms)

1. A diagnosis of probable prion disease according to CDC diagnostic criteria[97].
2. Documented symptoms of prion disease.
3. Age ≥18 at the time of informed consent.
4. MRC-PDRS ≥15 at the time of Screening
5. Provide written informed consent and be able and willing to comply with all study requirements (in the opinion of the Investigator), including travel to the Study Center, procedures, assessments, and visits. If the patient is not capable of providing consent, consent must be given by a legally authorized representative (LAR).
6. Study partner available to assist with study visits, who is sufficiently knowledgeable of the patient's condition to respond to inquiries about the patient for the duration of the trial.

Inclusion criteria (presymptomatic at-risk individuals)

1. A positive DNA test for a highly penetrant pathogenic *PRNP* mutation.
2. Age ≥40 at the time of informed consent
3. Provide written informed consent and be able and willing to comply with all study requirements (in the opinion of the Investigator), including travel to the Study Center, procedures, assessments, and visits.

Exclusion criteria (both populations, all arms)

1. Abnormal baseline labs. Platelet count < 50,000/mm3 , INR>1.5, alanine aminotransferase (ALT) or aspartate aminotransferase (AST) greater than upper limit of normal, estimated glomerular filtration (eGFR) of <60 mL/min/1.73m^2 at screening, or any other clinically significant laboratory abnormality at Screening that, in the opinion of the Investigator, would render a patient unsuitable for inclusion. The Investigator may choose to repeat a laboratory test that they suspect to have failed due to a technical failure, at their own discretion.
2. Abnormal baseline vital sign. Clinically significant vital sign or electrocardiogram (ECG) abnormality at Screening (including heart rate < 45 bpm; systolic blood pressure < 90 mmHg; confirmed blood pressure readings > 170/105 mmHg).
3. Infection. a) Active infection requiring systemic antiviral or antimicrobial therapy that will not be completed prior to Study Day 1. b) Viral infection within the past 30 days, COVID within the past 60 days. c) Known active infection with hepatitis C, hepatitis B or HIV diagnosed by initial serological testing and confirmed with RNA testing, or prior treatment for hepatitis C. Testing for these infections is not included in the screening process; only prior knowledge of these infections, for example, in the patient's medical record, will be considered. Patients positive by serology, but negative by RNA may be deemed eligible by the Investigator in consultation with the MSM.
4. Prior treatment. Participation in another prion disease clinical trial within the past 6 months.
5. Seropositivity. Prior receipt of an AAV gene therapy or anti-AAV antibody levels above a to be defined level. (Note that this exclusion criteria will not apply to the symptomatic observational arm.)
6. Immune confounders. a) Concomitant treatment with immune modulating therapy, b) Immunocompromised/immunodeficient individuals or anyone contraindicated for the immunosuppression paradigm.
7. LP contraindication. a) History of bleeding diathesis or coagulopathy, or current or anticipated use of warfarin, apixaban or other anti-coagulant or anti-platelet medication that cannot be safely held for the appropriate time prior to LP. b) Any contraindication to repeated LPs, including bleeding disorders, brain or spinal diseases or deformities. Examples include but are not limited to mass lesions, obstructive hydrocephalus, ventriculoperitoneal shunt, Chiari malformations, significant spondyloarthropathy and spinal stenosis, significant connective tissue disorders.
8. MRI contraindication. Any contraindication or unwillingness to undergo MRI, e.g., metal implants, claustrophobia, agitation, or motor symptoms that precludes scans) or MRI or CT results that suggest a different diagnosis or that trial participation would be unsafe.
9. Malignancy. Malignancy within 5 years, except for basal or squamous cell carcinoma of the skin or carcinoma in situ of the cervix that has been successfully treated. Patients with a history of other malignancies that have been treated with curative intent and which have no recurrence within 5 years may also be deemed eligible by the Investigator in consultation with the Medical Safety Monitor (MSM).

10. Compliance. Unwillingness to comply with study procedures, including follow-up, as specified by this protocol, or unwillingness to cooperate fully with the Investigator. This includes unwillingness to comply with study contraceptive requirements.
11. Pregnancy. Female patient who is breastfeeding, pregnant or planning a pregnancy.
12. Investigator judgment. Have concurrent non-prion CNS disease or any other condition, which, in the opinion of the Investigator would make the patient unsuitable for inclusion or could interfere with the patient participating in or completing the study.

12.4.5 Safety and tolerability

Safety will be monitored based on adverse event reporting and monitoring laboratory tests (hematology, serum chemistry, lymphocyte count, urinalysis), physical examination, vital sign measurement and neurological examination. In addition, monitoring and response protocols will be developed for safety considerations specific to a systemic AAV gene therapy, outlined at a high level in Table 17. For the FIH study, immediate safety monitoring after administration will include 4 days of in-patient observation with potential to expand to one week.

Below is a preliminary list of baseline assessments, with sub-bullets indicating key assessments for this modality or therapeutic hypothesis.

- Vitals
    - Heart rate
    - Blood pressure
- ECG
- Comprehensive metabolic panel
- Liver function (ALT, AST, bilirubin)
- Kidney function (eGFR)
- CBC (Day -7, Day -3, and Day 0 immediately prior to infusion)
    - Platelet count
    - Complement factors
- MRI
- Blood
    - Anti-capsid antibodies
    - Exploratory biomarkers
- CSF
    - PrP
    - Exploratory biomarkers

We will assemble a safety monitoring committee comprising core members and a team of specialists who will be consulted depending upon presenting symptoms:

- Core members:
    - Medical monitor
    - Clinical PI
    - Overall PI

- On call for safety consultations
    - Hepatologist
    - Immunologist
    - Hematologist
    - Nephrologist
    - Cardiologist
    - Cerebral edema response team

When reporting adverse events, site principal investigators will be asked to note their belief as to whether the AE is likely, possibly, or not drug-related. The medical monitor will have final authority for determining the drug relatedness of AEs based on all available data. Whenever a serious adverse event (SAE) is reported, dosing will be halted until the medical monitor can perform a safety assessment and determine whether dosing should continue. The medical monitor may choose to consult with the on-call specialists listed above. Note that prion disease is a rapidly progressive, fatal disease, and natural history data indicate that even with the inclusion criterion of MRC Score ≥15, approximately half of participants would be expected to die of disease progression within the first 24 weeks of the trial. Thus, SAEs are expected in this trial; decisions to stop dosing will only be made based on determinations of SAE relatedness to the investigational drug.

Participants will be encouraged to donate their brains to the National Prion Disease Pathology Surveillance Center in the event that they pass away during the trial. Because the CpG methylation marks installed by CHARM at the *PRNP* gene promoter are expected to be permanent and are not found in untreated individuals, methylation analysis of genomic DNA from brain tissue of deceased individuals can provide supplementary evidence of target engagement in addition to CSF PrP analyses in living individuals.

12.4.6 Preliminary immunosuppression protocol

Medications, doses and dose ranges are preliminary, and will be specified in our final clinical protocol along with ranges for number of doses/ dosing duration.

- Day -14: initiate Rituximab (2-3 doses totalling 1550 mg/m²)
- Day -7: initiate Sirolimus or Tacrolimus (target trough 4-8 ng/mL)
- Day -1: initiate corticosteroid (0.5 -1 mg/kg)

Table 17. Safety risks and response plans.

Risk	First clinical signs	Biomarkers	Monitoring frequency	Monitoring window	Response
Infusion related reactions (IRR)	Hives, nausea, allergic symptoms	Acute IRRs will be identified by clinical signs	during infusion	day 0	Pause infusion as needed Antihistamine
Cerebral edema	Vomiting, hypothermia, convulsions	MRI	daily	days 1-4	Consult cerebral edema response team
Acute immune response	Flu-like symptoms	Platelet counts, complement factors, cytokines, T cell response, liver enzymes	daily	days 1-10	Consult immunologist-high dose corticosteroids
Endothelial damage	Biomarkers likely to precede clinical signs	HA, vWF, and/or CD44 in blood, Platelets, CBC, LDH, Haptoglobin	daily	days -1 to 7	High dose corticosteroids
TMA	Fever, reduced urinary output, edema, hypertension, lethargy, pallor, pinpoint red spots or unusual bruising	Platelet counts	daily	days 1-7	Consult immunologist and hematologist– complement inhibitor
HLH	Fever, rash	Ferritin levels, cytopenia	daily	days 1-7	Consult hematologist and/or nephrologist Anakinra, high dose corticosteroids
Cardiac injury	Biomarkers likely to precede clinical signs	Troponin I in blood Echocardiogram	weekly	weeks 1-4	Consult cardiologist
Liver injury	Biomarkers likely to precede clinical signs	ALT, AST, bilirubin	weekly	weeks 1-12	Consult hepatologist

12.4.7 Statistical analysis plan overview

Unless specified otherwise, analyses will include all participants. Post-hoc analyses may stratify by *PRNP* genotype.

Preliminary analysis (all arms)

The primary endpoint is safety and tolerability, as measured by AEs. This will be reported descriptively. A table showing the percentage of participants reporting any type of AE and the overall count of AEs by arm, dose level, and severity category will be tallied and presented in tabular form. Tables will be provided overall, and by system organ class and preferred term. No statistical test will be conducted.

Secondary analyses

1. Pharmacodynamic (PD) Effects of Single Doses of PRNP-CHARM-001 (all arms)

The secondary endpoint is PD – CSF PrP concentration. Each individual's baseline CSF PrP concentration will be defined as that taken at the baseline / drug administration visit (Day 0). For this endpoint, each individual's CSF PrP concentration at each LP will be normalized to their individual concentration at baseline – i.e., normalized CSF PrP at each subsequent timepoint will be expressed as a percentage of the individual's own baseline level. This normalized value is termed residual CSF PrP. No statistical test will be conducted. Normalized values will be reported for each arm, timepoint, and dose level, descriptively by displaying means and 95% confidence intervals of PrP concentration (expressed as a percentage of individual baseline).

2. Compare Change in PrP Over Time (all arms)

For each individual, each CSF PrP concentration measurement will be normalized to individual pre-drug baseline to obtain a percentage residual. This percentage will then be compared across the groups, grouping by arm, timepoint, and dose level. CSF PrP measurements at weeks 8, 12, 24 and 48 provide an opportunity to descriptively characterize the stability of target engagement; no statistical test will be performed.

Because longitudinal CSF PrP data are not yet available for symptomatic individuals, individuals in the symptomatic treatment arm will also be compared to untreated individuals in the observational arm. For both symptomatic arms, a rank test will be used at the 4-week timepoint to compare the distribution of residual PrP levels between them. This will be assessed overall, and within each dose level of the treatment and observational arm.

Key exploratory analyses

1. Survival (Time to Death or Initiation Life-Extending Measures) (symptomatic treatment arm only)

For the key exploratory endpoint of survival (defined as time to death or initiation of life-extending measures) Kaplan-Meier curves will be plotted for each dose level. No formal statistical test will be conducted. Participants will be deemed to have an "event" at their time of death or on the date that life-extending measures were first initiated. Participants who do not meet either criteria will be censored at their last date of follow-up.

2. Medical Research Council Prion Disease Rating Scale (MRC-PDRS) (symptomatic treatment arm only)

The exploratory endpoint of MRC-PDRS score over time in the symptomatic treatment arm will be reported descriptively by displaying means and standard deviations of the MRC-PDRS by timepoint and dose level. No statistical test will be conducted.

3. Additional Exploratory Analyses (all arms)

Additional exploratory analyses are also planned but will not be included as part of the Final Study Report. Exploratory analyses for this study will include, but are not limited to, the following:

- Fluid biomarkers of prion seeding (CSF RT-QuIC)
- Fluid biomarkers of neurodegeneration
    - CSF neurofilament (NfL)
    - CSF T-tau
    - Plasma NfL

Missing Data

Missing data may occur, for example, if one or more symptomatic participants pass away before the 4-week timepoint. For the primary endpoint of AEs, all AEs will be reported. For the secondary endpoint of CSF PrP, missing values due to absent CSF samples will be omitted from summary descriptive statistics for secondary objectives 1 and 2.

Proposed Sample Size

We propose to include up to 15 patients in each arm: symptomatic treated, symptomatic observations, and presymptomatic treated.

If dose escalation proceeds as planned, the number of patients per dose level in each treatment arm will be 3, 3, and 9 patients, respectively. This may change depending on safety review. The presymptomatic arm may also change based on target engagement review as it will not be initiated until a dose is identified that appears to reach target engagement of 50%. Sentinel dosing will be employed as described above and shown in Figure 20. Depending on the outcomes at each dose level, the final number of individuals may differ from that denoted above.

12.5 Planned Regulatory and Clinical Development Path for PRNP-CHARM-001

Given the rare nature of prion disease, the academic Sponsor intends to be thoughtful and forward looking in the development of PRNP-CHARM-001 with the intention that efficacy data from the contemplated Phase 1/2 clinical study could be leveraged to support a future license application(s). The Sponsor intends to leverage existing and future generalized Agency guidance as well as direct Agency consultation to achieve this goal. The Sponsor believes that the Agency's existing guidance, some of which is listed below, provides both recognition of and support for the complex nature of developing sufficient clinical evidence for licensure in the context of rare disease such as prion disease.

- Expedited Programs for Regenerative Medicine Therapies for Serious Conditions - Finalized Guidance February 2019
- Human Gene Therapy for Rare Diseases - Finalized Guidance January 2020
- Human Gene Therapy for Neurodegenerative Diseases - Finalized Guidance October 2022
- Innovative Designs for Clinical Trials of Cellular and Gene Therapy Products in Small Populations - Draft Guidance September 2025
- Considerations for the use of the Plausible Mechanism Framework to Develop Individualized Therapies that Target Specific Genetic Conditions with Known Biological Cause - Draft Guidance Feb 2026
- Chemistry, Manufacturing, and Controls Flexibilities for Developing Human Cellular and Gene Therapy Products for a Biologics License Application - May 2026

PRNP-CHARM-001 CMC Development Plan

With the goals outlined above, the Sponsor recognizes the importance of a forward compatible CMC development plan fit for the immediate purpose of supporting initial clinical trials with the capability of meeting licensure requirements. The Sponsor appreciates that accelerated clinical development pathways do not reduce the burden of evidence to demonstrate manufacturing capability and meet quality requirements required for licensure. It is for this reason that the Sponsor selected Viralgen as a contract manufacturing organization, as we intend to leverage their significant platform manufacturing and testing knowledge to increase the probability of technical success in demonstrating control of the manufacturing and testing processes employed for PRNP-CHARM-001. Likewise, the Sponsor has already invested considerable time and attention to critical product specific potency and titer assays to ensure that early clinical evidence can be leveraged for licensure; we will endeavor to qualify and validate in a timely manner to support any potential accelerated clinical development opportunities.

Clinical CSF PrP assay

PrP levels in CSF will serve as a target engagement biomarker and secondary endpoint in the proposed first-in-human study and has been discussed with the Agency as a surrogate endpoint for Accelerated Approval of PrP-lowering drugs, particularly in the presymptomatic at-risk genetic prion disease population. Given centrality of CSF PrP in clinical assessment of PRNP-CHARM-001, we are currently working with the CRO MRIGlobal to develop a pharmacodynamic assay to quantify PrP in clinical CSF samples. This assay will be qualified at the time of first-in-human dosing and validated prior to submission of a license application. The Sponsor intends to directly and proactively engage the Agency such that the clinical biomarker data collected at all stages of clinical development are aligned with and capable of supporting both expedited program requirements and future licensure.

Relevant designations

The Sponsor intends to develop PRNP-CHARM-001 with the aid of the following designations and apply for the listed Agency sponsored program (Table 18). We believe that prion disease is in scope as a rare, serious and life-threatening condition and unmet medical need and, should the clinical evidence be sufficient, that PRNP-CHARM-001 would meet the requirements of all of the contemplated programs. We are eager to engage both through these existing pathways and through any new pathways developed for which prion disease and PRNP-CHARM-001 may be in scope.

Table 18. Planned designation applications for the PRNP-CHARM-001 gene therapy program

Application	Criteria	Does prion disease qualify?	Gating activities	Target submission timing
Orphan Drug designation	Upper limit of 200,000 people in the US affected	Yes. Prion disease is the cause of roughly 600 US deaths per year.	None	**Prior to IND**
Fast Track designation	Serious or life-threatening condition; unmet medical need	Yes. Prion disease is universally fatal with no standard of care.	Pre-IND	Prior to IND
Breakthrough Therapy designation	Serious or life-threatening condition; substantial improvement over existing therapies.	Yes, provided preliminary clinical evidence is positive. Yes, Prion disease is universally fatal; there is no existing therapy.	Phase I/II clinical dosing - responses from Patients	Prior to moving into the expansion cohort
Regenerative Medicine Advanced Therapy designation	Novel gene therapy; serious or life-threatening condition	Yes, provided preliminary clinical evidence is positive. Yes, PRNP-CHARM-001 is a novel gene therapy.	Phase I/II clinical dosing - responses from Patients	Same time as Breakthrough
Support for clinical Trials Advancing Rare disease Therapeutics (START)	Serious condition; unmet medical needs, show substantial clinical improvement	Potentially, depending on future program eligibility requirements. Yes, provided the START program is continued.	IND Allowance	Within 6 months of with IND allowance

Role of CSF PrP in PRNP-CHARM-001 future development path

The molecular aim of PRNP-CHARM-001 is to reduce PrP levels in the brain. PrP is the sole essential molecule in prion disease as it serves as substrate for the formation of pathogenic prions; brain PrP dosage is known to control prion formation, replication, and neurotoxicity.

Contemplating the development arc of PRNP-CHARM-001, in addition to serving as a target engagement biomarker in the presymptomatic at-risk population, CSF PrP may serve as a surrogate endpoint reasonably likely to predict clinical benefit. Because carriers are healthy and cross-sectionally lack prodromal signs of disease[90,92], modulation of symptoms or pathological biomarkers are not available as trial endpoints. Disease rarity and variability in age of onset combine to render it numerically infeasible to power a pre-approval trial to follow carriers to disease onset[100]. These considerations indicate that a path to approval in presymptomatic at-risk individuals must leverage a biomarker-based strategy. PrP is well established as the single causal molecule in prion disease and as a dose-dependent driver of all stages of the disease process, including prion formation, replication, and toxicity. As discussed in Section 12.1, CSF PrP levels have been shown to be readily measurable in humans (Figure 19) and drug responsive in rats (Figure 3D). A CSF PrP assay will be available, and stage-appropriately qualified and validated. We believe that the strategy of employing CSF PrP levels as a surrogate endpoint in the presymptomatic population is modality-agnostic and could broadly apply to therapies that lower PrP levels in the brain, and we have engaged CDER on this topic as well. We look forward to further engaging the Agency on this approach as PRNP-CHARM-001 moves forward.

13. Current Status of Product Development

As of the date of this submission, the Broad Institute has advanced multiple milestones for the PRNP-CHARM-001 program:

13.1 Manufacturing milestones

1.1. The Sponsor has selected Viralgen Vector Core (Viralgen) as the manufacturer of PRNP-CHARM-001 drug product. Viralgen is a reputable contract manufacturer with years of experience manufacturing AAV clinical material; a more detailed description of Viralgen's capabilities is provided in Section 11. Viralgen has developed expertise in the production of TfR1 CapX through active projects with different entities including Apertura Gene Therapy, Finding Hope for FRRS1L, and the Sponsor. As part of these efforts, Viralgen has demonstrated capability to execute TfR1 CapX clinical manufacturing at a 500L scale. Through a 50L-scale production, Viralgen has manufactured the PRNP-CHARM-001 material that will be administered in the PRNP-CHARM-001 GLP safety and biodistribution study. Viralgen will also manufacture a 500L batch of clinical material for the PRNP-CHARM-001 program's Phase I/II Clinical Trial.

1.2. Viralgen has initiated production of the PRNP-CHARM-001 test article that will be administered in the GLP PRNP-CHARM-001 safety and biodistribution study. Viralgen has produced this material in a 50L batch (batch no. T-POLARIS-123) to confirm the manufacturing scalability of the PRNP-CHARM-001 product. Furthermore, the 50L scale manufacturing process is representative of the 250L/500L manufacturing process available at Viralgen for clinical material production.

1.3. Production of plasmid material to support clinical manufacturing of the PRNP-CHARM-001 product is contracted, and plasmid production is underway. Gene-of-interest (GOI) plasmid (single-stranded AAV2-ITR.EFS-2XuORF-PRNP-CHARM-3XmiR-122-WPRE-SV40pA.AAV2-ITR payload) and AAV rep-cap plasmid (TfR1 CapX) will be produced as "GMP-like" plasmid grade. Production of both plasmids, as well as production of the AAV helper plasmid, will be managed by Viralgen. Specifications for the plasmids and their associated cell banks will be described in a future IND submission.

1.4. The clinical manufacturing of the PRNP-CHARM-001 product is contracted, and Work Statements are in process. Production will take place at Viralgen. A general description of Viralgen's manufacturing process and the proposed drug substance / drug product specifications are described in Section 11 of this submission.

13.2 Non-clinical studies

1.5. The Sponsor has completed non-GLP studies at the Broad Institute to define a PRNP-CHARM-001 transgene design for definitive safety and biodistribution assessments. The executed studies, described in Section 10, demonstrate efficacy of the PRNP-CHARM-001 transgene for lowering *PRNP* expression in translationally relevant mouse models expressing human *PRNP*. The Sponsor believes that the executed studies are sufficient to demonstrate the biological plausibility of the PRNP-CHARM-001 product for therapeutic reduction of *PRNP* expression.

1.6. The Sponsor has completed a series of *in vitro* assessments of potential off-target PRNP-CHARM-001 activity, using RNA-seq to measure changes in gene expression in response to transduction of increasing amounts of PRNP-CHARM. The outcomes of these analyses, as well as a proposed *in vivo* assessment of PRNP-CHARM off-target activity, is described in detail in Section 10.2 of this submission.

1.7. The in-life portion of the pilot PRNP-CHARM-001 Safety and Biodistribution (Pilot Tox) Study in the *hTFRC* KI mouse model has been completed, and post-life analyses are currently underway. The study, which is being executed at Labcorp (Madison, WI), is described in detail in Section 10.1.5 of this submission, along with interim data obtained for the 1- and 3-month dose cohorts. The Sponsor believes that this study will inform dose range selection for the PRNP-CHARM-001 product. The executed study protocol is presented in Appendix IX and the interim study report is presented in Appendix X.

1.8. The Sponsor has engaged Labcorp (Madison, WI) to develop a protocol for a GLP PRNP-CHARM-001 safety and biodistribution study (GLP Tox) using *hTFRC* KI mice and the T-POLARIS-123 50L-scale batch manufactured by Viralgen (San Sebastian, Spain). The GLP PRNP-CHARM-001 Safety and Biodistribution Study design is described in Section 10.1.6 of this submission, and the draft protocol is presented in Appendix XI.

14. Questions for the Agency and Sponsor Positions

14.1 Non-clinical

Question 1. *Does the Agency agree with the Sponsor's position that the proposed in silico, in vitro, and in vivo study designs to assess PRNP-CHARM-001 off-target activity are sufficient to proceed with an IND submission and subsequent clinical administration of the PRNP-CHARM-001 drug product?*

Sponsor Position Q1

The proposed off-target assessment comprising *in silico* ZF binding site prediction and *in vitro* dose-responsive RNA-seq in relevant human cell lines, supplemented by an *in vivo* long-term Differentially Expressed Genes (DEG) accumulation study is sufficient to characterize the off-target safety profile of PRNP-CHARM-001 and to support IND filing and initial clinical administration. The full scientific rationale and completed study data are provided in Appendix XII. We believe this off-target assessment approach to be sufficient based on available published Agency guidance.

PRNP-CHARM-001 encodes a ZF-CHARM fusion protein with two silencing effectors: a CHARM/DNMT3A-mediated epigenetic silencer and a KRAB-mediated transcriptional repressor. Any phenotypic off-target silencing/repression, regardless of whether it arises from CHARM or KRAB activity, will produce a change in gene expression detectable by RNA-seq. RNA-seq captures all biologically meaningful off-target events and is the appropriate and sufficient primary assay. By contrast, DNA methylation profiling, whether genome-wide or targeted, measures a mechanistic step that is neither necessary nor sufficient to characterize the safety-relevant endpoint.

The Sponsor's completed *in vitro* studies confirm PRNP-CHARM-001's specificity. Across five human cell lines (hCMEC/D3, SVG-p12, THLE-2, skeletal muscle, HEK293) treated with PRNP-CHARM-001 across a dose range straddling the IC50 for *PRNP* silencing/repression, *PRNP* was the only gene showing consistent dose-dependent downregulation across all cell lines. *In silico* ZF binding site analysis using Bowtie and BLASTN identified *PRNP* as the only gene with a perfect-match binding site; no other gene met the dual-evidence off-target designation criteria (PIP_CHARM > 0.99 in RNA-seq AND a predicted ZF binding site within 10,000 bp of the gene body as defined by GENCODE). These results provide strong evidence that PRNP-CHARM-001 is highly specific for its intended target.

§VI.B of the FDA April 2026 Draft Guidance on NGS-based safety assessment instructs sponsors to justify their off-target strategy based on mechanism of action.

CHARM acts through a dual mechanism: the D3L domain recruits DNMT3A for methylation, and the KRAB domain recruits Kap1 and represses transcription. Both events contribute to transcriptional repression, but only DNMT3A acts through methylation, with the result that the combined safety-relevant readout manifests at the level of the

transcriptome. §VI lines 194–198 of the same guidance define off-target sites at the transcriptome level for editors whose intended consequence is a change in gene expression. CHARM's intended consequence is a change in gene expression. The guidance's example suggesting methylation profiling for epigenome editors is qualified as one of multiple appropriate strategies; given CHARM's dual-effector mechanism and the analytical advantages of RNA-seq, the Sponsor's RNA-seq-focused approach is mechanistically justified and consistent with the guidance's stated flexibility.

Overview of the Off-Target Analysis Framework

The PRNP-CHARM-001 off-target analysis framework has two components:

Tier 1: *In silico* ZF binding site prediction. The CHARM ZF domain's 19-bp consensus binding site was searched against GRCh38 using Bowtie (exact/near-exact matching) and BLASTN (gapped, lower-stringency), identifying candidate off-target loci. Hits were filtered to those within 10,000 bp of a GENCODE annotated gene body, as ZF-CHARM requires promoter proximity to affect transcription. *PRNP* was the only gene with a perfect-match ZF site proximal to a TSS.

Tier 2: *In vitro* dose-responsive RNA-seq. PRNP-CHARM-001 was applied across six MOIs (0 to 1.28×10^5 vg/cell) in five human cell lines expressing *TFRC*, *PRNP*, and *DNMT3A*, representing CNS (hCMEC/D3, SVG p12), liver (THLE-2), muscle, and kidney (HEK293) tissues. Differential expression was assessed by a three-component Bayesian mixture model. Candidate off-targets were nominated based on PIP_CHARM > 0.99, then cross-referenced against Tier 1 to apply the dual-evidence designation criterion (dose-dependent RNA-seq signal + ZF site within 10,000 bp of gene body). *PRNP* was the only gene meeting both criteria across all cell lines.

The RNA-seq dose-response provides proof of mechanism and causal identification of off-target activity that we propose to be sufficient. A monotonic dose-response curve that mirrors *PRNP* silencing is mechanistically consistent with ZF-CHARM activity and provides stronger evidence of causality than a single-dose methylation comparison could provide. Selective methylation follow-up would add analytical complexity without improving the characterization of the safety-relevant endpoint.

***In Vivo* Long-Term Off-Target Accumulation Study (LOTAS)**

An *in vivo* study in *hTFRC KI;hPRNP* mice will assess whether the total number of DEGs increases over time following a single PRNP-CHARM-001 dose (Day 30 vs. Day 180), using RNA-seq in brain and liver tissue. This study addresses the question of whether persistent ZF-CHARM expression from stable AAV episomes drives progressive off-target accumulation. The interpretive scope is explicitly limited to aggregate DEG burden as a function of time. Individual DEGs identified in mouse tissues will not be confirmed by RT-PCR or methylation profiling and will not be assumed to represent relevant human off-targets, given that the ZF domain's human-genome-based binding predictions do not apply to the mouse transcriptome.

Clinical Gating

Day 30 necropsy data from the LOTAS *in vivo* off-target study are proposed as sufficient to support initiation of the symptomatic arm of the first-in-human clinical trial given the limited life expectancy of this population. Both Day 30 and Day 180 necropsies and analysis will be complete before dosing presymptomatic patients. An overview of the study design is provided in Section 10.2.3.

Question 2. *Does the Agency concur with the Sponsor's proposed plan for translating dosing based on IV administration of the PRNP-CHARM-001 product in hTFRC KI mice, where safety and toxicology assessments, together with vector biodistribution data, will be used to establish dose range selection for the clinical program?*

Sponsor Position Q2

The selection of PRNP-CHARM-001 human dose-levels will be guided by empirical demonstration of PRNP-CHARM-001 potency and safety in *hTFRC KI/hPRNP* and *hTFRC* KI models and scaled in a one-to-one allometric scaling ratio on a viral genome per kilogram of body weight basis from these mouse models to human subjects.

As described in the previous INTERACT submitted by the Sponsor for the PRNP-CHARM-001 product, and in accordance with Section III.B.2 (Animal Species Selection) of the FDA's Guidance for Industry, *Preclinical Assessment of Investigational Cellular and Gene Therapy Products*[104], we have evaluated available animal species and have concluded that because the TfR1 CapX capsid is specific for human TfR1 and not TfR1 from any other species (Figures 7, 10, 11), *hTFRC* KI mice are the appropriate and sole pharmacologically relevant species for definitive biodistribution and toxicology studies of PRNP-CHARM-001. No allometric step through NHP or other large animal species is informative; as expected, TfR1 CapX and BI-hTFR1, from which TfR1 CapX was derived, have no increased targeting of the CNS of NHPs relative to AAV9 (also see Apertura Gene Therapy AS-1801 NHP study report, Appendix IV).

PRNP-CHARM-001 enters the CNS via TfR1-mediated transcytosis across cerebral endothelium. The critical parameters (microvascular TfR1 expression[101] and capillary-to-neuron distances[102]) are conserved between mouse and human and capacity for transport of TfR1-binding shuttles is consistent across age[101], supporting a direct vg/kg extrapolation from mouse to human. This approach is consistent with established practice for IV-administered AAV CNS gene therapies[103] and with TfR1-shuttle biologics, where pharmacologically active human doses have been successfully predicted from h*TFRC* KI mouse data, including the antibody-iduronidase fusion pabinafusp alfa for MPS-II[70,71,104], the brain shuttle Fc fragment platform[63,65,69], and the TfR1-binding antibody RO7126209 (NCT04023994). Direct one-to-one scaling of vg/kg dosing in h*TFRC* KI mice is therefore proposed as the most physiologically grounded basis for human dose selection.

The *hTFRC* KI mouse model, described in Table 3 and Appendix I, supports TfR1 CapX-mediated CNS delivery and is a thoroughly established and characterized animal model commercial availability to support procurement of large, sex-balanced cohorts within

a tight age range. They are the most appropriate model for PRNP-CHARM-001 safety and biodistribution evaluations.

The *hTFRC KI;hPRNP* mouse model, built upon the *hTFRC* KI model, has been developed by the Sponsor, described in Table 3 and Appendix I, supports both TfR1 CapX-mediated CNS delivery and on-target PRNP-CHARM-001 silencing of human *PRNP*. They are the most translationally complete preclinical model for PRNP-CHARM-001 with evaluation of *PRNP* mRNA reduction and human PrP protein reduction serving as the relevant potency outcome. The model is not commercially available and is limited to use in non-GLP academic settings.

As noted in Section 10, preparations of AAV test articles used across all listed non-clinical studies have been subjected to a fit-for-purpose testing panel prior to use to ensure that material is adequately and sufficiently characterized. The Sponsor has endeavored to collect sufficient retains of critical preparations.

- All AAV test articles prepared by the Broad Institute were titered by the Broad Institute. All genomic titers listed for these materials throughout this document and associated appendices report the genomic titer and calculated dose-level using this Broad Institute titer unless otherwise specified.
- All AAV test articles prepared by Viralgen were titered by Viralgen using their qualified method. All genomic titers listed for these materials throughout this document and associated appendices report the genomic titer and calculated dose-level using this Viralgen titer unless otherwise specified.
- As discussed in subsequent sections, Viralgen produced AAV preparations and associated Viralgen titers will be used for the determination of human dose-levels.

Reasonable agreement has been found between Viralgen produced AAV preparations and Viralgen titer results with Broad Institute titer results from the same material. Nonetheless, should assessment of Broad Institute prepared materials be required, they will be tested at Viralgen using their qualified method.

The non-clinical studies that will inform dose selection are H3X, BRAV3, PRNP-CHARM-001 Safety and Biodistribution Study (Pilot tox), and GLP PRNP-CHARM-001 Safety and Biodistribution Study (GLP tox). These studies and their associated details are thoroughly described in Section 10 of this document and Appendices VII, VIII, IX, X, and XI.

Question 3. *Does the Agency agree with the Sponsor's position that the proposed design of the GLP PRNP-CHARM-001 safety and biodistribution study is sufficient to generate the necessary data to proceed with an IND submission and initial clinical administration of the PRNP-CHARM-001 product?*

Sponsor Position Q3

The Sponsor has developed the GLP PRNP-CHARM-001 safety and biodistribution study (GLP Tox) in alignment with Agency feedback from the Sponsor's INTERACT submission (PTS #PS009720), preclinical guidance "Preclinical Assessment of Investigational Cellular and Gene Therapy Products", and ICH S12 Nonclinical Biodistribution Considerations for Gene Therapy Guidance, as well as our experience with PRNP-CHARM-001 in the non-GLP safety and biodistribution study, as detailed in Appendix IX (executed study protocol) and Appendix X (interim study report).

The GLP safety and biodistribution study will be executed at Labcorp (Madison, WI), a GLP-compliant preclinical contract research organization (CRO) with substantial expertise in conducting *in vivo* nonclinical studies, including for AAV-based gene therapies. Through execution of the non-GLP safety and distribution study, we confirmed the capability of Labcorp to perform dose preparation and administration, manage animal care, and execute on the necessary post-life assessments for the GLP study. We have incorporated learnings from the non-GLP safety study into our GLP study design, including increasing the number of animals per group to obtain sufficient power for the planned panel of clinical chemistry and hematology assessments and improving randomization and assignment of animals to groups based on starting body weight. Additionally, we have shifted the range of dose levels evaluated downward to a range of 1.7E12 to 1.5E13 vg/kg based on observations of reduced weight gain in the 4.5E13 vg/kg cohort in the non-GLP study and to better bracket the anticipated clinical dose levels of PRNP-CHARM-001.

It is our position that in-life durations of 1 and 3 months in the GLP study are sufficient to capture safety outcomes associated with PRNP-CHARM-001 dosing. Based on the outcomes from the non-GLP PRNP-CHARM-001 safety and biodistribution study, all animals survived to their respective planned necropsies, which included a high-end dose level of 4.5E13 vg/kg which will not be used in the GLP study. Moreover, the Sponsor has executed studies with PRNP-CHARM-001 using a 12-week in-life (H3X, Appendix VII) and studies where animals dosed with mouse Prnp-CHARM packaged in AAV-PHP.eB have been taken out to 12 months (Figure 6).

14.2 CMC

Question 4. *Does the Agency agree that the proposed drug product manufacturing plan, stability testing plan, and draft release testing plan are appropriate for the PRNP-CHARM-001 product to be used in the program's initial Phase I/II clinical trial?*

Sponsor Position Q4

The Sponsor's position is that the proposed drug product manufacturing plan, stability testing plan, and draft release testing plan are appropriate to support the PRNP-CHARM-001 product to be used in the program's initial Phase I/II clinical trial. The plan leverages a qualified CDMO with established gene therapy manufacturing expertise, an extensively used proprietary AAV platform process, and direct prior experience producing TfR1 CapX–based clinical material at the same scale and using the same platform proposed for the PRNP-CHARM-001 clinical lot.

Selected manufacturer. The Sponsor has selected Viralgen Vector Core S.L.U (San Sebastián, Spain) as the GMP manufacturer of the PRNP-CHARM-001 drug product. Viralgen is dedicated exclusively to rAAV manufacturing. The facility holds GMP authorization (MIA reference 6711-E) from the Spanish Medicines Agency (AEMPS) and operates two complementary sites: a Clinical Facility (B1, 4,000 m² with 1,250 m² of cGMP space across four independent manufacturing suites capable of 250 L and 500 L batches) and a Commercial Facility (B2, ~28,000 m² across nine suites capable of batches up to 2,000 L). To date, Viralgen has supported authorization of 40 clinical trials with material it has manufactured across the United States, European Union, United Kingdom, and Canada. An overview of Viralgen and its facilities is provided in Section 11.2.

Platform manufacturing process. PRNP-CHARM-001 will be manufactured using Viralgen's proprietary Pro10™ suspension-cell AAV platform. The platform is based on triple-plasmid transient transfection of a suspension-adapted HEK293 cell line (Pro10™), using a helper plasmid, an AAV rep-cap plasmid encoding TfR1 CapX, and a single-stranded PRNP-CHARM-001 transgene plasmid co-formulated with PEI Max. Cell culture and purification use chemically defined materials, with no animal-origin components in either upstream or downstream processing. Downstream processing comprises chemical lysis, depth filtration, affinity chromatography, iodixanol gradient centrifugation for separation of full from empty capsids, anion-exchange polishing, tangential flow filtration into formulation buffer, and 0.2 µm sterile filtration, with the drug substance proceeding directly into fill/finish as a continuous process to the final container closure. A detailed process description and flow diagram are provided in Sections 11.3 through 11.7 and Figures 16 and 17.

Platform experience and TfR1 CapX-specific precedent. Viralgen's Pro10™ platform has been used to support authorization of 40 clinical trials to date, providing extensive demonstrated platform suitability for advancing AAV gene therapy products into the clinic. Of direct relevance to this submission, Viralgen has established specific manufacturing expertise with TfR1 CapX, the same capsid used in PRNP-CHARM-001 through three independent active programs: Apertura Gene Therapy (AGTX-1111, a TfR1 CapX-delivered therapeutic for Tuberous Sclerosis Complex), the Finding Hope for FRRS1L program, and the PRNP-CHARM-001 program. Through these efforts, **Viralgen has demonstrated capability to execute TfR1 CapX clinical manufacturing at the 500 L scale**, the same scale planned for the PRNP-CHARM-001 GMP clinical batch. This precedent provides direct, capsid-specific de-risking of the upstream and downstream unit operations proposed for the PRNP-CHARM-001 clinical lot, including productivity and full/empty separation behavior for TfR1 CapX particles in the Pro10™ process.

Status of PRNP-CHARM-001 manufacturing. Viralgen has completed a 50 L manufacturing run of PRNP-CHARM-001 (batch T-POLARIS-123, vialed 21 May 2026) that will supply the GLP biodistribution and toxicology study, the device-compatibility and in-use stability study, and the formal stability study. The 50 L process operated by Viralgen is representative of the 250 L and 500 L processes that will be used for clinical supply, supporting scale comparability for the planned 500 L GMP batch. GMP-like plasmid manufacturing for the GOI plasmid (AAV2-ITR-EFS-2×uORF-PRNP-CHARM-3×miR-122-WPRE-SV40pA-AAV2-ITR) and TfR1 CapX rep-cap plasmid is contracted and underway through Viralgen-managed suppliers, with helper plasmid produced by Aldevron (Fargo, ND) and the Pro10™ MCB and associated QC supplied by WuXi AppTec (Philadelphia, PA), as listed in Table 9.

Release and stability testing. The proposed drug substance and drug product release panels (Tables 10 and 12) cover identity (capsid SDS-PAGE/Western and genome Sanger sequencing), strength (ITR ddPCR and GOI ddPCR vector genome titer, TCID50 infectious titer, and infectious-to-genome ratio), safety (sterility, endotoxin, replication-competent AAV, mycoplasma, mycobacteria, adventitious virus, and bioburden), purity (residual host cell DNA by 18S qPCR and E1A ddPCR, residual plasmid DNA, residual process chemicals, residual immunoaffinity ligand, and full/empty ratio by CryoTEM), and general quality attributes (appearance, container closure integrity, subvisible particles, osmolality, pH, particle-size distribution, poloxamer 188, and extractable volume), with a fit-for-purpose transgene-expression biological-activity assay developed by the Sponsor and transferred to a CRO for qualification (Section 11.9). The stability program (Section 11.8 and Table 13) is being run on T-POLARIS-123 with sampling at 3, 6, 12, 18, and 24 months post-vialing and is designed to support the duration of the planned Phase I/II clinical trial, with a clinical-batch stability plan to be included in the IND.

For these reasons, the Sponsor believes the proposed drug product manufacturing, stability, and release plans are appropriate for the PRNP-CHARM-001 product to be used in the program's initial Phase I/II clinical trial, and seeks the Agency's concurrence.

Question 5. *Does the Agency agree that the proposed delivery-device compatibility and in-use stability study is appropriate for initial clinical administration of the PRNP-CHARM-001 product?*

Sponsor Position Q5

The Sponsor has developed a draft delivery-device compatibility and in-use stability study design for the PRNP-CHARM-001 clinical product, presented in Appendix XIII. The Sponsor believes that the study as outlined is fit for purpose for supporting Phase 1/2 clinical dosing. The PRNP-CHARM-001 product is formulated in a buffer used for other AAV gene therapy products, and will be handled with and will interact with product contacting surfaces that are in common use. The Sponsor therefore does not anticipate major differences in the

behavior of PRNP-CHARM-001 in this study from other AAV gene therapy products using similar delivery approaches.

As this study has not yet been executed, this pre-IND submission does not include associated data and results from this study. The Sponsor intends to present the completed delivery-device compatibility and in-use stability study protocol, methods, and results in the forthcoming IND submission, in alignment with the expectations in guidance published by the Agency.

Question 6. *We propose to use RT-qPCR-based quantification of PRNP RNA lowering in human HEK293T cells as a potency assay for release criteria. Does the Agency agree that the proposed potency assay will be adequate to support licensure-enabling studies?*

Sponsor Position Q6

Proposed assay summary: The proposed RT-qPCR-based *PRNP* mRNA potency assay in HEK293T cells directly measures the biological activity underlying PRNP-CHARM-001's intended therapeutic effect, is grounded in a well-characterized mechanism, is consistent with FDA guidance recommendations for gene therapy potency assays, and is supported by reproducible pilot data demonstrating dose-dependent, ZF-CHARM-specific *PRNP* silencing across three independent assay runs. We propose that when qualified at a GMP-compliant CRO this assay will be adequate to support IND filing and Phase 1/2 clinical investigations, and that when subsequently validated it will be adequate to support licensure.

Alignment with guidance: Section V.B.2 (Approaches to Potency Assay Selection and Design) of the FDA December 2023 Draft Guidance for Industry, Potency Assurance for Cellular and Gene Therapy Products, states that 'release testing for a gene therapy vector that expresses a transgene should generally include a potency assay that quantitates transgene mRNA or protein in transduced cells'[105]. The proposed assay measures *PRNP* mRNA reduction in TfR1 CapX-transduced HEK293T cells, directly fulfilling this recommendation. The assay measures the functional consequence of ZF-CHARM activity, transcriptional silencing of *PRNP*, which is the intended therapeutic effect of PRNP-CHARM-001, and is therefore directly linked to the product's mechanism of action and potency-related critical quality attribute.

The FDA's 2025 INTERACT response (PTS#PS009720) confirmed that RT-qPCR-based quantification of *PRNP* RNA in HEK293T cells is appropriate as the potency assay for product release. The Agency recommended that potency be reported relative to a well-characterized reference material lot, assessed in parallel with each test lot. The Sponsor will implement reference material-based relative potency reporting in the qualified assay, consistent with this recommendation.

Pilot assay results: The pilot assay data (Figure 18; three independent runs) demonstrate that the assay is capable of detecting dose-dependent *PRNP* silencing across a 256-fold MOI range (500 to 128,000 vg/cell). Residual *PRNP* mRNA decreases with increasing MOI in all three runs, reaching >97% reduction at the highest MOI tested. A no-CHARM control AAV

(identical capsid, non-coding stuffer payload) produced no reduction in *PRNP* mRNA at any dose across all runs, confirming that the assay signal is specific to ZF-CHARM activity and is not attributable to non-specific effects of TfR1 CapX transduction, cell lysis, or RNA extraction. Inter-run reproducibility was acceptable across all three runs. In the third run, both Broad Institute-manufactured and Viralgen-manufactured PRNP-CHARM-001 produced similar *PRNP* silencing curves, supporting the potency comparability of material from the two manufacturers.

Planned assay development: In accordance with the FDA's progressive potency assurance framework (§IV.G of the Potency Assurance Draft Guidance), the Sponsor's plan for potency assay development is as follows. For IND filing and Phase 1/2: The assay will be qualified at a GMP-compliant CRO, with qualification studies establishing assay precision (repeatability and intermediate precision), accuracy, linearity, and range across the working MOI range. Acceptance criteria for PRNP-CHARM-001 lot release will be established based on qualification data and will be designed to reject sub-potent lots. A reference material strategy will be implemented. For licensure (BLA): The assay will be validated in accordance with 21 CFR 211.165(e) and 21 CFR 211.194(a)(2), with full validation of precision, accuracy, linearity, range, specificity, and robustness. Acceptance criteria will be refined based on clinical and manufacturing experience accumulated during the Phase 1/2 program. The Sponsor requests Agency feedback on this progressive development plan and on whether the proposed qualification scope is adequate to support the initial IND filing.

14.3 Clinical

Question 7. *Does the Agency agree with the Sponsor's position that symptomatic prion disease patients and presymptomatic individuals at risk of genetic prion disease are separate populations and can be addressed in separate first-in-human studies?*

Sponsor position Q7

We propose that symptomatic prion disease patients and pre-symptomatic individuals at high risk for genetic prion disease represent two appropriate and complementary patient populations for a first-in-human study of PRNP-CHARM-001.

Section V.A (Study Population) of the FDA's Guidance for Industry, Human Gene Therapy Products Incorporating Human Genome Editing[106] recognizes the tradeoffs inherent in treating patients earlier versus later in disease course, stating "Subjects with severe or advanced disease may be more willing to accept the potential risks of an investigational human GE product. However, these subjects may be predisposed to experiencing more AEs or be receiving concomitant treatments, which could make the safety or effectiveness data difficult to interpret. Therefore, in some instances, subjects with less advanced or more moderate disease may be appropriate for inclusion in first-in-human clinical studies."

As described above, symptomatic prion disease patients and presymptomatic high-risk individuals are distinct populations with distinct challenges, opportunities for benefit and

prospects for data collection. We aim to honor these complex tradeoffs by performing parallel small N first-in-human studies in both populations.

The rapid decline and irreversible neuronal loss that characterize symptomatic prion disease patients may limit the potential for signals of efficacy in these patients from a drug expected to take four weeks to reach full effect at the protein level. We further accept that both safety and target engagement will be complex to read out in the context of disease.

By contrast, high-risk genetic carriers, though they do not reliably show prodromal molecular change in advance of symptom onset, face near certainty of fatal disease. Treatment of this population will allow full time for PrP protein reduction and reliable collection of both detailed safety and target engagement data. To balance the risk of the treatment with the risk of not treating in this population, we propose restricting enrollment to individuals age ≥40, who are on expectation nearer to age of onset[100].

Question 8. *Does the Agency agree that the clinical plan overview for first-in-human studies of PRNP-CHARM-001 in symptomatic prion disease patients and individuals at risk for presymptomatic genetic prion disease is reasonable, including general trial structure, staging of trial arms, and endpoints?*

Sponsor position Q8

We believe that in the context of prion disease, a rapidly fatal neurodegenerative disease with no standard of care, our overall data package, planned studies and CMC strategy support the overall clinical plan described above. Key attributes of this plan include:

Two patient populations: As described in Sponsor Position 7, we propose to enroll both symptomatic patients and pre-symptomatic individuals at high risk for genetic prion disease in our first-in-human clinical study.

Three arms: In addition to symptomatic and pre-symptomatic treatment arms, we propose a symptomatic observational trial arm. Symptomatic patients will have the option to enroll in this arm when unable to enroll in the symptomatic treatment arm due to structural pauses for observation and analysis or preexisting AAV seropositivity. The observational arm will serve as a concurrent natural history comparator for longitudinal trajectory of CSF PrP in symptomatic patients. If a spot becomes available in the symptomatic treatment arm, observational arm participants will have the option to cross over if eligible.

Staging: As overviewed in Figure 20, we propose a clinical study structure that encompasses the following stage gates:
1) A sentinel patient dosed at each dose level, per each population, and observed for a minimum of 8 weeks prior to dosing the next patient at that dose level.
2) Three patients dosed per dose level and observed for a minimum of 4-12 weeks prior to dose escalation within the same population.
3) Three symptomatic patients dosed per dose level and observed for a minimum of 10-18 weeks prior to dosing of a pre-symptomatic at-risk individual at that dose level.

4) Assessment of both safety and target engagement at all dose escalation decision points.
5) Demonstration of adequate safety and 50% target engagement based on totality of the evidence at a given dose prior to that dose level being administered to pre-symptomatic at-risk individuals.

Endpoints: The primary endpoint will be safety, and the secondary endpoint will be CSF PrP levels for both populations. Exploratory endpoints will include pathological biomarkers, rate of clinical decline (symptomatic only), time to death or life extending measures (symptomatic only); time to onset of genetic prion disease (presymptomatic only.)

Question 9. *Does the Agency concur with our proposed regulatory and clinical development engagement plan and staging of application for relevant designations for the PRNP-CHARM-001 gene therapy program?*

Sponsor position Q9

We propose that the plan outlined in Section 12.4 is appropriate to our drug and indication. Our temporal staging of qualification and validation of product-specific CMC assays, and of a CSF PrP quantification pharmacodynamic assay, appropriately meet the minimum criteria for a first-in-human trial while also providing a path to later licensure if appropriate, consistent with the Plausible Mechanisms Framework. Our intention to apply for Orphan Drug, Fast Track, Breakthrough, and Regenerative Medicine Advanced Therapy designations are appropriate to the nature of our drug and indication. Our intent to utilize the Accelerated Approval pathway for pre-symptomatic individuals based on surrogate biomarker CSF PrP is commensurate with the central role of PrP in our disease pathway and is consistent with previous conversations with the Agency.

We welcome Agency feedback on this overall approach to the development plan for PRNP-CHARM-001.

Conclusion

As a patient-scientist living with a known high risk for genetic prion disease, I (Dr. Vallabh) am highly motivated to seek the swift development of effective and safe PrP-lowering therapeutics.

The meeting will be a success if a path forward is identified for first-in-human treatment of prion disease patients and at-risk individuals with PRNP-CHARM-001.

15. References

1. Neumann, E. N. *et al.* Brainwide silencing of prion protein by AAV-mediated delivery of an engineered compact epigenetic editor. *Science* **384**, ado7082 (2024).

2. Qiao, C. *et al.* Liver-specific microRNA-122 target sequences incorporated in AAV vectors efficiently inhibits transgene expression in the liver. *Gene Ther.* **18**, 403–410 (2011).

3. Zanta-Boussif, M. A. *et al.* Validation of a mutated PRE sequence allowing high and sustained transgene expression while abrogating WHV-X protein synthesis: application to the gene therapy of WAS. *Gene Ther.* **16**, 605–619 (2009).

4. Vallabh, S. M., Minikel, E. V., Schreiber, S. L. & Lander, E. S. Towards a treatment for genetic prion disease: trials and biomarkers. *Lancet Neurol.* **19**, 361–368 (2020).

5. Prusiner, S. B. Prions. *Proc. Natl. Acad. Sci. U. S. A.* **95**, 13363–13383 (1998).

6. Hermann, P. *et al.* Biomarkers and diagnostic guidelines for sporadic Creutzfeldt-Jakob disease. *Lancet Neurol.* **20**, 235–246 (2021).

7. Minikel, E. V. *et al.* Quantifying prion disease penetrance using large population control cohorts. *Sci. Transl. Med.* **8**, 322ra9 (2016).

8. Will, R. G. & Ironside, J. W. Sporadic and Infectious Human Prion Diseases. *Cold Spring Harb. Perspect. Med.* **7**, a024364 (2017).

9. Franceschini, A. *et al.* High diagnostic value of second generation CSF RT-QuIC across the wide spectrum of CJD prions. *Sci. Rep.* **7**, 10655 (2017).

10. Büeler, H. *et al.* Mice devoid of PrP are resistant to scrapie. *Cell* **73**, 1339–1347 (1993).

11. Fischer, M. *et al.* Prion protein (PrP) with amino-proximal deletions restoring susceptibility of PrP knockout mice to scrapie. *EMBO J.* **15**, 1255–1264 (1996).

12. Brandner, S. *et al.* Normal host prion protein necessary for scrapie-induced neurotoxicity. *Nature* **379**, 339–343 (1996).

13. Mallucci, G. *et al.* Depleting neuronal PrP in prion infection prevents disease and reverses spongiosis. *Science* **302**, 871–874 (2003).

14. Safar, J. G. *et al.* Prion clearance in bigenic mice. *J. Gen. Virol.* **86**, 2913–2923 (2005).

15. Raymond, G. J. *et al.* Antisense oligonucleotides extend survival of prion-infected mice. *JCI Insight* **5**, e131175, 131175 (2019).

16. Minikel, E. V. *et al.* Prion protein lowering is a disease-modifying therapy across prion disease stages, strains and endpoints. *Nucleic Acids Res.* **48**, 10615–10631 (2020).

17. Büeler, H. *et al.* High prion and PrPSc levels but delayed onset of disease in scrapie-inoculated mice heterozygous for a disrupted PrP gene. *Mol. Med.* **1**, 19–30 (1994).

18. Prusiner, S. B. Novel proteinaceous infectious particles cause scrapie. *Science* **216**, 136–144 (1982).

19. Prusiner, S. B., Groth, D. F., Bolton, D. C., Kent, S. B. & Hood, L. E. Purification and structural studies of a major scrapie prion protein. *Cell* **38**, 127–134 (1984).

20. Dugger, B. N., Perl, D. P. & Carlson, G. A. Neurodegenerative Disease Transmission and Transgenesis in Mice. *Cold Spring Harb. Perspect. Biol.* **9**, a023549 (2017).

21. Bessen, R. A. *et al.* Non-genetic propagation of strain-specific properties of scrapie prion protein. *Nature* **375**, 698–700 (1995).

22. Telling, G. C. *et al.* Evidence for the conformation of the pathologic isoform of the prion protein enciphering and propagating prion diversity. *Science* **274**, 2079–2082 (1996).

23. Safar, J. *et al.* Eight prion strains have PrP(Sc) molecules with different conformations. *Nat. Med.* **4**, 1157–1165 (1998).

24. Mead, S., Lloyd, S. & Collinge, J. Genetic Factors in Mammalian Prion Diseases. *Annu. Rev. Genet.* **53**, 117–147 (2019).

25. Legname, G. *et al.* Synthetic mammalian prions. *Science* **305**, 673–676 (2004).

26. Deleault, N. R. *et al.* Protease-resistant prion protein amplification reconstituted with partially purified substrates and synthetic polyanions. *J. Biol. Chem.* **280**, 26873–26879 (2005).

27. Wang, F., Wang, X., Yuan, C.-G. & Ma, J. Generating a prion with bacterially expressed recombinant prion protein. *Science* **327**, 1132–1135 (2010).

28. Colby, D. W. *et al.* Design and construction of diverse mammalian prion strains. *Proc. Natl. Acad. Sci. U. S. A.* **106**, 20417–20422 (2009).

29. Mead, S. Prion disease genetics. *Eur. J. Hum. Genet. EJHG* **14**, 273–281 (2006).

30. Palmer, M. S., Dryden, A. J., Hughes, J. T. & Collinge, J. Homozygous prion protein genotype predisposes to sporadic Creutzfeldt-Jakob disease. *Nature* **352**, 340–342 (1991).

31. Shibuya, S., Higuchi, J., Shin, R. W., Tateishi, J. & Kitamoto, T. Codon 219 Lys allele of PRNP is not found in sporadic Creutzfeldt-Jakob disease. *Ann. Neurol.* **43**, 826–828 (1998).

32. Mead, S. *et al.* A novel protective prion protein variant that colocalizes with kuru exposure. *N. Engl. J. Med.* **361**, 2056–2065 (2009).

33. Scott, M. *et al.* Transgenic mice expressing hamster prion protein produce species-specific scrapie infectivity and amyloid plaques. *Cell* **59**, 847–857 (1989).

34. Watts, J. C. & Prusiner, S. B. Mouse models for studying the formation and propagation of prions. *J. Biol. Chem.* **289**, 19841–19849 (2014).

35. Mortberg, M. A. *et al.* Regional variability and genotypic and pharmacodynamic effects on PrP concentration in the CNS. *JCI Insight* **7**, e156532 (2022).

36. Richt, J. A. *et al.* Production of cattle lacking prion protein. *Nat. Biotechnol.* **25**, 132–138 (2007).

37. Benestad, S. L., Austbø, L., Tranulis, M. A., Espenes, A. & Olsaker, I. Healthy goats naturally devoid of prion protein. *Vet. Res.* **43**, 87 (2012).

38. Bremer, J. *et al.* Axonal prion protein is required for peripheral myelin maintenance. *Nat. Neurosci.* **13**, 310–318 (2010).

39. Küffer, A. *et al.* The prion protein is an agonistic ligand of the G protein-coupled receptor Adgrg6. *Nature* **536**, 464–468 (2016).

40. Skedsmo, F. S. *et al.* Demyelinating polyneuropathy in goats lacking prion protein. *FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol.* **34**, 2359–2375 (2020).

41. Minikel, E. V. *et al.* Evaluating drug targets through human loss-of-function genetic variation. *Nature* **581**, 459–464 (2020).

42. Lakkaraju, A. K. K. *et al.* Glial activation in prion diseases is selectively triggered by neuronal PrPSc. *Brain Pathol.* **32**, e13056 (2022).

43. Corridon, T. L. *et al.* PrP turnover in vivo and the time to effect of prion disease therapeutics. *PLoS Pathog.* **22**, e1014263 (2026).

44. Holtzman, L. & Gersbach, C. A. Editing the Epigenome: Reshaping the Genomic Landscape. *Annu. Rev. Genomics Hum. Genet.* **19**, 43–71 (2018).

45. Gilbert, L. A. *et al.* CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes. *Cell* **154**, 442–451 (2013).

46. Goell, J. H. & Hilton, I. B. CRISPR/Cas-Based Epigenome Editing: Advances, Applications, and Clinical Utility. *Trends Biotechnol.* **39**, 678–691 (2021).

47. Nakamura, M., Gao, Y., Dominguez, A. A. & Qi, L. S. CRISPR technologies for precise epigenome editing. *Nat. Cell Biol.* **23**, 11–22 (2021).

48. Park, M., Keung, A. J. & Khalil, A. S. The epigenome: the next substrate for engineering. *Genome Biol.* **17**, 183 (2016).

49. Kungulovski, G. & Jeltsch, A. Epigenome Editing: State of the Art, Concepts, and Perspectives. *Trends Genet. TIG* **32**, 101–113 (2016).

50. Liu, X. S. *et al.* Editing DNA Methylation in the Mammalian Genome. *Cell* **167**, 233-247.e17 (2016).

51. McDonald, J. I. *et al.* Reprogrammable CRISPR/Cas9-based system for inducing site-specific DNA methylation. *Biol. Open* **5**, 866–874 (2016).

52. O'Geen, H. *et al.* dCas9-based epigenome editing suggests acquisition of histone methylation is not sufficient for target gene repression. *Nucleic Acids Res.* **45**, 9901–9916 (2017).

53. Amabile, A. *et al.* Inheritable Silencing of Endogenous Genes by Hit-and-Run Targeted Epigenetic Editing. *Cell* **167**, 219-232.e14 (2016).

54. Mlambo, T. *et al.* Designer epigenome modifiers enable robust and sustained gene silencing in clinically relevant human cells. *Nucleic Acids Res.* **46**, 4456–4468 (2018).

55. Hofacker, D. *et al.* Engineering of Effector Domains for Targeted DNA Methylation with Reduced Off-Target Effects. *Int. J. Mol. Sci.* **21**, 502 (2020).

56. Kaluscha, S. *et al.* Evidence that direct inhibition of transcription factor binding is the prevailing mode of gene and repeat repression by DNA methylation. *Nat. Genet.* **54**, 1895–1906 (2022).

57. Nuñez, J. K. *et al.* Genome-wide programmable transcriptional memory by CRISPR-based epigenome editing. *Cell* **184**, 2503-2519.e17 (2021).

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

59. Pardridge, W. M., Eisenberg, J. & Yang, J. Human blood-brain barrier transferrin receptor. *Metabolism.* **36**, 892–895 (1987).

60. Moos, T. & Morgan, E. H. Transferrin and transferrin receptor function in brain barrier systems. *Cell. Mol. Neurobiol.* **20**, 77–95 (2000).

61. Maxfield, F. R. & McGraw, T. E. Endocytic recycling. *Nat. Rev. Mol. Cell Biol.* **5**, 121–132 (2004).

62. Kida, S. *et al.* Enzyme replacement with transferrin receptor-targeted α-L-iduronidase rescues brain pathology in mucopolysaccharidosis I mice. *Mol. Ther. Methods Clin. Dev.* **29**, 439–449 (2023).

63. Ullman, J. C. *et al.* Brain delivery and activity of a lysosomal enzyme using a blood-brain barrier transport vehicle in mice. *Sci. Transl. Med.* **12**, eaay1163 (2020).

64. Hammond, S. M. *et al.* Antibody-oligonucleotide conjugate achieves CNS delivery in animal models for spinal muscular atrophy. *JCI Insight* **7**, e154142 (2022).

65. Kariolis, M. S. *et al.* Brain delivery of therapeutic proteins using an Fc fragment blood-brain barrier transport vehicle in mice and monkeys. *Sci. Transl. Med.* **12**, eaay1359 (2020).

66. Rué, L. *et al.* Novel Human/Non-Human Primate Cross-Reactive Anti-Transferrin Receptor Nanobodies for Brain Delivery of Biologics. *Pharmaceutics* **15**, 1748 (2023).

67. Yu, Y. J. *et al.* Therapeutic bispecific antibodies cross the blood-brain barrier in nonhuman primates. *Sci. Transl. Med.* **6**, 261ra154 (2014).

68. JCR Pharmaceuticals Announces Approval of IZCARGO® (Pabinafusp Alfa) for Treatment of MPS II (Hunter Syndrome) in Japan. (2021).

69. BLA 761485: Corrected BLA Accelerated Approval.

70. Giugliani, R. *et al.* Iduronate-2-sulfatase fused with anti-hTfR antibody, pabinafusp alfa, for MPS-II: A phase 2 trial in Brazil. *Mol. Ther. J. Am. Soc. Gene Ther.* **29**, 2378–2386 (2021).

71. Sonoda, H. *et al.* A Blood-Brain-Barrier-Penetrating Anti-human Transferrin Receptor Antibody Fusion Protein for Neuronopathic Mucopolysaccharidosis II. *Mol. Ther. J. Am. Soc. Gene Ther.* **26**, 1366–1374 (2018).

72. Okuyama, T. *et al.* Iduronate-2-Sulfatase with Anti-human Transferrin Receptor Antibody for Neuropathic Mucopolysaccharidosis II: A Phase 1/2 Trial. *Mol. Ther. J. Am. Soc. Gene Ther.* **27**, 456–464 (2019).

73. Mortorana, A. Development of a scalable platform production process for AVB-406, a blood–brain barrier–crossing AAV vector for MAPT knockdown in Alzheimer's disease. Session title: AAV Downstream Manufacturing II. ASGCT 2026 Oral Presentation, Boston, MA.

74. Steinsapir, A. et al. Establishing scalable TfR1 CapX manufacturing platform to accelerate multiple CNS gene therapy programs. ASGCT 2026 Poster Presentation, Boston, MA.

75. Stecyk, A., Steinsapir, A., Trigueros, C., Martin, C. & Green, C. Viralgen: A Platform's Path to Rare Disease Patients: Accelerating AAV Manufacturing from Development to Commercialization. ASGCT 2026 Session, Boston, MA.

76. Davis, J. In vivo base editing extends the lifespan of a humanized mouse model of prion disease. ASGCT 2026 Oral Presentation, Boston, MA.

77. O'Connor, D. Preclinical development of AVB-406: An intravenous AAV-miRNA achieving sustained MAPT knockdown for the treatment of Alzheimer's disease. Session title: Neurologic diseases IV. ASGCT 2026 Oral Presentation, Boston, MA.

78. Joubert, R. Development and translational validation of AVB-406: an intravenous AAV-delivered miRNA targeting MAPT for the treatment of Alzheimer's disease. Session title: Neurologic diseases V. ASGCT 2026 Oral Presentation, Boston, MA.

79. Garza, I. Spatial single-cell assessment of CapX tropism, a second-generation human transferrin-targeted AAV capsid, at a sub-saturated dose in vivo. ASGCT 2026 Poster Session, Boston, MA.

80. Giannelli, S. Cell-type regulated Mecp2 gene therapy for the treatment of Rett syndrome. ASGCT 2026 Poster Session, Boston, MA.

81. Pardridge, W. M. Drug and gene delivery to the brain: the vascular route. *Neuron* **36**, 555–558 (2002).

82. Pocchiari, M. *et al.* Predictors of survival in sporadic Creutzfeldt-Jakob disease and other human transmissible spongiform encephalopathies. *Brain J. Neurol.* **127**, 2348–2359 (2004).

83. Thompson, A. G. B. *et al.* The Medical Research Council prion disease rating scale: a new outcome measure for prion disease therapeutic trials developed and validated using systematic observational studies. *Brain J. Neurol.* **136**, 1116–1127 (2013).

84. Appleby, B. S., Rincon-Beardsley, T. D., Appleby, K. K., Crain, B. J. & Wallin, M. T. Initial diagnoses of patients ultimately diagnosed with prion disease. *J. Alzheimers Dis. JAD* **42**, 833–839 (2014).

85. Rhoads, D. D. *et al.* Diagnosis of prion diseases by RT-QuIC results in improved surveillance. *Neurology* **95**, e1017–e1026 (2020).

86. Geschwind, M. D. *et al.* Quinacrine treatment trial for sporadic Creutzfeldt-Jakob disease. *Neurology* **81**, 2015–2023 (2013).

87. Haïk, S. *et al.* Doxycycline in Creutzfeldt-Jakob disease: a phase 2, randomised, double-blind, placebo-controlled trial. *Lancet Neurol.* **13**, 150–158 (2014).

88. Price, J. C., Guan, S., Burlingame, A., Prusiner, S. B. & Ghaemmaghami, S. Analysis of proteome dynamics in the mouse brain. *Proc. Natl. Acad. Sci. U. S. A.* **107**, 14508–14513 (2010).

89. Fornasiero, E. F. *et al.* Precisely measured protein lifetimes in the mouse brain reveal differences across tissues and subcellular fractions. *Nat. Commun.* **9**, 4230 (2018).

90. Vallabh, S. M. *et al.* Cerebrospinal fluid and plasma biomarkers in individuals at risk for genetic prion disease. *BMC Med.* **18**, 140 (2020).

91. Vallabh, S. M. *et al.* Prion protein quantification in human cerebrospinal fluid as a tool for prion disease drug development. *Proc. Natl. Acad. Sci. U. S. A.* **116**, 7793–7798 (2019).

92. Vallabh, S. M. *et al.* Fluid Biomarkers in Individuals at Risk for Genetic Prion Disease up to Disease Conversion. *Neurology* **103**, e209506 (2024).

93. Villar-Piqué, A. *et al.* Cerebrospinal Fluid Total Prion Protein in the Spectrum of Prion Diseases. *Mol. Neurobiol.* **56**, 2811–2821 (2019).

94. Minikel, E. V. *et al.* Domain-specific Quantification of Prion Protein in Cerebrospinal Fluid by Targeted Mass Spectrometry. *Mol. Cell. Proteomics MCP* **18**, 2388–2400 (2019).

95. Dorey, A. *et al.* Association of cerebrospinal fluid prion protein levels and the distinction between Alzheimer disease and Creutzfeldt-Jakob disease. *JAMA Neurol.* **72**, 267–275 (2015).

96. Abu Rumeileh, S. *et al.* Diagnostic Accuracy of a Combined Analysis of Cerebrospinal Fluid t-PrP, t-tau, p-tau, and Aβ42 in the Differential Diagnosis of Creutzfeldt-Jakob Disease from Alzheimer's Disease with Emphasis on Atypical Disease Variants. *J. Alzheimers Dis. JAD* **55**, 1471–1480 (2017).

97. Centers for Disease Control. Clinical Overview of Creutzfeldt-Jakob Disease (CJD). (2026).

98. Nagoshi, K., Sadakane, A., Nakamura, Y., Yamada, M. & Mizusawa, H. Duration of prion disease is longer in Japan than in other countries. *J. Epidemiol.* **21**, 255–262 (2011).

99. McNiven, K. *et al.* Enteral feeding is associated with longer survival in the advanced stages of prion disease. *Brain Commun.* **1**, fcz012 (2019).

100. Minikel, E. V. *et al.* Age at onset in genetic prion disease and the design of preventive clinical trials. *Neurology* **93**, e125–e134 (2019).

101. Torres, V. O. *et al.* Transferrin receptor-mediated transport at the blood-brain barrier is elevated during early development and maintained across aging and in an

Alzheimer's mouse model. *J. Cereb. Blood Flow Metab. Off. J. Int. Soc. Cereb. Blood Flow Metab.* **46**, 20–35 (2026).

102. Smith, A. F. *et al.* Brain Capillary Networks Across Species: A few Simple Organizational Requirements Are Sufficient to Reproduce Both Structure and Function. *Front. Physiol.* **10**, 233 (2019).

103. Mendell, J. R. *et al.* Single-Dose Gene-Replacement Therapy for Spinal Muscular Atrophy. *N. Engl. J. Med.* **377**, 1713–1722 (2017).

104. Okuyama, T. *et al.* A Phase 2/3 Trial of Pabinafusp Alfa, IDS Fused with Anti-Human Transferrin Receptor Antibody, Targeting Neurodegeneration in MPS-II. *Mol. Ther. J. Am. Soc. Gene Ther.* **29**, 671–679 (2021).

105. United States Food and Drug Administration. Potency Assurance for Cellular and Gene Therapy Products. (2023).

106. United States Food and Drug Administration. Human Gene Therapy Products Incorporating Human Genome Editing. (2024).

16. List of Abbreviations

Term	Definition
AA	Amino acid
AAV	Adeno-associated virus
AE	Adverse event
AEMPS	Agencia Española de Medicamentos y Productos Sanitarios (the Spanish Medicines Agency)
AGTX-1111	Apertura product using a hTfR1-binding capsid, TfR1 CapX (also known as TfR1Cap02), to deliver a therapeutic payload expressing cDNA for functional human TSC1
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
ASO	Antisense oligonucleotide
AST	Aspartate aminotransferase
BBB	Blood-brain barrier
BI-hTFR1	First generation TfR1 binding capsid (Huang et al. Science 2024) also known as TfR1Cap01 in referenced Apertura documents
C57BL/6J	Common strain of laboratory mice
CAG	Hybrid promoter: CMV enhancer-chicken beta-actin promoter-globin splice acceptor
CAP	AAV cap gene
CBC	Complete blood count
CBER	Center for Biologics Evaluation and Research
CDC	Centers for Disease Control
CDER	Center for Drug Evaluation and Research
CDMO	Contract development and manufacturing organization
CMV	Cytomegalovirus
cDNA	Complementary DNA; complementary deoxyribonucleic acid
CFU	Colony-forming units
cGMP	Current Good Manufacturing Practice
CHARM	Coupled Histone Tail for Autoinhibition Release of Methyltransferase
CHO	Chinese hamster ovary cells
CJD	Creutzfeldt-Jakob disease
CMC	Chemistry, Manufacturing, and Controls
CNS	Central nervous system
CpG	Cytosine-phosphate-guanine
CPIM	Critical Path Innovation Meeting
CRO	Contract research organization
CSF	Cerebrospinal fluid
CV	Coefficient of variation
ddPCR	Droplet digital polymerase chain reaction
DCN	Deep cerebellar nuclei
DEG	Differentially expressed gene
DNMT3A	DNA methyltransferase 3 alpha
DNMT3L	DNA Methyltransferase 3-Like

Term	Definition
DP	Drug product
dpi	Days post-inoculation
DRG	Dorsal root ganglia
DS	Drug substance
D3L	DNMT3L C-terminal domain
EFS	Elongation factor 1-alpha short
eGFR	Estimated glomerular filtration
ELISA	Enzyme-linked immunosorbent assay
EU	Endotoxin unit
FFI	Fatal familial insomnia
FIH	First-in-human
GLP	Good laboratory practices
GMP	Good manufacturing practices
GOI	Gene of interest
GSS	Gerstmann-Straussler-Scheinker
hBMVEC	Human brain microvascular endothelial cells
hCMEC/D3	Human cerebral microvascular endothelial cells
HEK293	Human embryonic kidney 293 cells
HLH	Hemophagocytic lymphohistiocytosis
HPLC	High-performance liquid chromatography
h*PRNP* mice	Transgenic mice with humanized *PRNP*
hTfR1	Human transferrin receptor 1
h*TFRC* KI	C57BL/6-Tfrc$^{tm1(TFRC)}$ knock in mice; humanized extracellular domain (exons 4-19) replacing mouse Tfrc exons 4-19.
H3L	DNMT3A autoinhibition release domain
IHC	Immunohistochemistry
IND	Investigational new drug
IRR	Infusion related reactions
ISH	In situ hybridization
ITR	Inverted terminal repeat
IV	Intravenous
KI	Knock-in
KO	Knock-out
KRAB	Kruppel-associated box repressor domain
LDH	Lactase dehydrogenase
LN-229	Human glioblastoma cells
LOF	Loss of function
LTFU	Long-term follow up
LP	Lumbar puncture
LUC	Luciferase
MCB	Master cell bank
miR122 TS	micro-RNA 122 target sequence
MOI	Multiplicity of infection
MRC-PDRS	Medical Research Council Prion Disease Rating Scale

Term	Definition
MRI	Magnetic resonance imaging
MSM	Medical safety monitor
NfL	Neurofilament light chain
NHP	Non-human primate
NIH	National Institutes of Health
NLS	Nuclear localization signal
OMIM	Online Mendelian Inheritance in Man, a compendium of human genes
PD	Pharmacodynamic
PIP	Posterior inclusion probability
polyA	Polyadenylation signal
PRNP	Gene encoding human prion protein
Prnp	Gene encoding mouse prion protein
PrP	Prion protein
QC	Quality control
rAAV	Recombinant adeno-associated virus
RLU	Relative light unit
RMT	Receptor-mediated transcytosis
RO	Retro-orbital
RT-qPCR	Reverse transcription quantitative polymerase chain reaction
SAD	Single ascending dose
SAE	Serious adverse event
SD	Standard deviation
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SEM	Standard error of the mean
siRNA	Short interfering RNA
SMA	Spinal muscular atrophy
SUB	Single-use production bioreactor
SVG-p12	SV40-immortalized human glial fetal cells
SV40 pA	SV40 polyadenylation signal
TAMC	Total aerobic microbial count
TBP	TATA box-binding protein
Tf	Transferrin
TFF	Tangential flow filtration
TfR1	Transferrin receptor 1; encoded by the *TFRC* gene
TfR1Cap01	Engineered AAV capsid derived from AAV9 capable of hTfR1-specific delivery to the CNS; also known as BI-hTFR1
TfR1Cap02	Engineered AAV capsid derived from AAV9 capable of hTfR1-specific delivery to the CNS, further engineered from TfR1Cap01; also known as TfR1 CapX
TfR1 CapX	Engineered AAV capsid derived from AAV9 capable of hTfR1-specific delivery to the CNS, further engineered from TfR1Cap01; also known as TfR1Cap02 and TfR1 CapX
TFRC	Gene encoding transferrin receptor 1
THLE-2	Transformed human liver epithelial cells
TMA	Thrombatic microangiopathy

Term	Definition
Tox	Toxicology
TSS	Transcription start site
TYMC	Total yeast and mold count
uORF	Upstream open reading frame
UTR	Untranslated region
U251MG	Human malignant glioblastoma cells
vg/dg	AAV viral genomes per diploid genomes
vg/kg	AAV viral genomes per kilogram of body weight
vWF	Von Willebrand factor
WPRE	Woodchuck hepatitis virus post-transcriptional regulatory element
WRO	Written Responses Only
WT	Wild-type
ZF	Zinc finger
ZFP	Zinc finger protein

17. Appendices

Appendix I: Mouse Models Used in PRNP-CHARM-001 Preclinical Studies

Appendix II: CDER Critical Path Innovation Meeting Minutes

Appendix III: Apertura Reference Memo Supporting PRNP-CHARM-001

Appendix IV: Apertura Gene Therapy AS-1801 NHP Study Report

Appendix V: Study Report: V1SION

Appendix VI: Study Report: CABS

Appendix VII: Study Report: H3X

Appendix VIII: Interim Study Report: BRAV3

Appendix IX: Executed Study Protocol: PRNP-CHARM-001 Safety and Biodistribution Study (Non-GLP toxicology study)

Appendix X: Interim Study Report: PRNP-CHARM-001 Safety and Biodistribution Study (Non-GLP toxicology study)

Appendix XI: Draft Study Protocol: GLP PRNP-CHARM-001 Safety and Biodistribution Study

Appendix XII: Study Report: PRNP-CHARM-001 In Vitro Off-target Analysis

Appendix XIII: Draft Study Protocol: PRNP-CHARM-001 Delivery-Device Compatibility and In-Use Stability Study

Appendix XIV: PRNP-CHARM-001 In Vitro Off-target Analysis RNA-seq Sample Information

Appendix I: Mouse Models Used in PRNP-CHARM-001 Preclinical Studies

1 BACKGROUND

As stated in Section III subsection B of the United States Food and Drug Administration (FDA) Center for Biologics Evaluation and Research (CBER) November 2013 Guidance for Industry titled "Preclinical Assessment of Investigational Cellular and Gene Therapy Products", regulatory guidance for gene and cell therapy advises use of animal species with pharmacological relevance to the investigational gene therapy product in preclinical studies[1]. Accordingly, mouse models with capability to demonstrate PRNP-CHARM potency and compatibility with different AAV capsids are leveraged in the PRNP-CHARM-001 preclinical program.

Four mouse models are used across the PRNP-CHARM-001 preclinical program, summarized in Table 1. Each model was selected to evaluate specific aspects of the therapeutic: the *hTFRC* KI model captures the pharmacologically relevant biodistribution of the TfR1 CapX capsid; the *hPRNP* models enable assessment of on-target PRNP-CHARM activity in the presence of human *PRNP*; and the *hTFRC KI*;*hPRNP* double-humanized model allows both capsid-dependent CNS delivery and human *PRNP* targeting to be evaluated simultaneously. Wild-type C57BL/6J or C57BL/6N mice were used in early proof-of-concept experiments. This appendix defines each model, describes its genetic composition, and summarizes which studies used it. These short names are used consistently throughout the main Pre-IND document and all study reports.

Table 1. Mouse lines used in the preclinical development of PRNP-CHARM-001

Short Name	*PRNP* Allele	*TFRC* Allele	Mouse Prnp CHARM ZF on-target	Human PRNP CHARM ZF on-target	TfR1 CapX CNS Delivery*	Studies
WT	Endogenous mouse *Prnp*	Endogenous mouse *Tfrc*	Yes	No	No	Historical PoC; background control
hTFRC KI	Endogenous mouse *Prnp*	Tfrctm1(*TFRC*) hom (humanized extracellular domain)	Yes	No	Yes	CABS; Pilot Tox (non-GLP); GLP Tox
hPRNP	Tg25109/+ (human *PRNP*, ~3 copies); $Prnp^{ZH3/ZH3}$	Endogenous mouse *Tfrc*	No	Yes	No (PHP.eB used)	V1SION (CMR-4033)
hTFRC KI; *hPRNP*	Tg26372/Tg26372 (human *PRNP*, ~6 copies); $Prnp^{ZH3/ZH3}$	Tfrctm1(*TFRC*) hom	No	Yes	Yes	H3X (CMR-4210); BRAV3

*All mouse lines are compatible with AAV-PHP.eB, leveraged as a surrogate capsid for TfR1 CapX

2 DESCRIPTION OF MOUSE MODELS

WT (Wild-type C57BL/6J or C57BL/6N)

- **Background:** Wild-type C57BL/6J or C57BL/6N mice.
- ***Prnp* allele:** Endogenous mouse *Prnp* (two copies).
- ***Tfrc* allele:** Endogenous mouse *Tfrc*.

- **Pharmacological relevance:** These mice express mouse *Prnp* and mouse *Tfrc*. The TfR1 CapX capsid does not achieve enhanced CNS biodistribution in WT mice.
- **Study usage:** WT mice were used as negative controls in capsid biodistribution comparisons with *hTFRC KI* mice and in historical proof-of-concept potency and efficacy experiments using the mouse-specific AAV-PHP.eB capsid and mouse *Prnp*-targeting CHARM constructs.

***hTFRC* KI (Humanized TfR1 knock-in mice)**

- **Background:** C57BL/6-Tfrc$^{tm1(TFRC)Bcgen}$/Bcgen mice (B-hTFR1 mice; Biocytogen) are referred to as *hTFRC* KI.
- ***Prnp* allele:** Endogenous mouse *Prnp* (two copies).
- ***TFRC* allele:** Homozygous humanized *TFRC* knock-in, in which mouse *Tfrc* exons 4–19 encoding the extracellular domain have been replaced with the corresponding human *TFRC* sequence (including human exons 4–19 and introns). *hTFRC* is expressed from the endogenous mouse *Tfrc* locus at physiologically normal levels[2]. The humanized extracellular domain supports TfR1 CapX binding and transcytosis into the CNS.
- **Pharmacological relevance:** The mice express mouse *Prnp*; therefore, PRNP-CHARM-001 has no on-target activity (*PRNP* promoter silencing/repression) in this model.
- **Study usage:** *hTFRC* KI mice were used for the 4-dose CABS TfR1 CapX - AAV-PHP.eB potency bridging study as well as the non-GLP safety and distribution study. *hTFRC* KI mice will be used for the GLP safety and distribution study where pharmacologically relevant TfR1 CapX CNS distribution is required. The FDA concurred with the use of this model as the sole species for definitive biodistribution and toxicology studies (INTERACT response, PTS#PS009720, February 2025).

***hPRNP* (Human *PRNP* transgenic mice on a mouse *Prnp* null background)**

- **Background:** Tg25109/+; Prnp$^{ZH3/ZH3}$ mice.
- ***PRNP* allele:** Heterozygous BAC transgene (Tg25109) carrying a 46 kb genomic fragment of the human *PRNP* locus (129M haplotype, approximately 3 copies of human *PRNP*) integrated at chromosome 12[3]; homozygous *ZH3* allele (8 bp frameshift deletion in mouse *Prnp*[4]) eliminating endogenous mouse PrP expression. These mice express human PrP from the human *PRNP* promoter and are therefore compatible with targeting by the human *PRNP*-targeting ZF-CHARM domain.
- ***Tfrc* allele:** Endogenous mouse *Tfrc*.
- **Pharmacological relevance:** These mice are not compatible with TfR1 CapX-mediated CNS delivery; the AAV-PHP.eB capsid (which uses the mouse LY6A receptor for transduction) was used for CNS delivery in studies with this model.
- **Study usage:** *hPRNP* mice were used in the V1SION study (CMR-4033) for transgene candidate selection, comparing construct designs for PRNP-CHARM potency in the presence of human *PRNP*.

***hTFRC KI*;*hPRNP* (Double-humanized mice on a mouse *Prnp* null background)**

- **Background:** Tg26372/Tg26372; Tfrc$^{tm1(TFRC)Bcgen}$/Bcgen/Tfrc$^{tm1(TFRC)Bcgen}$/Bcgen; Prnp$^{ZH3/ZH3}$ mice.
- ***PRNP* allele:** Homozygous BAC transgene (Tg26372) carrying a higher copy number of the human *PRNP* locus than Tg25109 (same 46 kb genomic fragment, 129M haplotype; homozygosity yields approximately 6 copies of human *PRNP* and correspondingly higher baseline PrP expression than the *hPRNP* model); homozygous *ZH3* allele eliminating endogenous mouse PrP expression.
- ***TFRC* allele:** Homozygous humanized *TFRC* KI (same Tfrc$^{tm1(TFRC)Bcgen}$/Bcgen allele as the *hTFRC* KI model).
- **Pharmacological relevance:** These mice support both TfR1 CapX-mediated CNS delivery and on-target PRNP-CHARM-001 silencing of human *PRNP*. They are the most translationally complete preclinical model for PRNP-CHARM-001.
- **Study usage:** *hTFRC*;*hPRNP* KI mice were used in the H3X study (CMR-4210) for lead construct confirmation with the TfR1 CapX capsid, and in the BRAV3 study for potency bridging of Broad vs. Viralgen-manufactured material.

3 ADDITIONAL NOTES

- WT mice were used only in historical proof-of-concept studies and as controls. They are not used in any of the formal study reports filed with this Pre-IND.
- The *hTFRC* KI and *hTFRC* KI;*hPRNP* models are homozygous for the same *TFRC* humanized allele ($Tfrc^{tm1(TFRC)Bcgen}$/Bcgen). The key difference is the presence or absence of a human *PRNP* transgene and the presence or absence of the wild-type mouse *Prnp* allele.
- The *hPRNP* and *hTFRC* KI;*hPRNP* models both carry the *ZH3* allele eliminating mouse PrP expression, but carry different human *PRNP* transgene insertions (Tg25109 vs. Tg26372), resulting in different copy numbers and baseline PrP expression levels as previously described[3]
- TfR1 CapX CNS delivery is pharmacologically relevant only in models carrying the humanized $Tfrc^{tm1(TFRC)Bcgen}$/Bcgen allele (*hTFRC* KI and *hTFRC* KI;*hPRNP*). In *hPRNP* and WT mice, TfR1 CapX does not achieve enhanced CNS biodistribution because TfR1 CapX does not bind mouse TfR1.

4 REFERENCES

1. United States Food and Drug Administration. Preclinical Assessment of Investigational Cellular and Gene Therapy Products. (2013).

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

3. Gentile, J. E. et al. Divalent siRNA for prion disease. Nucleic Acids Res. 54, gkag287 (2026).

4. Nuvolone, M. et al. Strictly co-isogenic C57BL/6J-Prnp-/- mice: A rigorous resource for prion science. J. Exp. Med. 213, 313–327 (2016).

Memorandum

Date: December 26, 2017

Subject: Critical Path Innovation Meeting: Genetic Prion Disease

Date of meeting: November 14, 2017

Requestor: Broad Institute

Note: Discussions at Critical Path Innovation Meetings are informal. All opinions, recommendations, and proposals are unofficial and nonbinding on FDA and all other participants.

FDA

Center for Drug Evaluation and Research

Office of the Center Director (OCD)
Bob Temple, M.D., Deputy Director for Clinical Science
Janet Woodcock, M.D., Center Director

Office of Translational Sciences (OTS) Immediate Office
Mitra Ahadpour, M.D., D.A.B.A.M., Deputy Director
Ru Chen, Ph.D., ORISE Fellow
Jacqueline Brooks-Leighton, GWCPM, Project Manager
Chekesha Clingman, Ph.D., M.B.A., Associate Director for Strategic Partnerships
Sarmistha Sanyal, Ph.D., Staff Fellow

OTS, Office of Clinical Pharmacology
Mike Pacanowski, Pharm.D., M.P.H., Associate Director for Genomics and Targeted Therapies
Hobart Rogers, Pharm.D., Ph.D., Reviewer, Genomics and Targeted Therapies

Office of New Drugs (OND) Immediate Office
Yujin Chung
Jonathan Goldsmith, M.D., Associate Director, Rare Disease Program
Chris Leptak, M.D., Ph.D., Associate Director, Biomarker Development and Regulatory Science
Parima Ghafoori
Peter Stein, M.D, Deputy OND Office Director

OND Immediate Office, Clinical Assessment Staff (COA)
Elektra Papadopoulos, M.D., M.P.H., Acting Associate Director, Clinical Outcomes Assessments Staff

OND Office of Antimicrobial Products
Eric Donaldson, Ph.D., Microbiologist, Division of Antiviral Products

Pat Harrington, Ph.D., Microbiologist, Division of Antiviral Products
Jules O'Rear, Ph.D., Supervisory Microbiologist, Division of Antiviral Products
Mary Singer, M.D., Ph.D., Division of Antiviral Products

OND Office of Drug Evaluation I
Eric Bastings, M.D., Deputy Director, Division of Neurology Products
Billy Dunn, M.D., Director, Division of Neurology Products

Center for Biologics Evaluation and Research
Office of Biostatistics and Epidemiology, Office of Blood Research and Review
David Asher, M.D., Chief, Lab of Bacterial and Transmissible Spongiform, Division of Emerging and Transfusion Transmitted Diseases
Chintamani Atreya, Associate Director for Research and Review
Luisa Gregori

Center for Radiological Health and Devices
Reena Phillip, Ph.D., Director, Division of Molecular Genetics and Pathology

REQUESTER:
Broad Institute
Steven Arnold, M.D.
Eric Lander, Ph.D.
Eric Mikel, MS
Margaret Orseth, M.B.A.
Sonia Vallabh, JD

1. BACKGROUND

Prion disease is a fatal, rare neurodegenerative disease caused by the misfolding of the prion protein (PrP). Familial prion disease accounts for approximately 15% of all human occurrences of the disease. Scientists from the Broad Institute of MIT and Harvard requested a CPIM to discuss their preliminary work to develop a therapeutic strategy for genetic prion disease.

2. DISCUSSION

The research team from the Broad Institute presented data on the pathogenesis of prion disease. All cases of prion disease in humans and animals are due to the same causative factor, a gain-of-function mutation in the prion protein gene (*PRNP)* which produces a misfolded version of the prion protein (PrP) known as a "prion". The prion acts as an infectious agent that can interact with normal prion proteins leading to the aberrant form known as PrP Scrapie (PrP^{SC}) and can spread across the brain. The research team indicated that less than 1% of prion disease cases are acquired through infection and that 85% of cases occur spontaneously, with no known environmental or genetic cause. They further noted that the remaining 15% of prion disease cases are due to a genetic cause — protein-altering variants in *PRNP*, with three high-

penetrance mutations comprising the majority of genetic cases. Prion disease progression is extremely rapid, with death occurring approximately 6 months from onset of initial symptoms of disease. The age of onset in genetic prion disease is highly variable for any given allele. Previously published biochemical and human and animal genetic data support a central role for PrP in prion disease. The research team presented data from mouse models showing that PrP knockout mice are resistant to prion disease, and postnatal suppression of PrP can delay or prevent prion disease. The adverse effects of PrP loss appeared to be minimal. These results indicate that therapeutic strategies which turn off or substantially reduce PrP expression by targeting *PRNP*, RNA or PrP in the brain may provide protection against the disease. Preclinical studies of compounds shown to extend survival in mouse models, but not effective against human prion disease stains, suggest that treatment can be highly effective before disease symptoms occur.

Broad Institute team proposed antisense oligonucleotides (ASOs) against PrP RNA as therapeutic candidates for prion disease. Results from preclinical and clinical studies of ASO targets against other diseases can be leveraged to support the safety, tolerability, and pharmacodynamics and pharmacokinetics of ASOs in humans (i.e. Huntington's disease model). The Broad Institute is collaborating with a pharmaceutical company on proof-of-concept studies in mice to assess the effectiveness of ASOs in reducing PrP mRNA levels in mouse cortex and spinal cord. Preliminary data in mice show that the ASOs are capable of reducing PrP RNA levels in the brain by 50%. Survival studies in mice intracerebrally infected with prions and treated with ASOs are ongoing. The Broad Institute team anticipates preliminary results from these studies in January 2018. FDA recommended that the Broad Institute team conduct natural history, safety and pharmacokinetics studies in non-human primates before moving to human studies.

The Broad Institute research team provided rationale for the use of cerebrospinal fluid (CSF) PrP measurement as a surrogate endpoint for evaluating efficacy of ASOs in lowering PrP levels in humans. CSF PrP levels can be quantified with ELISA in presymptomatic individuals who are more likely to benefit from therapy than symptomatic individuals. Studies indicate that CSF PrP measurement is stable over time. In addition, the Broad Institute team is exploring the use of mass spectrometry to quantify PrP in CSF. FDA recommended the conduct of studies to assess the distribution of PrP in different cell types in the brain, and how effective ASOs are in reducing PrP expression in these different cell types. The FDA also recommended evaluating knockdown of PrP in NHPs to assess pharmacodynamics and the impact of loss of PrP in a primate model, as well as evaluation of candidate ASOs in symptomatic human individuals. The Broad Institute team and their commercial partner are working to identify ASO candidates for human studies. FDA expressed support of the evaluation of CSF PrP as a surrogate endpoint and recommend the use of registries to identify prion disease kindreds. The Broad Institute team noted that the National Prion Disease Surveillance Center can be a resource to identify potential patients for clinical trials.

NEXT STEPS

- Broad Institute will share results of their survival studies in mice with FDA.

- Broad Institute will share information on data being collected in current or planned patient registries. Chris Leptak in OND will help to coordinate feedback on general registry design elements.
- FDA encourages subsequent communication with the Broad Institute around targeted topics to advance development of ASOs for treatment of prion disease. Topics include preclinical studies, assay validation, pharmtox, and registries/databases. The Division of Neurology Products will serve as the point of contact for additional discussions with the Broad Institute.
- FDA will share information on a bioinformatic tool to assist with ASO candidate selection.

Bioinformatics Analysis of ASO Targets

To characterize the potential off-targets of antisense oligonucleotides (ASO), we request that the following analyses be performed in a bioinformatics study using BLAST or other tools that compare the ASO target to genes in an appropriate database:

1. Please provide a list of all potential off-target matches in the human transcriptome, including those matches identified for the sense and antisense strands of each ASO with relaxed matching criteria. For each of these, describe available information on mouse knockouts and human genetic disease. Please comment on how any significant off-target effects would be monitored.
2. Please provide a list of potential off-target matches in the human mitochondrial transcriptome (example: https://www.mitomap.org/foswiki/bin/view/MITOMAP/WebHome).
3. Please determine the variation within the off-target matches in the transcriptomes of different populations in the US. Are different populations more susceptible to off-target effects than others?
4. Please determine the conservation among the candidate off-target human genes with their respective mouse and nonhuman primate genes. Are these sites sufficiently different in mouse and nonhuman primate such that toxicities related to off-target matches would not be present in these animals?

Docusign Envelope ID: D8A85DAC-F886-8700-8224-32BA2E18AC8E

Apertura Gene Therapy, LLC
[redacted]
New York, NY 10010

APPENDIX III: Apertura Reference Memo Supporting PRNP-CHARM-001

Apertura GENE THERAPY

June 2, 2026

Julia Russell, MS
Regulatory Project Manager (RPM)
United States Food and Drug Administration
Division of Review Management and Regulatory Review
Office of Review Management and Regulatory Review
Office of Therapeutic Products
Center for Biologics Evaluation and Research

Re: PRNP-CHARM-001 Program, PTS #PS009720, Type B Pre-IND Meeting
Subject: Authorization to Reference Previously Submitted Apertura Information

Dear Ms. Russell,

On behalf of the Apertura team, and pursuant to 21 CFR 312.23(b), Apertura Gene Therapy, LLC ("Apertura") hereby authorizes the Sponsor of the PRNP-CHARM-001 program, Dr. Sonia M. Vallabh at the Broad Institute of MIT and Harvard, to reference the following information previously submitted by Apertura to FDA in support of Apertura's AGTX-1111 program, "*Intravenous AAV-based Gene Therapy for Tuberous Sclerosis Complex Caused by TSC1 Gene Mutation*":

1. AGTX-1111 INTERACT Meeting Request, submitted January 31, 2024, PTS # PS009041;
2. FDA Meeting Denied correspondence for AGTX-1111, dated February 16, 2024, PTS # PS009041;
3. Apertura correspondence responding to the AGTX-1111 INTERACT Meeting Denial, dated February 21, 2024, PTS # PS009041;
4. AGTX-1111 INTERACT Meeting Request Resubmission, submitted March 20, 2024, referencing original submission PTS # PS009041 and assigned PTS # PS009147;
5. FDA correspondence accepting the AGTX-1111 INTERACT Meeting Request Resubmission, dated April 10, 2024, PTS # PS009147;
6. FDA Written Responses Only for the AGTX-1111 INTERACT meeting, dated June 4, 2024, PTS # PS009147, Meeting ID # 15601;
7. Apertura's clarification request regarding the AGTX-1111 INTERACT Written Responses Only, dated June 23, 2024, PTS # PS009147, Meeting ID # 15601; and
8. FDA's response to Apertura's clarification request, dated July 12, 2024, PTS # PS009147, Meeting ID # 15601.

This authorization is provided to permit FDA's review staff to reference the above-listed Apertura submissions and associated FDA correspondence in connection with the PRNP-CHARM-001 Type B Pre-IND meeting request and related FDA review activities for PTS # PS009720.

Thank you, and please contact me if the Agency requires any additional information regarding this authorization.

Sincerely,

[redacted]

Jorge Santiago-Ortiz, Ph.D.
Vice President, CMC & Regulatory Affairs
Apertura Gene Therapy, LLC
[redacted]

Appendix IV: APERTURA GENE THERAPY AS-1801 NHP STUDY REPORT

1 AS-1801 EXECUTIVE SUMMARY

The goal of this study was to evaluate the tissue distribution and tolerance of the human transferrin receptor (hTfR1)-binding capsid TfR1Cap01 in non-human primates (NHPs), specifically cynomolgus macaques (*Macaca fascicularis*), after being administered individually or co-administered with two other comparator capsids. Based on *in silico*, *in vitro*, and *in vivo* evaluations, TfR1Cap01 and TfR1Cap02 (also referred to as TfR1 CapX) specifically engage the human transferrin receptor and do not bind orthologs of transferrin receptor present in model systems such as wild type mice and cynomolgus macaques. This study was executed to empirically demonstrate the human specificity of TfR1Cap01 in cynomolgus macaques. TfR1Cap01 is a relevant capsid for this evaluation in NHPs as it contains the same peptide insertion needed for hTfR1 binding as TfR1Cap02, while TfR1Cap02 differs in flanking modifications. This study was performed at Labcorp (Madison, WI), a contract research organization (CRO), as study number 8517271 according to established protocols approved by the CRO's IACUC committee and standard operation procedures. Dosing of TfR1Cap01, both alone and in combination with two other AAV capsids, was well tolerated in cynomolgus macaques. Clinical chemistry performed following dosing revealed transient increases in liver enzyme levels that resolved over the course of the study, consistent with published studies profiling clinical chemistry responses in NHPs, including cynomolgus macaques, dosed with AAV[1]. Distribution analyses using *in situ* hybridization to detect viral episomes showed lack of episome signal in the brain for TfR1Cap01, while episome signal was detected in the liver, providing confirmation of successful test article dosing and ISH-based episome detection. The distribution of TfR1Cap01 was consistent with the findings by Huang et al.[2]. That is, the TfR1Cap01 capsid does not efficiently distribute to cynomolgus macaque CNS tissues.

2 AS-1801 STUDY SCOPE

As stated in Section III subsection B of the United States Food and Drug Administration (FDA) Center for Biologics Evaluation and Research (CBER) November 2013 Guidance for Industry titled "Preclinical Assessment of Investigational Cellular and Gene Therapy Products", regulatory guidance for gene and cell therapy advises use of animal species with pharmacological relevance to the investigational gene therapy product in preclinical studies[3]. To this end, we sought to evaluate the distribution of the human transferrin receptor (hTfR1)-binding capsid TfR1Cap01 in cynomolgus macaques (*Macaca fascicularis*) to determine the suitability of this species for preclinical evaluations. The TfR1Cap01 capsid has been engineered to specifically bind to hTfR1, and as a result it has an enhanced central nervous system (CNS) tropism when hTfR1 is expressed[2]. Through complementary findings from extensive *in silico*, biochemical, and *in vitro* characterizations of TfR1Cap01 and TfR1Cap02 (also referred to as TfR1 CapX), Apertura has confirmed these capsids are not responsive to transferrin receptors from other commonly used laboratory animal species, including NHPs, due to differences in the amino acid sequences between NHP and human transferrin receptors. The compatibility of TfR1Cap01 and TfR1Cap02 with different model species based on hTfR1 expression is summarized in Table 1. TfR1Cap01 is a relevant capsid for this evaluation in NHPs as it contains the same peptide insertion needed for hTfR1 binding as TfR1Cap02, while TfR1Cap02 differs in flanking modifications. Based on in vitro evaluations, Apertura does not expect the TfR1Cap01 capsid to exhibit enhanced CNS tropism in cynomolgus macaques or other NHPs in vivo.

Table 1. Model systems and anticipated compatibility with TfR1Cap01 and TfR1Cap02 based on humanized TfR1 receptor expression.

System	Scientific name	Expresses humanized TfR1	Compatible with TfR1Cap01 and TfR1Cap02
Human	*Homo sapiens*	Yes	Yes
B-hTFR1 mouse (Biocytogen)	*Mus musculus*	Yes	Yes
C57BL/6J mouse	*Mus musculus*	No	No
Rat	*Rattus norvegicus*	No	No
Pig	*Sus domesticus*	No	No
Cynomolgus macaque	*Macaca fascicularis*	No	No

The goal of this study was to evaluate the biodistribution of the TfR1Cap01 capsid in cynomolgus macaque tissues after being administered individually or being co-administered with two other comparator capsids – AAV9 and BI-93, an engineered AAV capsid derived from AAV9; BI-93 was identified in a prior NHP library study as a capsid that was enriched in the CNS tissues at a 4-hour timepoint and serves as an additional comparator. Evaluation of a pool of capsids that can be distinguished using sequence-specific reagents (e.g. *in situ* hybridization probes) allows comparison of TfR1Cap01 and comparator capsid distribution within the same animal, controlling for subject-to-subject variability in dosing and viral episome detection. Accordingly, this pooled capsid approach reduces the number of NHPs needed for distribution analyses, in line with the FDA and International Council for Harmonization (ICH) "3R" principles (reduce, refine, and replace) of animal research, specifically reducing the number of animals necessary for a study while maintaining rigor. The transgene cassettes leveraged in this study are based on the referenced publication[2] and consist of a modified CAG (CEG) promoter driving expression of the human Glucosylceramidase Beta 1 (GBA1) gene with a C-terminal influenza virus hemagglutinin (HA) tag; a woodchuck hepatitis virus post-transcriptional regulatory element (WPRE), and human growth hormone (hGH) polyadenylation signal were included. GBA1 constructs encode the same protein but were codon-optimized (co) differently such that they differ at the nucleotide level; as such, each can be distinguished from one another at the DNA- and RNA-sequence level using sequence-specific probes for *in situ* hybridization (ISH).

The study evaluated one timepoint of IV vector administration (AAV9-seronegative female cynomolgus macaques 20-29 months in age), one dose level per each vector administration (Table 2), and one post-dose study duration (28 days). Test articles (TAs), each packaging a differently codon-optimized (co) GBA1 construct (Table 2), were produced at Apertura Gene Therapy and tested to meet minimal requirements for safety, strength, potency, and purity. TAs were dosed using a single IV bolus administration via saphenous vein. A total of two (2) cynomolgus macaques were dosed: one cynomolgus macaque receiving an administration of TfR1Cap01 as a single capsid, and one cynomolgus macaque receiving a multi-capsid administration of TfR1Cap01, AAV9, and BI-93. Notably, TA-2 is formulated with a ~10-fold higher dosage of TfR1Cap01 relative to the AAV9 comparator. This was done to ensure that sufficient product was administered to allow for distribution to be detected and provide further confidence that a lack of observed TfR1Cap01 CNS distribution is not simply driven by an insufficiently high capsid dosage.

Table 2. Test articles evaluated in AS-1801.

TA #	AAV Capsid	Payload	Capsid Dose (vg/kg)	Total AAV Dose (vg/kg)
TA-1	TfR1Cap01	CEG.GBA1co6.HA.WPRE.hGHpA	9.6E13	9.6E13
TA-2	AAV9	CEG.GBA1co5.HA.WPRE.hGHpA	7.2E12	2.3E14
	TfR1Cap01	CEG.GBA1co6.HA.WPRE.hGHpA	9.6E13	
	BI-93	CEG.GBA1co7.HA.WPRE.hGHpA	1.3E14	

3 AS-1801 ANALYSIS PLAN

The AS-1801 study analysis plan was predefined and determined prior to study initiation. The analysis plan was executed as detailed below without change or amendment.

AAV9 Neutralizing antibody titer analysis: As per Labcorp standard practice, prior to dosing, NHPs will be screened to identify AAV9 sero-negative subjects for study inclusion. The AAV9 neutralizing antibody assessment is appropriate for TfR1Cap01 as this capsid is derived from the AAV9 serotype; accordingly, we anticipate neutralizing antibodies specific to AAV9 identified in potential study subjects would also neutralize TfR1Cap01. In this same vein, we anticipate that neutralizing antibodies generated in response to TfR1Cap01 dosing would also neutralize the AAV9 reporter vector used in the titering assay due to the high degree of similarity in capsid sequence between TfR1Cap01 and AAV9. AAV9 neutralizing antibody titers will be measured from up to 21 cynomolgus macaques from Worldwide Primates Inc. (Miami, FL) to identify study subjects. Additionally, AAV9 neutralizing antibody titers will be measured from dosed subjects to confirm successful dosing, as evidenced by an increase in AAV9 neutralizing antibody titers relative to pre-dose titers. For AAV9 neutralizing antibody analysis, blood will be collected from NHPs via femoral vein into serum separator tubes, and serum will be harvested into three approximately equal aliquots and frozen on dry ice. Serum will be shipped to VRL Diagnostics (San Antonio, TX) for AAV9 neutralizing antibody titer analysis. The AAV9 neutralizing antibody titer analysis will use a cell-based assay where an AAV9 reporter vector expressing GFP is pre-incubated with a serial dilution of the test sample before in vitro transduction of a permissible cell line. The reported titer will be the serum sample dilution for which 50% inhibition of transduction is observed. A summary result of “Negative” or “Positive” will be provided, where a “Negative” result indicates lack of inhibition for the least dilute sample tested (1:5 dilution of serum). Subjects will be eligible for study inclusion if a “Negative” result is obtained from pre-dose AAV9 neutralizing antibody titer analysis.

Hematology, coagulation, and clinical chemistry analysis: Hematology, coagulation, and clinical chemistry analysis will be used to assess tolerability and safety of the Test Articles when administered to cynomolgus macaques at the indicated dose levels. Blood samples for hematology (approximately 1 mL), coagulation (approximately 1.8 mL), and clinical chemistry (approximately 1 mL) will be collected from animals fasted overnight via a femoral vein. Blood samples will be collected twice prior to dosing and once on Days 1, 3, 8, 15, 22, and 29 after dosing. The specific tests to be included in the hematology, coagulation, and clinical chemistry panels are provided in Table 3.

Table 3. Hematology, coagulation, and clinical chemistry tests performed on subject samples.

Test panel	Tests performed	
Hematology	● Red blood cell (erythrocyte) count ● Hemoglobin ● Hematocrit ● Mean corpuscular volume ● Mean corpuscular hemoglobin ● Mean corpuscular hemoglobin concentration ● Red cell distribution width ● Absolute reticulocyte count	● Absolute neutrophil count ● Absolute lymphocyte count ● Absolute monocyte count ● Absolute eosinophil count ● Absolute basophil count ● Absolute large unstained cell count ● Blood smear ● Platelet count ● White blood cell (leukocyte) count
Coagulation	● Prothrombin time ● Fibrinogen	● Activated partial thromboplastin time
Clinical chemistry	● Glucose ● Urea nitrogen ● Creatinine ● Total protein ● Albumin ● Globulin ● Albumin:globulin ratio ● Total cholesterol ● Triglycerides ● Total bilirubin	● Aspartate aminotransferase ● Alanine aminotransferase ● Alkaline phosphatase ● Gamma glutamyltransferase ● Creatine kinase ● Calcium ● Inorganic phosphorus ● Sodium ● Potassium ● Chloride

***In situ* hybridization (ISH) distribution analysis**: Biodistribution of each AAV capsid will be evaluated through ISH analysis of formalin-fixed, paraffin embedded (FFPE) tissues harvested 28 days after dosing. All ISH work will be executed by Advanced Cell Diagnostics, Inc. (ACD; Newark, CA) under study number SP-015498. ISH work will be performed through ACD's Professional Assay Services (PAS), which provides good clinical laboratory practice-compliant ISH assay services in support of preclinical and clinical studies. Sequence-specific ISH probes will be developed to target viral episomal DNA (each differently codon-optimized GBA1 payload) as a marker of transduction. Probes designed to detect the GBA1 payload present in viral episomal DNA will lack sequence specificity to the endogenously expressed cynomolgus macaque *GBA1* ortholog. To assess cell type specific

transduction in brain samples, the neuronal marker RNA binding fox1 homolog 3 (RBFOX3) and the astrocytic marker SRY-box containing gene 9 (SOX9) will be probed in conjunction with the viral episome. Cell-type markers will be probed using both ISH probes to detect RNA, as well as immunohistochemistry to detect the proteins which are nuclearly localized and will enable higher confidence in quantification. Initial histology analysis will probe brain and liver tissues using two approaches. The first approach will be to use ISH for AAV DNA episomes using the GBA probes in combination with the SOX9 and RBFOX3 RNA probes in brain tissue, and ISH for AAV DNA episomes using the GBA probes in liver tissue. The second approach will be to use the combined ISH probes for AAV DNA episomes in combination with immunohistochemistry (IHC) for SOX9 and RBFOX3 proteins in the brain samples. ISH analysis also will include semi-quantitative scoring of evaluated tissues to generate approximate transduction levels. Other tissues that may also be analyzed include spinal cord (cervical, thoracic, lumbar), dorsal root ganglia (DRG), heart, muscle (biceps femoris), kidney, spleen, and eyes. AAV capsid distribution may also be evaluated by droplet digital polymerase chain reaction (ddPCR) analysis of DNA extracted from collected tissue.

4 AS-1801 IN-LIFE PROCEDURES SUMMARY

The study was executed at a CRO – Labcorp (Madison, WI) – as study number 8517271 according to established protocols approved by the CRO's IACUC committee and standard operation procedures, as described in the study protocol. Labcorp has extensive experience in execution of GLP and non-GLP preclinical studies in support of gene and cell therapy development, where their lab network has supported over 80% of all FDA-approved cell and gene therapies. Labcorp is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

AAV9 Neutralizing antibody titer analysis: Prior to dosing, cynomolgus macaques were screened to identify AAV9 sero-negative subjects for study inclusion. At least 3 weeks prior to shipment to the test facility, blood samples (up to 1 mL) were collected via a femoral vein from up to 21 females at Worldwide Primates Inc. (Miami, FL). Blood was collected into serum separator tubes (without anticoagulant), allowed to clot at room temperature, and centrifuged within 1 hour of collection. Serum was harvested in screw-capped tubes and placed on dry ice until stored in a freezer, set to maintain-60 to -80°C. Samples were shipped on dry ice to VRL Diagnostics (San Antonio, TX) and analyzed for AAV9 neutralizing antibodies. Animals were selected for shipment based on neutralizing AAV9 antibody titer results of ≤1:5 ("Negative" result). Eight naïve female cynomolgus macaques were received at Labcorp (Madison, WI) from Worldwide Primates Inc. (Miami, FL). AAV9 neutralizing antibody titer results are presented in Table 4 for the eight animals received at Labcorp. Animals were acclimated to the test facility for at least 5 weeks prior to study initiation.

Table 4. AAV9 neutralizing antibody (NAb) titer analysis screening for sero-negative NHPs for study inclusion

Pre-test subject ID	AAV9 NAb titer result	Study usage	Study subject ID	Age at TA dosing (months)
PM231236	Negative	AS-1901 (capsid library study)	P0001	23-24
PM231237	Negative	AS-1901 (capsid library study)	P0002	23-24
PM231238	Negative	AS-1801 (this study)	P0201	28-29

Pre-test subject ID	AAV9 NAb titer result	Study usage	Study subject ID	Age at TA dosing (months)
PM231239	Negative	Transferred to Labcorp stock colony	N/A	Not dosed
PM231240	Negative	AS-1901 (capsid library study)	P0101	23-24
PM231241	Negative	AS-1901 (capsid library study)	P0102	23-24
PM231242	Negative	Transferred to Labcorp stock colony	N/A	Not dosed
PM231243	Negative	AS-1801 (this study)	P0301	28-29

Dosing procedures: TA and diluent were supplied to CRO by Apertura along with detailed formulation instructions. The diluent used was the same as the viral formulation buffer and consisted of 20 mM Tris, 200 mM sodium chloride, 1 mM magnesium chloride, 0.005% Pluronic F-68, pH 8.0. TAs were shipped on dry ice with a temperature monitoring device and stored at -80°C until formulation. Starting seven days before TA dosing and continuing throughout the study, animals were dosed with the immunosuppressant Prednisolone (1mg/kg/day) via oral gavage. Administration of corticosteroids prior to AAV dosing is consistent with immunosuppression approaches used for clinical dosing of AAV. While more sophisticated immunosuppression approaches exist (e.g. inclusion of rituximab and mTOR inhibitors like sirolimus[4]), this was not feasible nor necessary to implement to achieve the goals of this study. IV injections of TA occurred for all animals via saphenous vein as required for each study group. Animals were observed daily post-dose for 28 days.

Blood collection: Blood samples were collected twice prior to dosing and once on Days 1, 3, 8, 15, 22, and 29 after dosing. The Day 3 post-dosing clinical pathology samples were stored in refrigerated conditions following collection, instead of the normal frozen conditions required for storage. As the Day 3 post-dosing samples were stored incorrectly, they were not considered stable at the time of analysis and were excluded from clinical chemistry analysis. An additional blood sample was collected on Day 13 post-dosing and subjected to hematology, coagulation, and clinical chemistry analyses to provide closer monitoring of elevated ALT, AST, and total bilirubin levels in subjects. This additional sample was included in study analyses.

Veterinary observations: TA-related clinical observations included signs of vomitus observed 5 days (TA-1) and 3 and 5 days (TA-2) after TA administration. Signs of dehydration were also observed by the veterinary staff 8 days after dosing in both animals. TA-related veterinary interventions included the administration of subcutaneous fluids and Denamarin on Day 8 after dosing to both animals, due to signs of dehydration and abnormal clinical pathology values on Day 8; the animal administered TA1 was noted with moderately increased alanine aminotransferase (ALT) while the animal administered TA2 was found to have markedly increased ALT, moderately increased aspartate aminotransferase (AST), and total bilirubin. Subsequent clinical pathology results were found to be mild or unremarkable and thus, no additional veterinary treatment was needed beyond the initial treatment. The transient elevation and subsequent resolving of acute liver injury markers has been documented to occur in NHPs several days after IV administration of AAV9[5]. No TA-related alterations in body weight, body weight change, or food consumption were noted. No TA-related

effects were identified in hematology or coagulation test results. Overall, TAs were considered to be well tolerated in NHPs.

5 AS-1801 TERMINAL PROCEDURES SUMMARY

Animals were euthanized twenty-eight days after dosing. Animals were anesthetized with sodium pentobarbital, perfused with chilled nuclease-free phosphate-buffered saline (PBS) until fluid ran sufficiently clear, and necropsied. Prior to perfusion, blood samples were collected from subjects for AAV9 neutralizing antibody titer, hematology, coagulation, and clinical chemistry analyses. Terminal body weights were recorded for sacrificed animals. A macroscopic examination of the external features of the carcass; external body orifices; abdominal, thoracic, cranial, pelvic, and oral cavities; organs; and tissues was performed. No TA-related macroscopic findings were observed during the terminal evaluations. During sacrificing, tissue samples were collected for histology and for ddPCR analysis.

Histology tissue collections: Brain tissues samples from each animal were collected from the left hemisphere of the brain. Coronal sections were collected across the entire NHP brain in accordance with the Bolon Trim Method[6]. The Bolon trim sections corresponding to specific brain regions are listed in Table 5. Peripheral tissue samples from each animal listed in Table 5 were collected according to a predefined study protocol, where specific tissues were prioritized for collection according to the collection tier provided in Table 5. Tissue samples were prepared for histology analysis according to a predefined study protocol and stored until shipped to Advanced Cell Diagnostics (ACD) for processing and ISH analysis.

Table 5. Tissues collected for *in situ* hybridization distribution analysis.

Tissue	Bolon trim brain section	Collection tier
Brain, brainstem	10, 11, 12, 13	1
Brain, caudate	3, 4, 5	1
Brain, deep cerebellar nuclei	10, 11, 12, 13	1
Brain, frontal cortex	2	1
Brain, hippocampus	7, 8, 9	1
Brain, hypothalamus	7, 8	1
Brain, motor cortex	6, 7	1
Brain, putamen	3, 4, 5	1
Brain, substantia nigra	8	1
Brain, thalamus	7, 8	1
Brain, inferior colliculus	10	2
Brain, corpus callosum	3, 4, 5, 6, 7, 8	2
Brain, temporal cortex	5, 6, 7, 8, 9	2
Brain, visual cortex	12, 13	2
Brain, periventricular regions	5, 6, 7, 8, 9	2

Tissue	Bolon trim brain section	Collection tier
Dorsal root ganglion, cervical	N/A	1
Dorsal root ganglion, lumbar	N/A	1
Dorsal root ganglion, thoracic	N/A	1
Eye, left	N/A	1
Heart	N/A	1
Kidney, cortex	N/A	1
Liver, left lateral lobe	N/A	1
Muscle, left biceps femoris	N/A	1
Spinal cord, cervical	N/A	1
Spinal cord, lumbar	N/A	1
Spinal cord, thoracic	N/A	1
Spleen	N/A	2

ddPCR tissue collections: Tissue samples listed in Table 6 were harvested according to a predefined study protocol, where specific tissues were prioritized for collection according to the collection tier provided in Table 6. The number of individual samples collected for each tissue is provided in Table 6. Tissues were successfully collected, flash-frozen in liquid nitrogen, and stored in a freezer set to maintain -60 to -80°C.

Table 6. Tissues collected for ddPCR distribution analysis.

Tissue	Number of samples collected	Collection tier
Brain, brainstem	6	1
Brain, caudate/putamen	6	1
Brain, cerebellar cortex	6	1
Brain, corpus callosum	6	1
Brain, deep cerebellar nuclei	6	1
Brain, frontal cortex	6	1
Brain, hippocampus	6	1
Brain, hypothalamus	6	1
Brain, inferior colliculus	6	1
Brain, motor cortex	6	1
Brain, thalamus	6	1

Tissue	Number of samples collected	Collection tier
Brain, visual cortex	6	1
Brain, remaining right hemisphere	1	1
Eye, right retina	1	1
Heart, apex	6	1
Heart, left ventricle	6	1
Heart, right ventricle	6	1
Kidney, right cortex	6	1
Kidney, right medulla	6	1
Liver, left lateral lobe	10	1
Liver, right lateral lobe	10	1
Muscle, diaphragm	6	1
Muscle, right quadriceps	6	1
Muscle, right triceps	6	1
Spinal cord, cervical	6	1
Spinal cord, lumbar	6	1
Spinal cord, thoracic	6	1
Adrenal glands	2	2
Dorsal root ganglion, cervical	6	2
Dorsal root ganglion, lumbar	6	2
Dorsal root ganglion, thoracic	6	2
Lung, right caudal lobe	6	2
Lung, right middle lobe	6	2
Lung, right cranial lobe	6	2
Lymph node, mandibular	2	2
Lymph node, mesenteric	6	2
Muscle, right gastrocnemius	6	2
Muscle, right latissimus dorsi	6	2
Muscle, right trapezius	6	2
Nerve, right optic	1	2
Nerve, sciatic	6	2
Ovary	1	2

Tissue	Number of samples collected	Collection tier
Pancreas	6	2
Spleen	6	2
Thymus gland	6	2
Thyroid gland	6	2

6 AS-1801 POST-LIFE ANALYSIS AND RESULTS SUMMARY

AAV9 Neutralizing antibody titer analysis: AAV9 neutralizing antibody titering of pre-dosing samples from subjects P0201 and P0301 showed both subjects tested negative at the least dilute sample titer (1:5) and were therefore suitable for study inclusion and test article dosing. Post-dosing serum samples from both subjects tested positive for AAV9 neutralizing antibodies, consistent with the expectation that AAV dosing would prompt generation of neutralizing antibodies. Accordingly, observation of AAV9 neutralizing antibodies in post-dosing samples provides confirmation of successful systemic AAV test article administration for both study subjects. AAV9 neutralizing antibody titer results for all study subject samples are provided in Table 7.

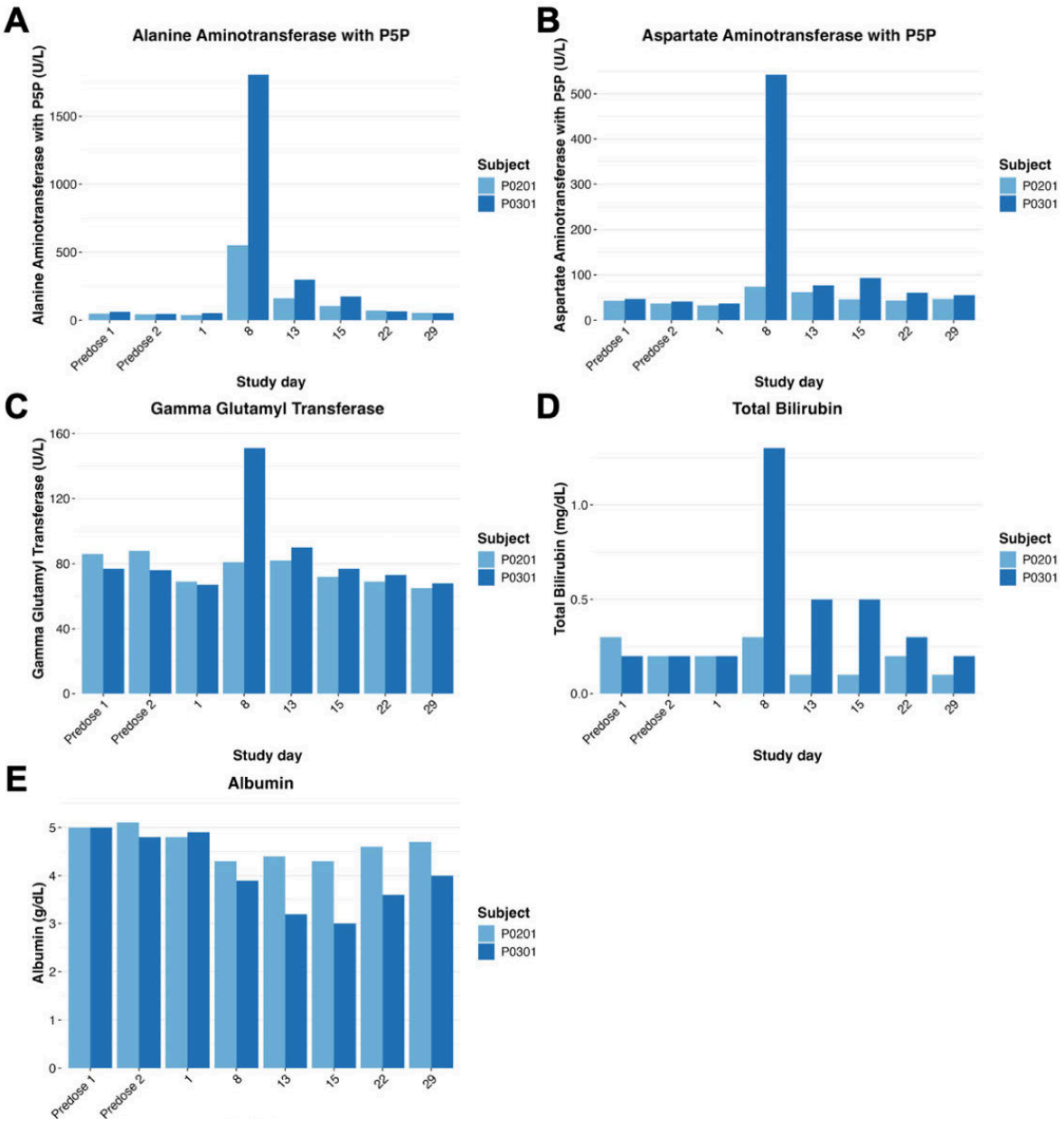
Table 7. AAV9 neutralizing antibody titer analysis for pre- and post-dosing serum samples

Sample description	Sample receipt date	Results for P0201/PM231238	Results for P0301/PM231243
Pre-dosing sample	07-Jun-2023	Negative	Negative
Pre-dosing sample	12-Oct-2023	Negative	Negative
Pre-dosing sample	21-Dec-2023	Negative	Negative
Post-dosing sample	23-Jan-2024	Positive (titer of 1:710)	Positive (titer of 1:2327)

Hematology, coagulation, and clinical chemistry analysis: Hematology, coagulation, and clinical chemistry test panels were performed for blood samples collected from study subjects over the course of the study to better understand safety and tolerability of the TfR1Cap01-based test article. For subject P0201 (single capsid), test article-related effects consisted of transient, mildly to moderately increased alanine aminotransferase (ALT) activity on Days 8, 13, and 15 after dosing. All other differences in clinical chemistry test results between the pre-dose phase and post-dosing phase test results were consistent with normal variation and considered incidental. For subject P0301 (multi-capsid), test article-related effects were transient and included minimally to mildly decreased albumin concentration on Days 13, 15, and 22 after dosing and mildly to markedly increased aspartate aminotransferase (AST), ALT, and gamma glutamyltransferase (GGT) activities and total bilirubin concentration on Days 8, 13, and 15 after dosing. The levels of ALT, AST, GGT, total bilirubin, and albumin for both subjects over the course of the study are shown in Figure 1. We speculate that the higher magnitude changes in clinical chemistry parameters for subject P0301 relative to subject P0201 is linked to the higher total vector load dosed in subject P0301 compared to

P0201 (see Table 2 for dose level comparison). All other differences in clinical chemistry test results between the pre-dose phase and post-dosing phase test results were consistent with normal variation and considered incidental. No test article-related effects were identified in hematology or coagulation test results.

Figure 1. Clinical chemistry results showing transient test article-dependent changes and resolution of alanine aminotransferase (A), aspartate aminotransferase (B), gamma glutamyltransferase (C), total bilirubin (D), and albumin (E) levels in subjects over course of study.



***In situ* hybridization distribution analysis**: Fixed brain and liver tissues from study subjects were used to evaluate test article distribution using in situ hybridization (ISH), as performed by Advanced Cell Diagnostics (ACD). Prior to evaluation with ISH probes specific to viral episomes, brain and liver tissue samples were subjected to ISH to measure expression of *PPIB* and *UBC* as positive controls and

expression of the bacterial gene *dapB* as a negative control. *PPIB* transcripts were detected in brain samples, *UBC* transcripts were detected in liver samples, and no signal was detected for *dapB*, as expected. These analyses confirmed the suitability of tissues for ISH analysis using transgene-specific probes.

Test article distribution was evaluated using ISH probes specific to each of the codon-orthogonal versions of the transgene payload, GBA1, that target the viral episome. Episome distribution was quantified using semi-quantitative scoring performed by ACD. Semi-quantitative scoring is reported as a score from 0-4, indicating the number of dots detected per cell (where a dot reflects a target detected by the ISH probe), where a score of 0 indicates no signal or <1 dot per 10 cells and a score of 4 indicates >15 dots per cell. Additionally, semi-quantitative scoring provides a fraction of cells with at least one dot (% positive cells), binned as <1%, 1-10%, 11-25%, 26-50%, 51-75%, or 76-99%. For brain regions, scoring is performed over an entire region of interest (thalamus, hippocampus, substantia nigra, temporal cortex, and periventricular regions), as defined based on H&E staining. Results of ISH semi-quantitative scoring are presented in Table 8 and discussed below.

Distribution in brain samples from study subjects was compared to a negative control NHP cortex sourced by ACD, which was not dosed and should show no test article-derived signal. As the goal of the study is to assess the absence of TfR1Cap01 distribution to the brain, use of this negative control sample provides a measure of any background signal resulting from transgene-specific ISH. As anticipated for this undosed negative control, no signal was observed for any of the three episome-specific probes. ISH analysis of tissues from P0201 and P0301 indicates lack of detectable TfR1Cap01 or AAV9 episome distribution in the brain, whereas episome signal is detected for both capsids in the liver. BI-93 was also not detected in brain tissue, though conclusions regarding its distribution are not the primary focus of this study. Episome detection in liver tissue provides confirmation that ISH probes are capable of detecting viral episomes when present. As the levels of episome signal in the brain are the same for AAV9 and TfR1Cap01, TfR1Cap01 provides no enhancement of CNS distribution relative to AAV9 in NHPs. Moreover, as TfR1Cap01 is present at a ~10-fold higher dosage relative to the AAV9 comparator in the dosed test article, this lack of enhanced CNS distribution is not simply driven by an insufficiently high TfR1Cap01 dosage. The lack of episome signal in brain tissue is consistent with our expectation that TfR1Cap01 would be unable to distribute to the brain after intravenous delivery due to lack of expression of the cognate human receptor.

ISH analysis of episome distribution was also performed in combination with probes measuring expression of the neuronal marker RNA binding fox1 homolog 3 (RBFOX3) and the astrocytic marker SRY-box containing gene 9 (SOX9). These cell-type markers were probed using both ISH probes to detect RNA, as well as immunohistochemistry to detect the proteins which are nuclearly localized and enable higher confidence in cell type-specific quantification. RBFOX3-positive and SOX9-positive cells were successfully detected in NHP brain tissue when probed in combination with episome ISH probes. As no episome signal was detected in the brain, these cell type-specific measures of episome distribution were not informative and not included in this report.

Based on the ISH analysis conducted, we did not observe enhanced CNS distribution of TfR1Cap01 relative to AAV9, while we observed the anticipated peripheral distribution of both TfR1Cap01 and AAV9 to the liver. Given these clear results, we did not prioritize evaluation of other peripheral tissues using ISH or test article distribution using ddPCR. Tissues collected for ISH and ddPCR distribution analysis are retained in storage for analysis if necessary.

Table 8. Semi-quantification of episome distribution in brain and liver using *in situ* hybridization

Subject	Tissue	Region of interest	AAV9 ISH (GBA1co5)		TfR1Cap01 ISH (GBA1co6)		BI-93 ISH (GBA1co7)	
			Score	% Positive cells	Score	% Positive cells	Score	% Positive cells
Negative control	Brain	Cortex	0	0%	0	0%	0	0%
P0201 (single capsid)	Brain	Thalamus	N/A	N/A	0	0%	N/A	N/A
		Hippocampus	N/A	N/A	0	0%	N/A	N/A
		Substantia nigra	N/A	N/A	0	0%	N/A	N/A
		Temporal cortex	N/A	N/A	0	0%	N/A	N/A
		Periventricular regions	N/A	N/A	0	0%	N/A	N/A
	Liver	Whole tissue	N/A	N/A	1-2	76-99%	N/A	N/A
P0301 (multi-capsid)	Brain	Thalamus	0	0%	0	0%	0	0%
		Hippocampus	0	0%	0	0%	0	0%
		Substantia nigra	0	0%	0	0%	0	0%
		Temporal cortex	0	0%	0	0%	0	0%
		Periventricular regions	0	0%	0	0%	0	0%
	Liver	Whole tissue	1	51-75%	1-2	76-99%	1-2	51-75%

7 AS-1801 CONCLUSIONS

AS-1801 was conducted to assess the distribution and tolerability of the TfR1Cap01 capsid in cynomolgus macaques after intravenous dosing. A single dose of 9.6×10^{13} vg/kg of TA1 – administration of the TfR1Cap01 capsid individually – or 2.3×10^{14} vg/kg of TA2 – administration of the TfR1Cap01 capsid in combination with two other capsids – was well tolerated in cynomolgus macaques. In support of this conclusion, clinical chemistry monitoring demonstrated transient changes in alanine aminotransferase, aspartate aminotransferase, total bilirubin, gamma glutamyltransferase, and albumin levels after dosing which resolved over the course of the study. No test article-dependent effects were observed for the hematology and coagulation parameters examined.

Data collected and analyzed to date from other studies show the TfR1Cap01 capsid does not engage with the cynomolgus macaque homolog of the transferrin receptor, a result of the specific nature of the binding of the TfR1Cap01 capsid to the human transferrin receptor and the differences in the amino acid sequences of cynomolgus macaque and human transferrin receptors. Based on these data, we do not anticipate enhanced CNS distribution of TfR1Cap01 in cynomolgus macaques. In situ hybridization was used to measure TfR1Cap01 episome distribution in the brain and liver in dosed subjects and compared to AAV9 distribution. Control analyses confirmed suitability of ISH probes for episome detection and suitability of tissue samples for analysis. Episome signal was detected in the liver for both TfR1Cap01 and AAV9, providing confirmation of successful test article dosing and ISH-based episome detection. Distribution analyses showed lack of episome signal in the brain for TfR1Cap01 and AAV9. From these data, we conclude that TfR1Cap01 does not provide enhanced distribution to the CNS relative to AAV9 in NHPs, which we attribute to lack of cognate human receptor expression in NHPs.

Overall, the results generated from this study further support the existing body of data for the TfR1Cap01 capsid and are consistent with the findings by Huang et al.[2] describing TfR1Cap01. That is, the TfR1Cap01 capsid does not efficiently distribute to cynomolgus macaque CNS tissues.

8 AS-1801 REFERENCES

1. Hordeaux, J. *et al.* High-dose systemic adeno-associated virus vector administration causes liver and sinusoidal endothelial cell injury. *Molecular Therapy* **32**, 952–968 (2024).
2. Huang, Q. *et al.* An AAV capsid reprogrammed to bind human transferrin receptor mediates brain-wide gene delivery. *Science (1979)* **384**, 1220–1227 (2024).
3. FDA. *Guidance for Industry: Preclinical Assessment of Investigational Cellular and Gene Therapy Products*. (2013).
4. Salabarria, S. M. *et al.* Thrombotic microangiopathy following systemic AAV administration is dependent on anti-capsid antibodies. *J Clin Invest* **134**, (2024).
5. Hudry, E. *et al.* Liver injury in cynomolgus monkeys following intravenous and intrathecal scAAV9 gene therapy delivery. *Molecular Therapy* **31**, 2999–3014 (2023).
6. Bolon, B. Regulatory Forum Opinion Piece*: Effective Brain Trimming for Regulatory-type Nonclinical Toxicity Studies. *Toxicol Pathol* **46**, 115–120 (2018).

Appendix V: STUDY REPORT: V1SION

1 EXECUTIVE SUMMARY

V1SION was conducted to assess the potency of CHARM candidates, with the evaluated test articles enabling comparisons of transgene plasmid backbones, exploratory self-silencing motifs, and inclusion of upstream ORFs (uORFs) and miR-122 target sites to reduce overall and peripheral transgene expression. This non-GLP study was executed at the Broad Institute using test articles prepared at the Broad Institute. Test articles were packaged in AAV-PHP.eB and evaluated in the *hPRNP* mouse model, allowing for assessment of human *PRNP* targeted ZF-CHARM transgene candidates. Key readouts for the study included measurement of *PRNP* mRNA in brain and liver, prion protein (PrP) levels in brain, and body weight over the course of the study.

hPRNP mice were dosed intravenously with test articles at 1E13 and 5E13 vg/kg and monitored until necropsy at 6 and 8 weeks post-dosing. No clear test article-dependent impact on body weight was observed over the course of the study, suggesting test articles were tolerated in this mouse model at the dose levels evaluated. Comparison of test article impact on brain PrP and *PRNP* mRNA levels demonstrated that ZF-CHARM transgene potency was consistent across Broad and Viralgen AAV genome plasmids, confirming the suitability of the Viralgen AAV genome for use in the development candidates. Test articles packaging transgenes with exploratory self-silencing motifs (PRNP162 and PRNP163) achieved reduced potency compared to transgene designs lacking these motifs. Self-silencing was therefore not included in the lead candidate design. *PRNP* mRNA knockdown assessment in liver tissue confirmed the ability of miR-122 target sites in PRNP164 to reduce transgene expression and activity in hepatocytes. Overall, these data supported selection of PRNP164 as the lead candidate for further development, providing a favorable balance of CNS efficacy and peripheral tissue selectivity, while maintaining potency in the plasmid backbone to be used for clinical-scale manufacturing.

2 STUDY SCOPE

V1SION was designed to support selection of a lead construct for advancement into IND-enabling toxicology studies, with particular emphasis on achieving robust *PRNP* silencing throughout the CNS while reducing transgene translation broadly and further reducing ZF-CHARM activity in hepatocytes, where reduction of *PRNP* expression is not anticipated to have a therapeutic benefit. V1SION compared the potency of candidate ZF-CHARM vector designs in a translationally relevant *hPRNP* mouse model across two dose levels.

V1SION included comparison of ZF-CHARM vector design in the context of plasmid backbones originating from the Broad Institute or from Viralgen, the CDMO selected for clinical-scale manufacturing of PRNP-CHARM-001. This comparison was included to confirm that ZF-CHARM potency was maintained after transitioning to a new transgene plasmid backbone.

Additionally, V1SION included potency evaluations of two ZF-CHARM designs with different self-silencing motifs (PRNP162 and PRNP163) designed according to the principles in Neumann and Bertozzi et al. 2024[1]. The objective of this experiment was to determine whether these ZF-CHARM designs can confer potent *PRNP* gene silencing while also

reducing or eliminating long-term transgene expression after *PRNP* methylation and silencing is established. The self-silencing motifs consisted of binding sites for the *PRNP*-targeting zinc finger present in CHARM. The self-silencing motifs were positioned upstream of the EFS promoter in either the forward orientation (PRNP162) or reverse orientation (PRNP163) relative to the directionality of the transgene cassette. Additionally, PRNP163 used a modified EFS promoter incorporating additional cytosine-guanosine dinucleotides (CpG), with the goal of increasing the number of target sites for CHARM-mediated DNA methylation.

Lastly, V1SION evaluated PRNP164, which lacked a self-silencing motif but incorporated (1) two uORFs between the transcriptional start site and transgene ATG to broadly lower translation and (2) three tandem miR-122 target sites in the 3' untranslated region (UTR) designed to reduce transgene expression and silencing activity in hepatocytes. PRNP161 served as a ZF-CHARM comparator in the Viralgen plasmid backbone, as it lacks uORFs and miR-122 binding sites but is otherwise identical to PRNP164. Constructs were tested using PHP.eB in *hPRNP* mice (see Appendix I for more information on mouse models).

Test articles: Test articles were manufactured at the Broad Institute (Cambridge, MA). Test articles were packaged in AAV-PHP.eB, a mouse-specific blood-brain barrier (BBB)-crossing capsid. AAV-PHP.eB has been demonstrated to traffic through the BBB via the LY6A receptor, resulting in broad, efficient delivery to the central nervous system (CNS) of mice that express the LY6A receptor. AAV-PHP.eB is a suitable BBB-crossing surrogate capsid to model anticipated distribution of TfR1 CapX, the capsid intended for clinical dosing, in mouse lines lacking expression of hTfR1. AAV preparations were loaded into iodixanol step gradients and genome-containing AAV particles were separated from empty capsids and cellular debris by ultracentrifugation. The AAVs were buffer exchanged into phosphate-buffered saline with magnesium and calcium, supplemented with Pluronic F-68 to a final concentration of 0.001% (volume/volume). Test articles were subjected to an analytical panel consisting of ddPCR-based titering and SDS-PAGE to assess vector purity. A summary of the test articles evaluated is presented in Table 1.

Table 1. Test articles evaluated in V1SION study

Test Article ID	AAV Capsid	Transgene plasmid backbone source	Transgene	Purpose
PRNP150	AAV-PHP.eB	Broad Institute	EFS-PRNP-CHARM-WPRE-SV40pA	Comparator ZF-CHARM transgene design with no uORFs, no miR-122 binding sites, and no self-silencing motif in Broad Institute transgene plasmid backbone
PRNP161	AAV-PHP.eB	Viralgen	EFS-PRNP-CHARM-WPRE-SV40pA	Evaluates ZF-CHARM transgene design with no uORFs, no miR-122 binding sites, and no self-silencing motif in Viralgen transgene plasmid backbone; comparator for other Viralgen backbone plasmids
PRNP162	AAV-PHP.eB	Viralgen	EFS-FSS-2XuORF-PRNP-CHARM-3XmiR-122-WPRE-SV40pA	Evaluates candidate ZF-CHARM transgene design with uORFs, miR-122 binding sites, and forward orientation self-silencing motif (FSS) in Viralgen transgene plasmid backbone
PRNP163	AAV-PHP.eB	Viralgen	EFS(hCpG)-RSS-2XuORF-PRNP-CHARM-3XmiR-122-WPRE-SV40pA	Evaluates candidate ZF-CHARM transgene design with high CpG EFS promoter (EFS(CpG)), uORFs, miR-122 binding sites, and reverse orientation self-silencing motif (RSS) in Viralgen transgene plasmid backbone
PRNP164	AAV-PHP.eB	Viralgen	EFS-2XuORF-PRNP-CHARM-3XmiR-122-WPRE-SV40pA *(chosen as lead candidate)*	Evaluates candidate ZF-CHARM transgene design with uORFs, miR-122 binding sites, and no self-silencing motif in Viralgen transgene plasmid backbone

Study groups: Study groups were set to evaluate test article efficacy at two dose levels, 1E13 and 5E13 vg/kg, relative to cohorts of untreated control mice. Study groups were planned to include even numbers of female and male mice (2 each, 4 mice total per AAV-dosed group). Study groups were set to evaluate test article efficacy across two in-life durations: 6 weeks post-dosing and a second later timepoint based on interim analysis of self-silencing transgene efficacy in the 6-week cohort. If the test articles with self-silencing transgenes (PRNP162 and PRNP163) showed equivalent efficacy to test articles without self-silencing transgenes (PRNP161 and PRNP164) in the 6-week cohort, groups 12-20

would be examined after a longer in-life duration to better understand the time frame in which transgene self-silencing occurs. A range of ages were considered acceptable for study enrollment in the study to allow for available mice to be utilized. A summary of the planned study groups, dose levels, and target group sizes is presented in Table 2.

Table 2. Planned study groups, dose levels, and target group sizes

Group	Test Article ID	Dose level (vg/kg)	Age at dosing (weeks)	In-life duration (weeks)	Total mice	Female mice	Male mice
1	N/A (untreated)	0	9-16	6	8	4	4
2	PRNP150	1E13	9-16	6	4	2	2
3	PRNP150	5E13	10-16	6	4	2	2
4	PRNP161	1E13	10-16	6	4	2	2
5	PRNP161	5E13	11-16	6	4	2	2
6	PRNP164	1E13	10-16	6	4	2	2
7	PRNP164	5E13	10-16	6	4	2	2
8	PRNP162	1E13	9-16	6	4	2	2
9	PRNP162	5E13	10-16	6	4	2	2
10	PRNP163	1E13	9-16	6	4	2	2
11	PRNP163	5E13	10-16	6	4	2	2
12	N/A (untreated)	0	10-16	Pending	8	4	4
13	PRNP161	1E13	11-15	Pending	4	2	2
14	PRNP161	5E13	11-15	Pending	4	2	2
15	PRNP164	1E13	10-16	Pending	4	2	2
16	PRNP164	5E13	10-16	Pending	4	2	2
17	PRNP162	1E13	10-16	Pending	4	2	2
18	PRNP162	5E13	10-17	Pending	4	2	2
19	PRNP163	1E13	10-16	Pending	4	2	2
20	PRNP163	5E13	10-16	Pending	4	2	2

Study readouts: The ZF-CHARM payload was designed to silence *PRNP* expression through targeted methylation of the *PRNP* locus. Primary readouts for the study included measurement of *PRNP* mRNA in brain and liver and prion protein (PrP) levels in brain. Brain samples were also collected for exploratory measurement of *PRNP* methylation. Body weight was monitored over the course of the study as a preliminary evaluation of test article safety and tolerability across dose levels.

3 ANALYSIS PLAN

The V1SION study analysis plan was predefined and determined prior to study initiation.

Body weights: Body weight will be recorded for each animal on study prior to dosing to determine test article dosage, and at least once per week after dosing. Terminal body weights will be recorded for animals prior to necropsy.

Tissue collection: During necropsy, tissue samples will be collected for ELISA, RT-qPCR, and exploratory methylation analyses. The left hemisphere of the brain will be collected, frozen on dry ice, and stored at -80C for ELISA and RT-qPCR analyses. The right hemisphere of the brain will be embedded in Tissue-Tek OCT Compound (Sakura), frozen on dry ice, and stored at -80C for methylation analysis. The right lobe of the liver will be collected, frozen on dry ice, and stored at -80C for RT-qPCR analyses. The left half of the medial lobe of the liver, spleen, heart, and lungs will be collected from animals in the 6-week in-life cohort, frozen on dry ice, and stored at -80C for future exploratory analyses.

ELISA: ELISA analysis will be used to measure levels of PrP present in brain tissue samples collected from study subjects[2]. The left hemisphere of the brain will be homogenized in CHAPS buffer. A portion of brain homogenate will be reserved for RNA isolation for RT-qPCR analysis. PrP abundance will be measured in dilutions of brain homogenate, comparing to recombinant PrP standards. Brain PrP abundance in dosed subjects will be normalized to levels measured in uninjected control mice to assess the magnitude of PrP reduction.

RT-qPCR: RT-qPCR will be used to measure transgene expression and the extent of human *PRNP* mRNA knockdown in brain and liver tissue samples. RNA will be isolated from brain homogenate reserved from ELISA analysis and from frozen liver samples using the QIAGEN RNEasy Lipid RNA Isolation Kit with on-column DNase digestion. RT-qPCR will leverage primers specific to human *PRNP*, the WPRE portion of the transgene RNA, and mouse *Tbp* as a reference. Control samples without reverse transcription will be included in analyses to measure transgene expression. *PRNP* RNA levels in dosed subjects will be normalized to levels measured in uninjected control mice to assess the magnitude of *PRNP* knockdown.

4 IN-LIFE PROCEDURES SUMMARY

V1SION was executed as a non-GLP study at the Broad Institute (Cambridge, MA) according to established protocols approved by the institute's IACUC committee and standard operation procedures. The Broad Institute is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

Animals were dosed with test articles via retro-orbital intravenous injection according to the planned study enrollment. All animals were dosed successfully, and no issues were noted during dosing.

Over the duration of the study, animals were monitored for health issues, and body weights were recorded weekly. Three unscheduled deaths occurred over the course of the study. Dermatitis was noted for one animal (Animal ID 161226.2, Group 3), which was subsequently euthanized due to reaching a humane endpoint after unsuccessful veterinary

treatment; brain, liver, spleen, heart, and lungs were collected from this animal. One animal (Animal ID 164032.4, Group 3) was euthanized due to reaching a humane endpoint after a 29% body weight loss six days after dosing; brain and liver were collected from this animal. One animal (Animal ID 164033.1, Group 20) was found dead; no tissues were collected from this animal. All other animals survived to their respective necropsy timepoints.

5 TERMINAL PROCEDURES SUMMARY

Animals in groups 1-11 were euthanized 6 weeks after dosing according to their group enrollment. After interim potency analysis in the 6-week cohort (see Section 6, Post-Life Analysis and Results Summary), it was observed that test articles with self-silencing transgenes (PRNP162 and PRNP163) showed reduced potency compared to test articles with transgenes lacking self-silencing (PRNP161 and PRNP164). Accordingly, a longer duration assessment of self-silencing transgene potency in groups 12-20 was deprioritized, and animals in these groups were sacrificed 8 weeks after dosing.

Terminal body weights were recorded for all sacrificed animals. During necropsy, tissue samples were collected for ELISA, RT-qPCR, and methylation analyses. The number of individual samples collected for each tissue and analysis is provided in Table 3. The left hemisphere of the brain was collected, frozen on dry ice, and stored at -80C for ELISA and RT-qPCR analyses. The right hemisphere of the brain was embedded in Tissue-Tek OCT Compound (Sakura), frozen on dry ice, and stored at -80C for methylation analysis. The right lobe of the liver was collected, frozen on dry ice, and stored at -80C for RT-qPCR analyses. The left lobe of the liver was collected, frozen on dry ice, and stored at -80C for future exploratory analyses. For the mice taken down at 6 weeks, the heart, spleen and lungs were collected, frozen on dry ice, and stored at -80C for future exploratory analyses. All tissues were successfully collected for study analyses from animals reaching terminal necropsy.

6 POST-LIFE ANALYSIS AND RESULTS SUMMARY

Animals excluded from analysis: A total of 3 animals were not included in specific study analyses. No tissues were collected from animal 164033.1 (Group 20) as detailed above, precluding tissue analyses. Animals 161226.2 (Group 3) and 164032.4 (Group 3) reached humane endpoints prior to necropsy and were only included in brain potency analyses. Final study enrollment for analysis is detailed in Table 4.

Table 3. Tissues collected for study analyses.

Tissue	Necropsy Timepoint	Number of samples collected per animal	Total samples collected	Purpose
Brain, left hemisphere	6 weeks	1	47	ELISA, RT-qPCR
	8 weeks	1	40	
Brain, right hemisphere	6 weeks	1	47	Reserved for methylation analysis
	8 weeks	1	40	Reserved for methylation analysis
Liver, right lobe	6 weeks	1	47	RT-qPCR
	8 weeks	1	40	
Liver, left lobe	6 weeks	1	47	Reserved for exploratory analyses
	8 weeks	1	40	
Heart	6 weeks	1	47	Reserved for exploratory analyses
Lung	6 weeks	1	47	Reserved for exploratory analyses
Spleen	6 weeks	1	47	Reserved for exploratory analyses

Table 4. Final enrollment in study groups

Group	TA ID	Dose level (vg/kg)	Age at dosing (weeks)	In-life duration (weeks)	Total mice	Female mice	Male mice
1	N/A (untreated)	0	9-16	6	8	4	4
2	PRNP150	1E13	9-16	6	4	2	2
3	PRNP150	5E13	10-16	6	2	0	2
4	PRNP161	1E13	10-16	6	4	2	2
5	PRNP161	5E13	11-16	6	4	2	2
6	PRNP164	1E13	10-16	6	4	2	2
7	PRNP164	5E13	10-16	6	4	2	2
8	PRNP162	1E13	9-16	6	4	2	2
9	PRNP162	5E13	10-16	6	4	2	2
10	PRNP163	1E13	9-16	6	4	2	2
11	PRNP163	5E13	10-16	6	4	2	2
12	N/A (untreated)	0	10-16	8	8	4	4
13	PRNP161	1E13	11-15	8	4	2	2
14	PRNP161	5E13	11-15	8	4	2	2
15	PRNP164	1E13	10-16	8	4	2	2
16	PRNP164	5E13	10-16	8	4	2	2
17	PRNP162	1E13	10-16	8	4	2	2
18	PRNP162	5E13	10-17	8	4	2	2
19	PRNP163	1E13	10-16	8	4	2	2
20	PRNP163	5E13	10-16	8	3	2	1

Body weight analysis: Body weights recorded weekly over the course of the study were analyzed to identify any test article-dependent effects on body weight. Mean weekly body weights for all dose groups are shown in Figure 1. Over the course of the study, all test article-treated groups showed similar trajectories of weight gain as compared to untreated control mice. Based on these outcomes, there did not appear to be a dose-dependent impact of test article dosing on body weight.

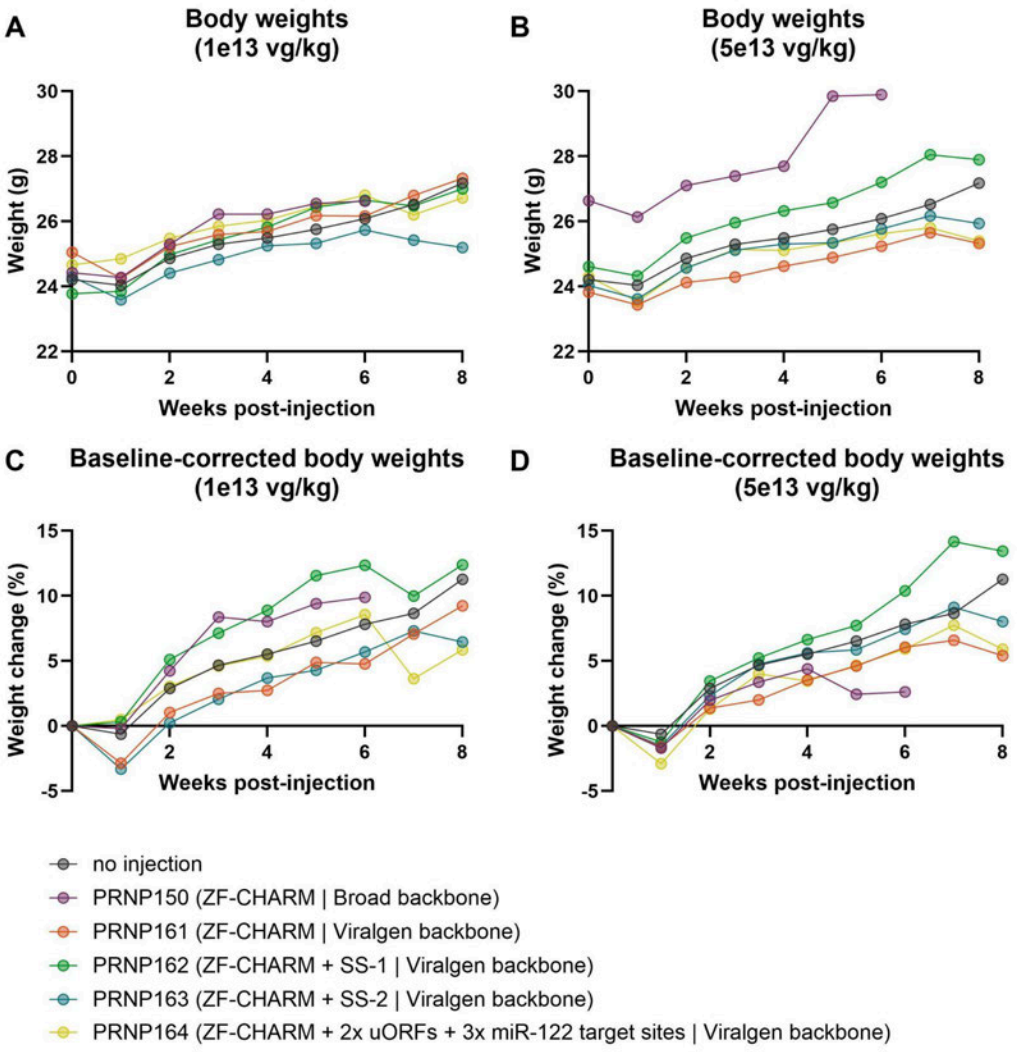


Figure 1. Body weights across the study for mice dosed with CHARM. *hPRNP* mice were dosed with different ZF-CHARM constructs at 1E13 vg/kg (A, C) or 5E13 vg/kg (B, D) and then weighed weekly across the 6 or 8-week in-life period. Baseline-corrected body weights were calculated for individual mice by subtracting their baseline weight and dividing by their baseline weight for each value (Formula: 100*(Value–Baseline/Baseline)) (C, D).

ELISA analysis: ELISA analysis was used to measure levels of PrP present in brain tissue samples collected from study subjects, and the results of this analysis are shown in Figure 2. PrP levels in treated mice are normalized to the uninjected controls. All test articles showed varying extents of dose-dependent decrease in brain PrP levels, which was less apparent for test articles with self-silencing transgenes (PRNP162 and PRNP163). PRNP162 and PRNP163 showed overall reduced potency in lowering PrP levels compared to test articles lacking self-silencing (PRNP150, PRNP161, and PRNP164). No significant differences in PrP levels were observed between PRNP161 (Viralgen backbone) and PRNP150 (Broad backbone).

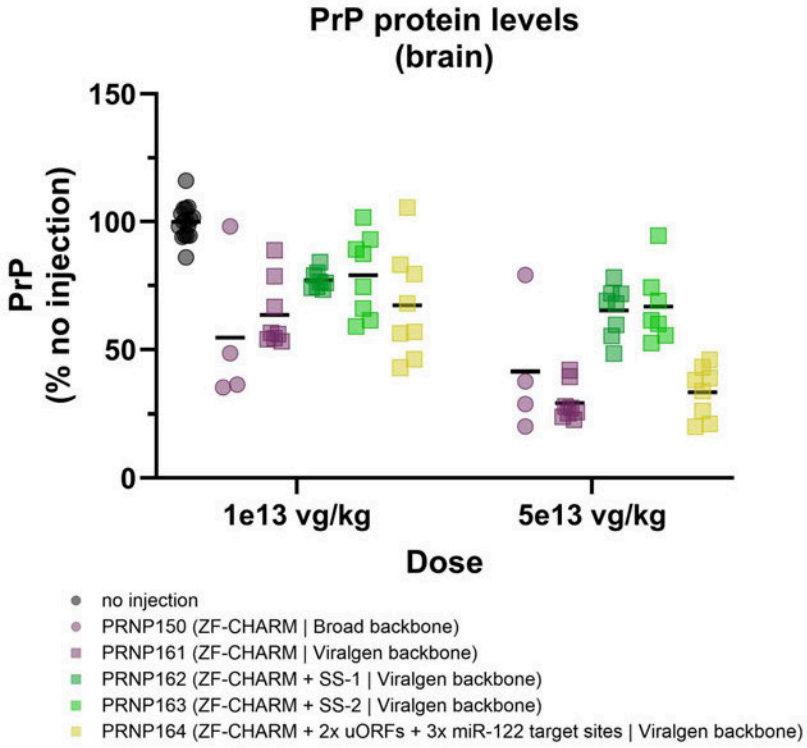


Figure 2. Lowering of PrP in the brain by human PRNP ZF-CHARM candidates. *hPRNP* mice were injected with different ZF-CHARM constructs at one of two doses (1e13 vg/kg, or 5e13 vg/kg) at 9-17 weeks of age. Whole brain hemispheres were analyzed for PrP protein levels by ELISA at 6 or 8 weeks post-dose (groups combined).

RT-qPCR analysis: RT-qPCR was used to measure transgene expression and the extent of human *PRNP* lowering in brain and liver tissue samples. The results of this analysis are shown in Figures 3 and 4. *PRNP* mRNA levels in treated mice were normalized to *PRNP* levels in uninjected control mice. Treatment of mice with either PRNP150, PRNP161, PRNP162, or PRNP164 led to a dose-dependent decrease in brain *PRNP* mRNA levels, while a dose-dependent reduction of brain *PRNP* mRNA was not observed for PRNP163. Similar to the results from ELISA analysis, both test articles using self-silencing transgenes (PRNP162 and PRNP163) showed reduced *PRNP* silencing potency compared to test articles with transgenes lacking self-silencing (PRNP150, PRNP161, and PRNP164). RT-qPCR analyses in liver tissue focused on test articles showing potency in brain tissue (PRNP150, PRNP161, and PRNP164). The extent of *PRNP* mRNA reduction was similar between PRNP161 (Viralgen backbone) and PRNP150 (Broad backbone).

In liver tissue, PRNP150 and PRNP161 achieved *PRNP* silencing at both dose levels evaluated. However, animals treated with PRNP164 demonstrated reduced *PRNP* silencing in liver at both dose levels relative to those treated with PRNP150 or PRNP161, consistent with effective liver detargeting mediated by inclusion of miR-122 target sites included in PRNP164.

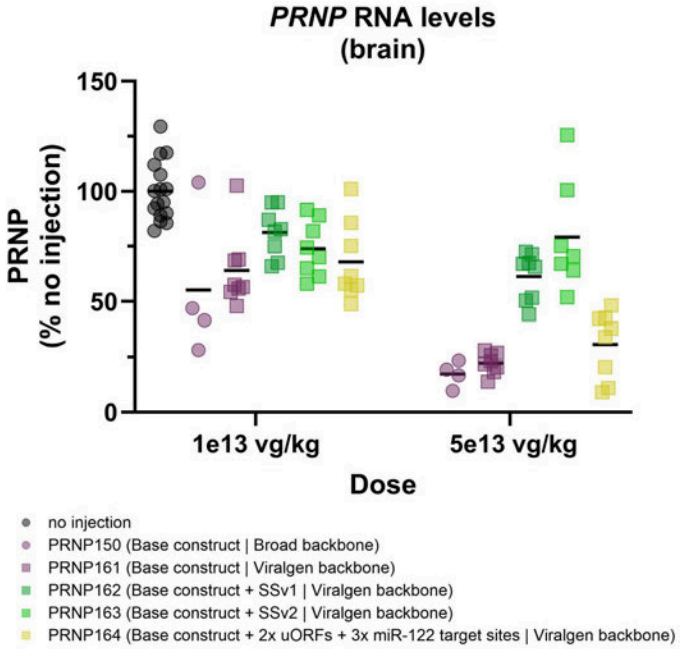


Figure 3. **Lowering of *PRNP* mRNA in the brain by human PRNP ZF-CHARM candidates.** *hPRNP* mice were injected with different ZF-CHARM constructs at one of two doses (1e13 vg/kg, or 5e13 vg/kg) at 9-17 weeks of age. Whole brain hemispheres were analyzed for *PRNP* RNA by RT-qPCR (housekeeping gene TBP) at 6 weeks or 8 weeks post-dose (groups combined).

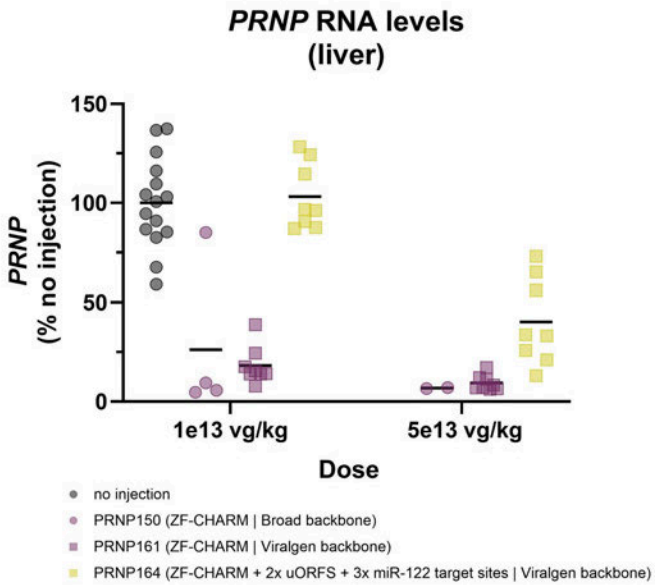


Figure 4. *PRNP* knockdown in liver. *hPRNP* mice were injected with different ZF-CHARM constructs at one of two doses (1e13 vg/kg, or 5e13 vg/kg) at 9-17 weeks of age. Livers were analyzed for *PRNP* RNA by RT-qPCR (housekeeping gene TBP) at 6 weeks post-dose.

7 CONCLUSIONS

V1SION was conducted to assess the potency of ZF-CHARM candidates, with the evaluated test articles enabling comparisons of transgene plasmid backbones, self-silencing motifs, and inclusion of upstream ORFs and miR-122 target sites to reduce overall and peripheral transgene expression. Test articles were packaged in AAV-PHP.eB and evaluated in the *hPRNP* mouse model, allowing for evaluation of human *PRNP*-targeted ZF-CHARM transgene candidates. Key readouts for the study included measurement of *PRNP* mRNA in brain and liver, prion protein (PrP) levels in brain, and body weight over the course of the study.

PRNP150, PRNP161, PRNP162, and PRNP164 treatment led to dose-dependent decreases in brain PrP and *PRNP* mRNA levels. In addition, by comparing PRNP150 and PRNP161, we conclude that the vector designs had similar potency in the Broad and Viralgen plasmid backbones. The Viralgen plasmid backbone was therefore deemed suitable for use in the PRNP-CHARM-001 product.

In liver tissue, reductions in *PRNP* mRNA were observed for animals treated with PRNP150, PRNP161, and PRNP164. However, animals treated with PRNP164 demonstrated less potent *PRNP* silencing in liver at both dose levels relative to those treated with PRNP150 or PRNP161, consistent with effective liver detargeting mediated by inclusion of miR-122 target sites in PRNP164. These data indicated that liver detargeting can be incorporated into the transgene design without negatively impacting ZF-CHARM activity in the brain, the key target tissue for *PRNP*-targeted ZF-CHARM therapeutic activity.

Test articles packaging transgenes with self-silencing motifs (PRNP162 and PRNP163) achieved reduced potency compared to transgene designs lacking these motifs as evaluated through PrP ELISA and *PRNP* mRNA RT-qPCR at the 6-week timepoint. Given their reduced *PRNP* silencing potency, self-silencing motifs will not be incorporated into the lead candidate construct. No clear test article-dependent impact on body weight was observed over the course of the study, suggesting test articles were tolerated in this mouse model at the dose levels evaluated. A more detailed assessment of the impact of test article dosing on body weight will be obtained in the ongoing and planned toxicology studies.

Overall, these data supported selection of PRNP164 as the lead candidate for further development, providing a favorable balance of CNS potency and peripheral tissue selectivity, while maintaining potency in the plasmid backbone used for clinical scale manufacturing.

8 REFERENCES

1. Neumann, E. N. et al. Brainwide silencing of prion protein by AAV-mediated delivery of an engineered compact epigenetic editor. Science 384, ado7082 (2024).

2. Mortberg, M. A. et al. Regional variability and genotypic and pharmacodynamic effects on PrP concentration in the CNS. JCI Insight 7, e156532 (2022).

Appendix VI: STUDY REPORT: CABS

1 EXECUTIVE SUMMARY

The objective of this study was to compare the biodistribution and efficacy of a mouse-*Prnp* targeted ZF-CHARM transgene as delivered by AAV-PHP.eB or TfR1 CapX in *hTFRC* KI mice. Biodistribution and potency data were generated with the goal of enabling dose translation between CHARM transgene designs delivered using AAV-PHP.eB, a mouse-specific capsid leveraged for CHARM preclinical development, and TfR1 CapX, the hTfR1-specific capsid intended for clinical use. This non-GLP study was executed at the Broad Institute using test articles prepared at the Broad Institute. Key readouts for the study included measurement of *Prnp* mRNA and vector genome content in brain, liver, heart, quadriceps, and spinal cord tissue samples and prion protein (PrP) levels in the brain.

hTFRC KI mice were dosed intravenously with test articles at 6.67E11, 2.0E12, 6.0E12, or 1.8E13 vg/kg, and monitored until necropsy at 6 weeks post-dosing. Both the AAV-PHP.eB- and TfR1 CapX-based test articles achieved robust and dose-dependent reduction of mouse *Prnp* mRNA and mouse PrP in the brain. Both test articles showed dose-dependent increases in vector genome distribution in brain, spinal cord, liver, and heart, but not quadriceps. While vector genome content in brain was roughly higher across the range of AAV-PHP.eB dose levels compared to TfR1 CapX, TfR1 CapX achieved greater *Prnp* silencing in brain than AAV-PHP.eB relative to their respective vector genome abundance. Overall, these results demonstrate greater potency of *Prnp* lowering when delivered with TfR1 CapX relative to AAV-PHP.eB, but support the use of AAV-PHP.eB as a surrogate for TfR1 CapX in mouse models with mouse *Tfrc*.

2 STUDY SCOPE

CABS was designed to enable dose translation between human *PRNP* directed CHARM transgenes delivered using AAV-PHP.eB, a mouse-specific surrogate capsid leveraged for CHARM preclinical development, and TfR1 CapX, the hTfR1-specific capsid intended for clinical use. AAV-PHP.eB has been demonstrated to traffic through the blood-brain barrier (BBB) by engaging LY6A, resulting in broad, efficient delivery to the central nervous system (CNS) of mice that express the LY6A receptor[1]. The TfR1 CapX capsid engages the hTfR1 receptor to effectively cross the BBB leading to widespread CNS distribution. Accordingly, AAV-PHP.eB is anticipated to be a suitable BBB-crossing surrogate capsid to model anticipated TfR1 CapX distribution in mouse lines lacking expression of hTfR1. The distribution and efficacy data generated in this study enabled a direct comparison of these capsids in a mouse model expressing the cognate receptor for each capsid.

Animal model justification: We evaluated test article efficacy in homozygous *hTFRC* KI mice, which express a humanized TfR1 used for enhanced CNS distribution of TfR1 CapX, and WT mouse LY6A. See Appendix I for more information on the mouse model.

Test articles: Test articles were manufactured at the Broad Institute (Cambridge, MA) in HEK293T cells by triple transfection with PEI. AAV particles were recovered from cell clarified cell lysates. Clarified lysates were treated with nuclease and loaded into iodixanol step gradients. Genome-containing AAV particles were separated from empty capsids and

cellular debris by ultracentrifugation. The AAVs were buffer exchanged into phosphate-buffered saline with magnesium and calcium, supplemented with Pluronic F-68 to a final concentration of 0.001% (volume/volume) and filtered through a 0.2 μm filter. Test articles were subjected to an analytical panel consisting of ddPCR-based titering and SDS-PAGE to assess vector purity. A summary of the test articles evaluated is presented in Table 1.

Table 1. **Test articles evaluated in CABS study**

Test Article ID	AAV Capsid	Transgene	Purpose
PRNP216	TfR1 CapX	EFS-Prnp-CHARM	Evaluates mouse-specific Prnp-CHARM transgene packaged in intended clinical capsid
PRNP217	AAV-PHP.eB	EFS-Prnp-CHARM	Comparator mouse-specific Prnp-CHARM transgene packaged with mouse-specific BBB-crossing capsid used in preclinical development

Study groups: Study groups were set to evaluate test article efficacy and distribution at four dose levels, 6.67E11, 2.0E12, 6.0E12, and 1.8E13 vg/kg, relative to cohorts of untreated control mice. Study groups were planned to include even numbers of female and male mice (3 each, 6 mice total per AAV-treated group). Study groups were set to evaluate test article efficacy and distribution after an in-life duration of 6 weeks post-dosing. A range of ages were used to capture any variance in biodistribution across ages. A summary of the planned study groups, dose levels, and target group sizes is presented in Table 2.

Table 2. Planned study groups, dose levels, and target group sizes

Group	Test Article ID	Capsid	Dose level (vg/kg)	Age at dosing (weeks)	In-life duration (weeks)	Total mice	Female mice	Male mice
1	N/A	N/A	0	8-33.5	6	12	6	6
2	PRNP217	AAV-PHP.eB	6.67E11	8-33.5	6	6	3	3
3	PRNP217	AAV-PHP.eB	2.00E12	8-33.5	6	6	3	3
4	PRNP217	AAV-PHP.eB	6.00E12	8-33.5	6	6	3	3
5	PRNP217	AAV-PHP.eB	1.80E13	8-33.5	6	6	3	3
6	PRNP216	TfR1 CapX	6.67E11	8-33.5	6	6	3	3
7	PRNP216	TfR1 CapX	2.00E12	8-33.5	6	6	3	3
8	PRNP216	TfR1 CapX	6.00E12	8-33.5	6	6	3	3
9	PRNP216	TfR1 CapX	1.80E13	8-33.5	6	6	3	3

Study readouts: The Prnp-CHARM payload was designed to silence and repress mouse *Prnp* expression through targeted methylation and repression of the mouse *Prnp* locus. Primary efficacy readouts for the study included measurement of *Prnp* mRNA in brain, liver, heart, quadriceps, and spinal cord and prion protein (PrP) levels in the brain. qPCR was used to measure vector genome abundance relative to mouse genome content in brain, liver, heart, quadriceps, and spinal cord tissue samples. Brain samples were also collected for RNAscope assessment of *Prnp* and transgene expression alongside NeuN immunohistochemistry to identify neurons, providing a readout of neuron-specific distribution and CHARM-Krab activity. Body weight was monitored over the course of the study as a preliminary evaluation of test article safety and tolerability across dose levels.

3 ANALYSIS PLAN

The CABS study analysis plan was predefined and determined prior to study initiation.

Body weights: Body weight will be recorded for each animal on study prior to dosing to determine test article dosage, and at least once per week after dosing. Terminal body weights will be recorded for animals prior to necropsy.

Tissue collection: During necropsy, tissue samples will be collected for ELISA, RT-qPCR, and RNAscope analyses. The left hemisphere of the brain will be collected, frozen on dry ice, and stored at -80C. The left hemisphere will then be cryopulverized and aliquoted for use in ELISA, RT-qPCR and vector distribution assays. The right hemisphere of the brain will be embedded in Tissue-Tek OCT Compound (Sakura), frozen on dry ice, and stored at -80C for RNAscope analysis. The right lobe of the liver, heart, quadriceps, and spinal cord will be collected, frozen on dry ice, and stored at -80C for RT-qPCR analyses.

ELISA: ELISA analysis will be used to measure levels of PrP present in the brain tissue samples collected from study subjects[2]. An aliquot of pulverized brain tissue will be homogenized in CHAPS buffer. PrP abundance will be measured in dilutions of brain homogenate, comparing to recombinant PrP standards. Brain PrP abundance in dosed subjects will be normalized to levels measured in uninjected control mice to assess the magnitude of PrP reduction.

RT-qPCR: RT-qPCR will be used to measure the extent of mouse *Prnp* mRNA lowering in the brain, liver, heart, quadriceps, and spinal cord tissue samples. RNA will be isolated from pulverized brain hemisphere tissue and from frozen tissue samples using the QIAGEN RNEasy 96 well kit (QIAGEN catalog number 74881) and DNase digestion with QIAGEN DNase set (QIAGEN catalog number 79254). RT-qPCR will leverage primers specific to mouse *Prnp* and mouse *Gapdh* as a reference. *Prnp* RNA levels in dosed subjects will be normalized to levels measured in uninjected control mice to assess the magnitude of *Prnp* knockdown.

Vector distribution qPCR: qPCR will be used to measure vector genome abundance relative to mouse genome content in the brain, liver, heart, quadriceps, and spinal cord tissue samples. DNA will be isolated from pulverized brain tissue and from frozen tissue samples using the QIAGEN DNeasy 96 Blood & Tissue Kit (QIAGEN catalog number 69581).

Vector genome content will be measured using primers specific to the WPRE portion of the transgene, and mouse genome content will be measured using primers specific to *Gapdh*. Standard curves of known quantities of vector genomes and *Gapdh* target DNA will be used to convert Ct values for WPRE and *Gapdh* to vector genome (vg) copy number and diploid genome (dg) copy number, respectively.

RNAscope: RNAscope will be used to assess the spatial distribution and neuronal specificity of test article transduction and *Prnp* knockdown. The right hemisphere of the brain will be embedded in Tissue-Tek OCT Compound (Sakura), frozen on dry ice, and stored at -80C for RNAscope analysis. Sequence-specific RNAscope probes will be used to detect the WPRE portion of the transgene RNA and *Prnp* RNA. RNAscope will be performed jointly with NeuN immunohistochemistry to identify neurons, providing a qualitative readout of neuron-specific transgene distribution and CHARM activity.

4 IN-LIFE PROCEDURES SUMMARY

The study was executed as a non-GLP study at the Broad Institute (Cambridge, MA) under study name CABS according to established protocols approved by the institute's IACUC committee and standard operation procedures. The Broad Institute is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

Animals were dosed with test articles via retro-orbital (intravenous) injection according to the planned study enrollment. All animals were dosed successfully; no issues were noted during dosing.

Over the duration of the study, animals were monitored for health issues, and body weights were recorded weekly. One unscheduled death occurred over the course of the study. One animal (Animal ID 145251.23, Group 4) was found dead 72 hours after dosing; no tissues were collected from this animal. All other animals survived to the terminal necropsy timepoint.

5 TERMINAL PROCEDURES SUMMARY

Animals were euthanized 6 weeks after dosing and terminal body weights were recorded for sacrificed animals. During necropsy, tissue samples were collected for ELISA, RT-qPCR, vector distribution qPCR, and RNAscope analyses. The number of individual samples collected for each tissue and analysis is provided in Table 4. The left hemisphere of the brain was collected, frozen on dry ice, and stored at -80C for ELISA, RT-qPCR, and vector distribution qPCR analyses. The right hemisphere of the brain was embedded in Tissue-Tek OCT Compound (Sakura), frozen on dry ice, and stored at -80C for RNAscope analysis. The right hemispheres were collected as planned, but were processed in a manner that severely damaged the brain tissue morphology, rendering them unusable for RNAscope as intended. A supplementary study was performed to generate tissue for RNAscope-based assessment of *Prnp* and transgene mRNA (see below, RNAscope analysis section and Figure 6). The right lobe of the liver, heart, quadriceps, and spinal cord were collected, frozen on dry ice, and stored at -80C for RT-qPCR and vector distribution qPCR analyses. All tissues were successfully collected for study analyses from animals reaching terminal necropsy.

Table 4. Tissues collected for study analyses.

Tissue	Number of samples collected per animal	Total samples collected	Purpose
Brain, left hemisphere	1	59	ELISA, RT-qPCR, vector distribution qPCR
Brain, right hemisphere	1	59	RNAscope
Liver, right lobe	1	59	RT-qPCR, vector distribution qPCR
Heart	1	59	RT-qPCR, vector distribution qPCR
Quadriceps	1	59	RT-qPCR, vector distribution qPCR
Spinal cord	1	59	RT-qPCR, vector distribution qPCR

6 POST-LIFE ANALYSIS AND RESULTS SUMMARY

Animals excluded from analysis: One animal was not included in study analyses. No tissues were collected from animal 145251.23 (Group 4) as detailed above, precluding tissue analyses. Final study enrollment for analysis is detailed in Table 5.

Table 5. Final enrollment in study groups

Group	TA ID	Capsid	Dose level (vg/kg)	Age at dosing (weeks)	In-life duration (weeks)	Total mice	Female mice	Male mice
1	N/A	N/A	0	8-33.5	6	12	6	6
2	PRNP217	PHP.eB	6.67E11	8-33.5	6	6	3	3
3	PRNP217	PHP.eB	2.00E12	8-33.5	6	6	3	3
4	PRNP217	PHP.eB	6.00E12	8-33.5	6	5	3	2
5	PRNP217	PHP.eB	1.80E13	8-33.5	6	6	3	3
6	PRNP216	CapX	6.67E11	8-33.5	6	6	3	3
7	PRNP216	CapX	2.00E12	8-33.5	6	6	3	3
8	PRNP216	CapX	6.00E12	8-33.5	6	6	3	3
9	PRNP216	CapX	1.80E13	8-33.5	6	6	3	3

ELISA analysis: ELISA analysis was used to measure levels of PrP present in brain tissue samples collected from study subjects, and the results of this analysis are shown in Figure 1. PrP levels in treated mice are normalized to the uninjected controls. Treatment of mice with EFS-Prnp-CHARM delivered by either AAV-PHP.eB or TfR1 CapX led to a dose-dependent decrease in brain PrP levels. AAV-PHP.eB-mediated delivery of EFS-Prnp-CHARM resulted in residual PrP levels in the brain of 78% in mice treated with 6.67E11 vg/kg, 67% in mice treated with 2.00E12 vg/kg, 36% in mice treated with 6.00E12 vg/kg, and 49% in mice treated with 1.80E13 vg/kg. TfR1 CapX delivery of EFS-Prnp-CHARM resulted in residual PrP levels in the brain of 73% in mice treated with 6.67E11 vg/kg, 51% in mice treated with 2.00E12 vg/kg, 35% in mice treated with 6.00E12 vg/kg, and 16% in mice treated with 1.80E13 vg/kg. Similar levels of PrP depletion were observed at equivalent dose levels of AAV-PHP.eB and TfR1 CapX, except for the 1.8E13 vg/kg dose level where TfR1 CapX showed more potent PrP reduction.

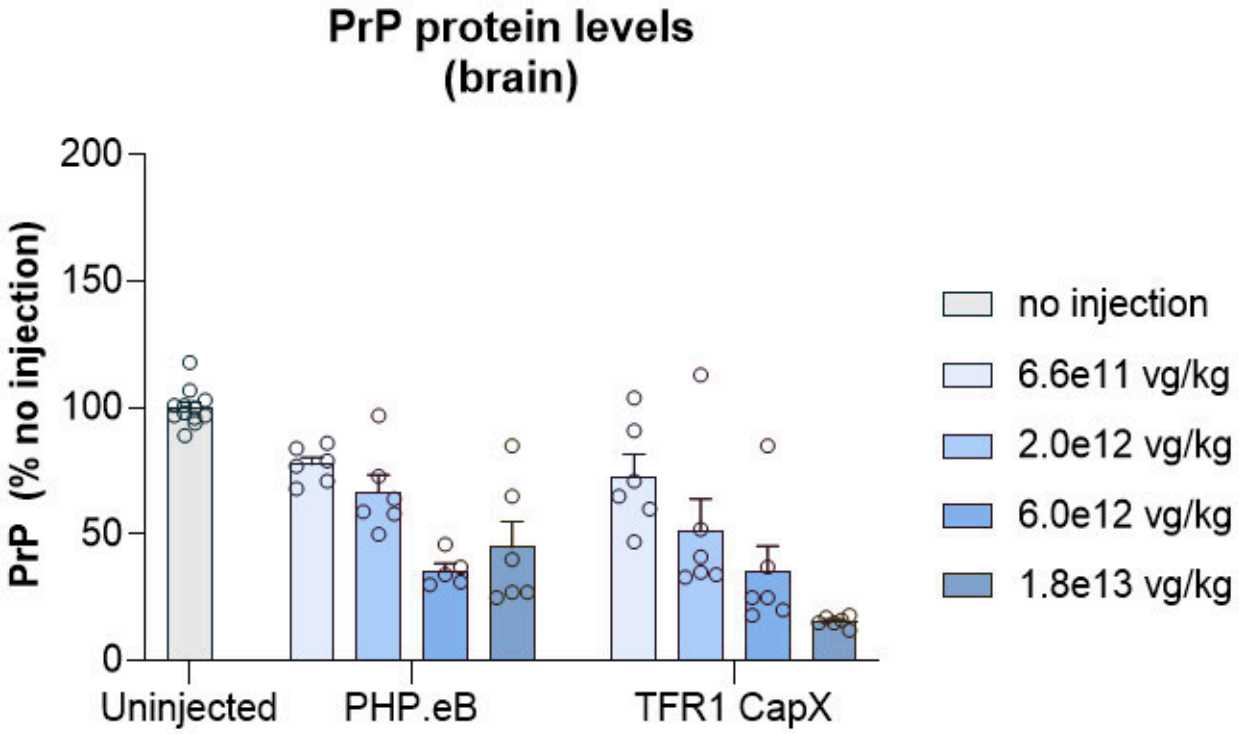


Figure 1. ELISA readout of CHARM-mediated PrP reduction delivered with PHP.eB or TfR1 CapX. *hTFRC* KI mice were injected with 6.6e11 vg/kg, 2.0e12 vg/kg, 6.0e12 vg/kg, or 1.8e13 vg/kg AAV-PHP.eB or AAV-TfR1 CapX at 8-33.5 weeks of age. Whole brain hemispheres were analyzed for PrP protein levels by ELISA at 6 weeks post-dose.

RT-qPCR analysis: RT-qPCR was used to measure the extent of mouse *Prnp* RNA knockdown in brain and liver tissue samples, and the results of this analysis are shown in Figures 3 and 4. *Prnp* levels in treated mice were normalized to *Prnp* levels in uninjected control mice. Treatment of mice with either AAV-PHP.eB or TfR1 CapX led to a

dose-dependent decrease in brain and liver *Prnp* RNA levels. AAV-PHP.eB delivery of EFS-Prnp-CHARM resulted in residual *Prnp* levels in the brain of 81% in mice treated with 6.67E11 vg/kg, 71% in mice treated with 2.00E12 vg/kg, 27% in mice treated with 6.00E12 vg/kg, and 37% in mice treated with 1.80E13 vg/kg. TfR1 CapX delivery of EFS-Prnp-CHARM resulted in residual *Prnp* levels in the brain of 85% in mice treated with 6.67E11 vg/kg, 55% in mice treated with 2.00E12 vg/kg, 24% in mice treated with 6.00E12 vg/kg, and 4% in mice treated with 1.80E13 vg/kg. Similar levels of *Prnp* knockdown in brain were observed at equivalent dose levels of AAV-PHP.eB and TfR1 CapX, except for the 1.8E13 vg/kg dose level where TfR1 CapX showed more potent *Prnp* reduction (Figure 2), consistent with ELISA results measuring PrP depletion. In liver tissue, similar levels of *Prnp* knockdown were observed at equivalent dose levels of AAV-PHP.eB and TfR1 CapX (Figure 3).

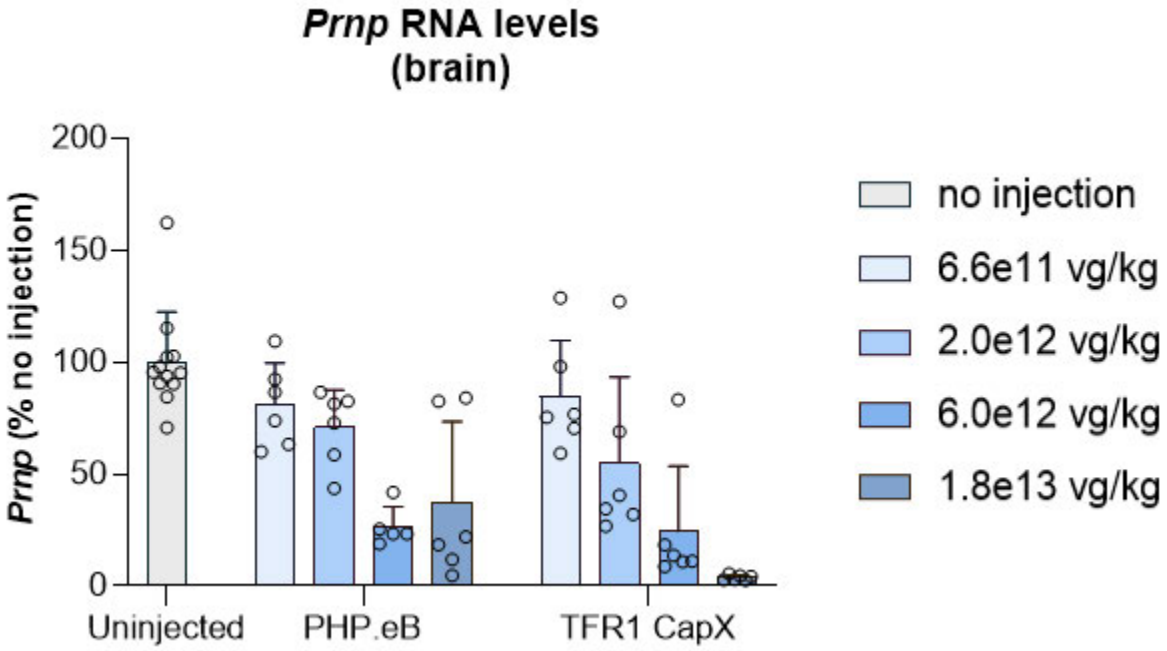


Figure 2. RT-qPCR readout of CHARM-mediated *Prnp* silencing delivered with PHP.eB or TfR1 CapX. *hTFRC* KI mice were injected with 6.6e11 vg/kg, 2.0e12 vg/kg, 6.0e12 vg/kg, or 1.8e13 vg/kg AAV-PHP.eB or AAV-TfR1 CapX at 8-33.5 weeks of age. Whole brain hemispheres were analyzed for Prnp mRNA levels by RT-qPCR at 6 weeks post-dose.

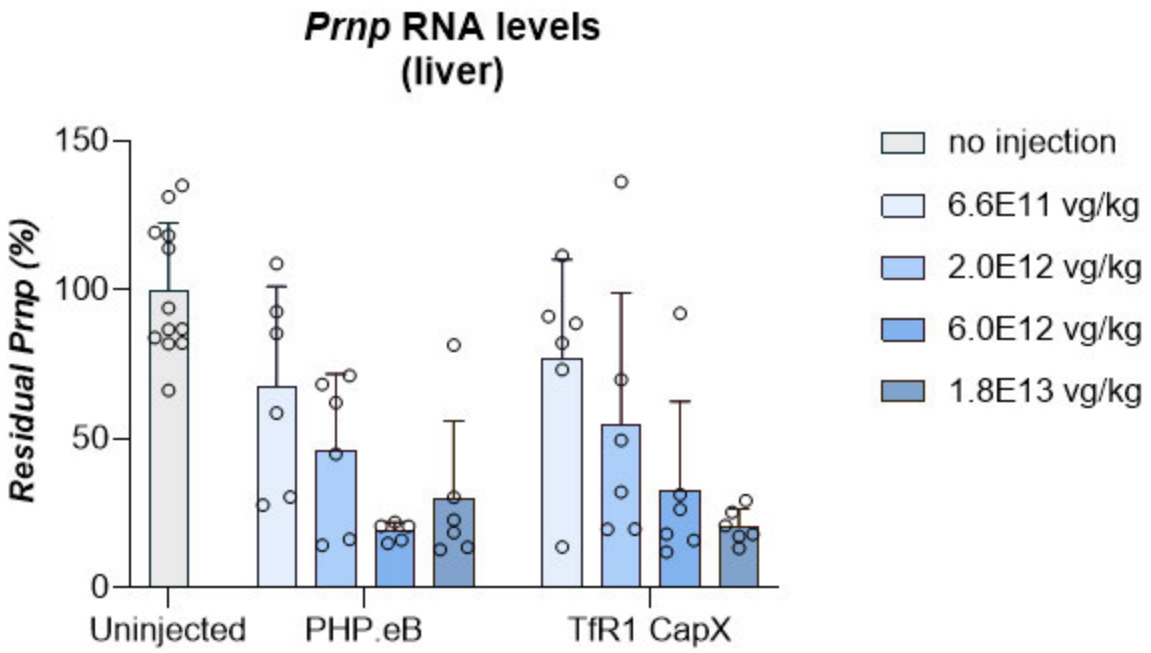


Figure 3. CHARM-mediated *Prnp* silencing delivered with PHP.eB or TfR1 CapX. *hTFRC* KI mice were injected with 6.6e11 vg/kg, 2.0e12 vg/kg, 6.0e12 vg/kg, or 1.8e13 vg/kg of Prnp-CHARM encapsidated in AAV-PHP.eB or AAV-TfR1 CapX at 8-33.5 weeks of age. Liver was analyzed for *Prnp* mRNA levels by RT-qPCR at 6 weeks post-dose.

Vector distribution qPCR: qPCR was used to measure vector genome abundance relative to mouse genome content in brain, liver, heart, quadriceps, and spinal cord tissue samples. The results of these analyses are shown in Figure 4. Both test articles showed a dose-dependent increase in vector genome distribution in brain, spinal cord, liver, and heart, while this trend was not apparent in quadriceps. At equivalent dose levels, TfR1 CapX (PRNP216) showed lower brain and spinal cord distribution compared to AAV-PHP.eB (PRNP217), except for the 1.8E13 vg/kg dose level where both test articles showed similar CNS distribution. In liver and heart, TfR1 CapX showed higher vector genome distribution relative to AAV-PHP.eB at the 1.8E13 vg/kg dose level; however, this increased relative distribution of TfR1 CapX was not consistently observed at lower dose levels. In quadriceps, TfR1 CapX showed lower vector genome distribution relative to equivalent dose levels of AAV-PHP.eB, though a clear dose-response of distribution was not observed in this tissue.

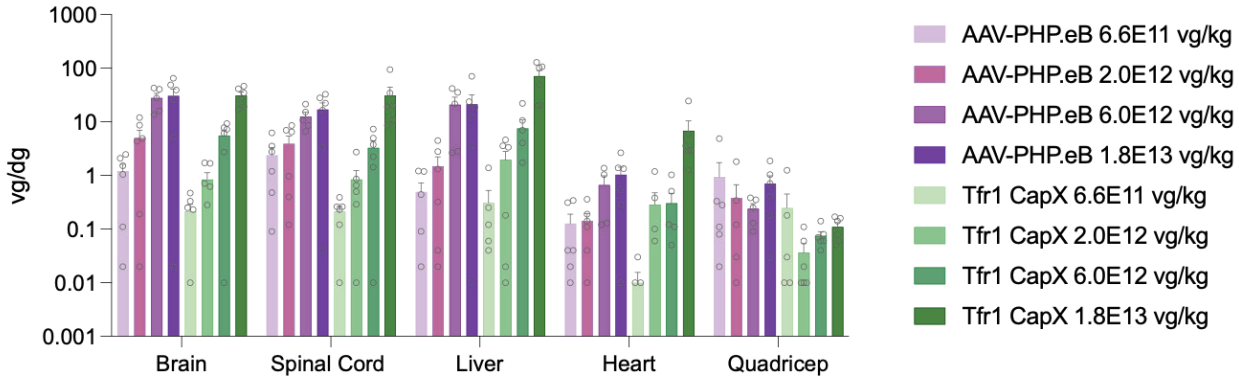


Figure 4. qPCR analysis of PHP.eB and TfR1 CapX biodistribution. *hTFRC* KI mice were injected with 6.6e11 vg/kg, 2.0e12 vg/kg, 6.0e12 vg/kg, or 1.8e13 vg/kg AAV-PHP.eB or AAV-TfR1 CapX at 8-33.5 weeks of age. Brain, spinal cord, liver, heart and quadricep were analyzed for vector genome abundance (WPRE) relative to mouse genome content (mouse glucagon) by qPCR.

The relationship between vector genome distribution and *Prnp* RNA or PrP reduction efficacy was further examined, as shown in Figure 5. While AAV-PHP.eB showed higher CNS distribution compared to TfR1 CapX, TfR1 CapX yielded superior efficacy of *Prnp* RNA knockdown and PrP reduction in the brain as a function of vector genome distribution. In the liver, TfR1 CapX and AAV-PHP.eB showed a similar relationship between vector genome distribution and *Prnp* RNA knockdown, indicating the increased potency of TfR1 CapX was specific to brain tissue.

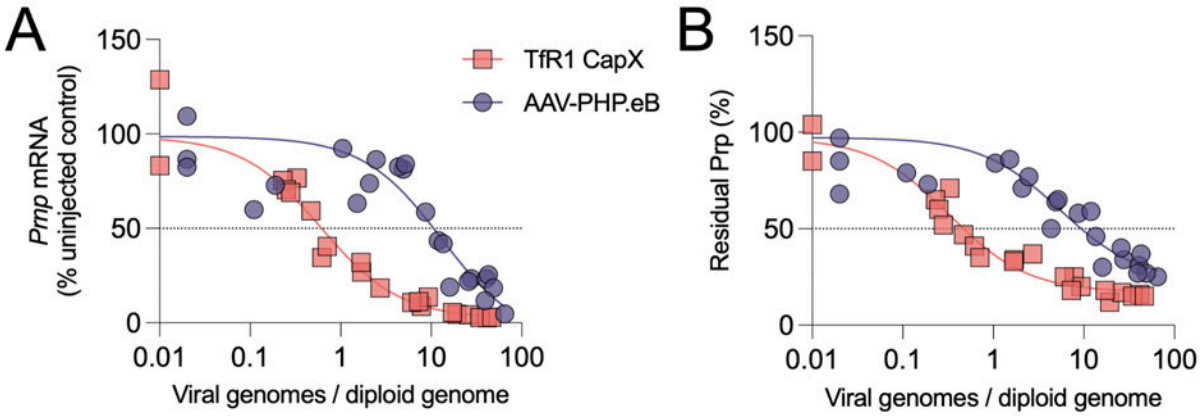


Figure 5. Brain *Prnp* silencing as a function of viral genomes/diploid genomes. *hTFRC* KI mice were injected with 6.6e11 vg/kg, 2.0e12 vg/kg, 6.0e12 vg/kg, or 1.8e13 vg/kg AAV-PHP.eB or TfR1 CapX at 8-33.5 weeks of age. Whole brain hemispheres were analyzed for (**A**) *Prnp* RNA by RT-qPCR (housekeeping gene TBP) or (**B**) PrP protein (ELISA). Biodistribution (viral genomes/diploid genome) was measured by qPCR (WPRE for viral genomes, mouse glucagon for mouse genomes) at 6 weeks post-dose.

RNAscope analysis: RNAscope analysis of study samples was not performed as the brain tissue samples collected for this purpose were not suitable for analysis. An additional 8 uninjected *hTFRC* KI mice (4 males, 4 females) were taken down to serve as controls for RNAscope analysis. An additional 12 mice were dosed with 1.8E13 vg/kg AAV-PHP.eB (3 males, 3 females) or 1.8E13 vg/kg AAV-TfR1 CapX (3 males, 3 females) for RNAscope analysis. All mice were 8-12 weeks old at dosing.

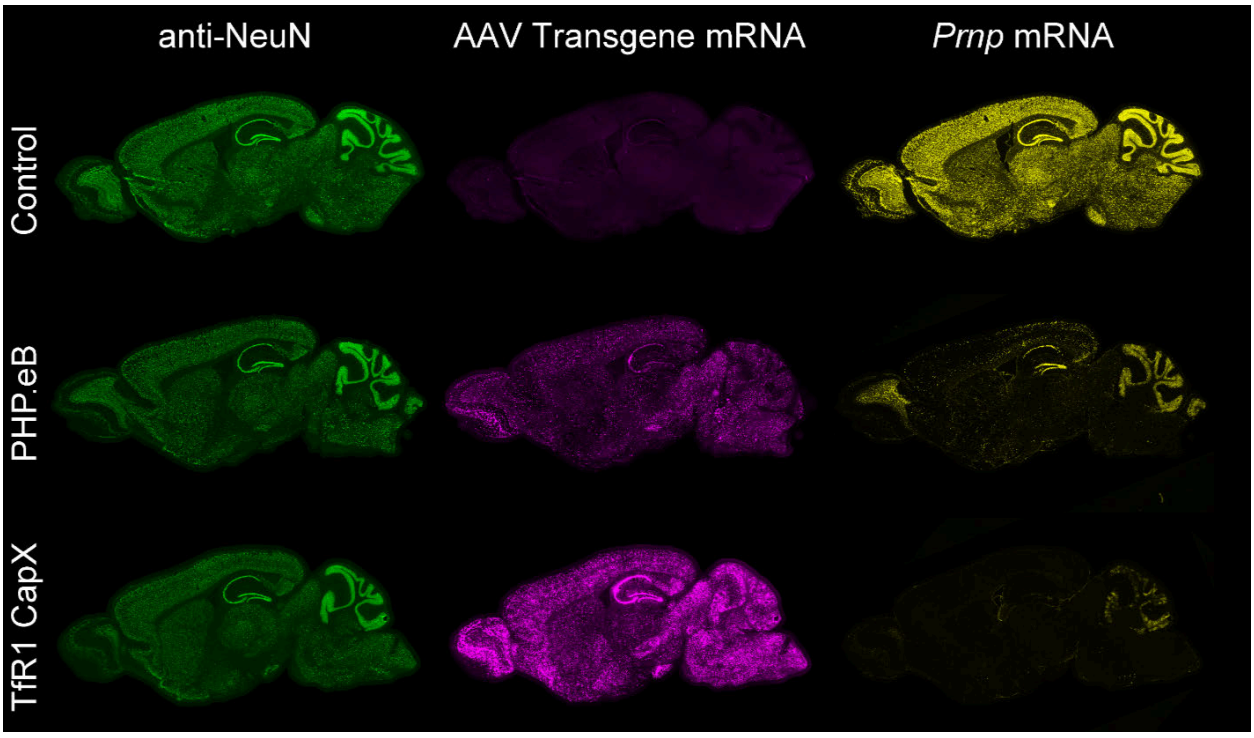


Figure 6. RNAscope analysis of WPRE (transgene) and *Prnp* mRNA in brain. *hTFRC* KI mice were injected with 1.8E13 vg/kg AAV-PHP.eB or TfR1 CapX at 8-33.5 weeks of age. Control mice were not injected. Whole brain hemispheres were analyzed for WPRE (*Prnp* ZF-CHARM transgene) and *Prnp* RNA by RNAscope at 6 weeks post-dose.

7 CONCLUSIONS

CABS was conducted to compare the biodistribution and efficacy of a mouse-targeted Prnp-CHARM transgene as delivered by the AAV-PHP.eB or the hTfR1-targeting capsid TfR1 CapX in *hTFRC* KI mice. Both the AAV-PHP.eB- and TfR1 CapX-based test articles achieved robust and dose-dependent reduction of *Prnp* mRNA and PrP in the brain. Both test articles showed a dose-dependent increase in vector genome distribution in brain, spinal cord, liver, and heart, while this trend was not apparent in quadriceps. While vector genome content in the brain was roughly higher with the AAV-PHP.eB-based test article compared to the TfR1 CapX-based test article, TfR1 CapX achieved stronger *Prnp* lowering in the brain than AAV-PHP.eB as a function of vector genome delivery.

Overall, these results indicated stronger, but comparable potency of *Prnp* lowering when delivered with TfR1 CapX relative to AAV-PHP.eB supporting the use of AAV-PHP.eB as a surrogate capsid for TfR1 CapX in models lacking human TfR1. Therefore, the vector distribution and efficacy data generated in this study can be leveraged for dose translation between CHARM transgene designs delivered using AAV-PHP.eB, as implemented in preclinical studies for CHARM transgene development, and TfR1 CapX, the capsid intended for clinical use.

8 REFERENCES

1. Chan, K. Y. et al. Engineered AAVs for efficient noninvasive gene delivery to the central and peripheral nervous systems. Nat. Neurosci. 20, 1172–1179 (2017).

2. Mortberg, M. A. et al. Regional variability and genotypic and pharmacodynamic effects on PrP concentration in the CNS. JCI Insight 7, e156532 (2022).

Appendix VII: STUDY REPORT: H3X

1 EXECUTIVE SUMMARY

The objective of H3X was to evaluate the potency of the candidate PRNP176 (ultimately chosen as the lead candidate for PRNP-CHARM-001) relative to a comparator PRNP177 following systemic administration in a translationally relevant *hTFRC* KI;*hPRNP* mouse model using TfR1 CapX. The mouse model allows evaluation of both the *PRNP*-targeted ZF-CHARM transgene and AAV capsid intended for clinical use (see Appendix I for more information on mouse lines).

This non-GLP study was executed at the Broad Institute using test articles prepared at the Broad Institute. PRNP177 is a single stranded AAV genome with a EFS-PRNP-CHARM-WPRE-SV40pA expression cassette packaged into TfR1 CapX. PRNP176 shares the same overall design as PRNP177 but incorporates upstream open reading frames (uORFs) in the 5' UTR and miR-122 target sites in the 3' untranslated region (UTR) designed to reduce overall transgene expression and detarget hepatocytes, where reduction of *PRNP* expression is not anticipated to have a therapeutic benefit and reduced transgene expression is expected to improve tolerability. Key readouts for the study included measurement of *PRNP* mRNA in brain and liver, prion protein (PrP) levels in brain, and body weight over the course of the study.

hTFRC KI;*hPRNP* mice were dosed intravenously with test articles at 5E12, 1.5E13, and 4.5E13 vg/kg and monitored until necropsy at 6 and 12 weeks post-dosing. Both PRNP176 and PRNP177 achieved robust and dose-dependent reduction of *PRNP* mRNA and PrP in brain tissue. Knockdown was durable, persisting through the 12-week timepoint. In liver tissue, *PRNP* mRNA knockdown was observed for both constructs. However, PRNP176 demonstrated reduced knockdown at low and mid doses in liver relative to PRNP177, consistent with effective liver detargeting mediated by inclusion of miR-122 target sites in PRNP176.

Across the 12-week study duration, untreated control mice exhibited expected weight gain (10-20% increase relative to starting weight), while low-dose treatment groups demonstrated modest weight gain (5-10% relative to starting weight). Mid-dose groups exhibited minimal weight gain at 6 weeks post-dosing, and high-dose groups showed little to no weight gain over the study period, indicating a dose-dependent impact on body weight.

Overall, both test articles exhibited strong potency and durability of silencing through 12 weeks in CNS tissues. PRNP176 additionally demonstrated improved tissue selectivity through reduced hepatocyte activity, supporting its advancement as the lead candidate. PRNP176 produced by Viralgen will be referred to as PRNP-CHARM-001.

2 STUDY SCOPE

H3X was designed to support selection of a lead AAV genome design for advancement into IND-enabling toxicology studies, with particular emphasis on achieving robust CNS knockdown while minimizing activity in peripheral tissues. H3X compared the potency of two candidate AAV genomes in a translationally relevant *hTFRC KI*;*hPRNP* mouse model across multiple dose levels and in-life durations. PRNP177 comprises AAV2 ITRs, an EFS promoter, mouse-alpha globin 5' UTR sequence, ZF-CHARM (CHARM with human *PRNP*-

targeting ZF-KRAB with two bipartite NLSs, WPRE, SV40pA, and all synthetic linkers). PRNP176 comprises all components of PRNP177 with the addition of two tandem two-amino acid upstream open reading frames (uORFs) within the 5'UTR and three tandem miR-122 target sites in the 3' untranslated region (UTR) designed to reduce overall transgene expression and silencing activity in hepatocytes, where reduction of *PRNP* expression is not anticipated to have a therapeutic benefit and reduced transgene expression is expected to improve tolerability. These AAV genomes were tested using TfR1 CapX in *hTFRC KI*;*hPRNP* mice (see Appendix I for additional information on the mouse model).

Test articles: Test articles were manufactured at the Broad Institute (Cambridge, MA). Test articles were formulated in phosphate-buffered saline with magnesium and calcium, supplemented with Pluronic F-68 to a final concentration of 0.001% (volume/volume). Prior to dosing, genome-containing AAV particles were separated from empty capsids and cellular debris by ultracentrifugation and subjected to an analytical panel consisting of ddPCR-based titering and SDS-PAGE to assess vector purity. A summary of the test articles evaluated is presented in Table 1.

Table 1. Test articles evaluated in H3X study

Test Article ID	AAV Capsid	Transgene	Purpose
PRNP176	TfR1 CapX	EFS-2XuORF-PRNP-CHARM-3XmiR-122-WPRE-SV40pA	Evaluates candidate PRNP-CHARM-001 transgene design with uORFs and miR-122 binding sites
PRNP177	TfR1 CapX	EFS-PRNP-CHARM-WPRE-SV40pA	PRNP-targeting ZF-CHARM transgene design lacking uORFs and miR-122 binding sites

Study groups: Study groups were set to evaluate test article potency at three dose levels (5E12, 1.5E13, and 4.5E13 vg/kg), relative to cohorts of untreated control mice. Study groups were planned to include even numbers of female and male mice (3 each, 6 mice total per group). Study groups were set to evaluate test article potency across two in-life durations: 6 and 12 weeks post-dosing. A range of ages were considered acceptable for study enrollment in the study to allow for available mice to be utilized. A summary of the planned study groups, dose levels, and target group sizes is presented in Table 2.

Table 2. Planned study groups, dose levels, and target group sizes

Group	Test Article ID	Dose level (vg/kg)	Age at dosing (weeks)	In-life duration (weeks)	Total mice	Female mice	Male mice
1	N/A (untreated)	0	9-17	6	6	3	3
2	N/A (untreated)	0	13-17	12	6	3	3
3	PRNP176	5E12	9-17	6	6	3	3
4	PRNP176	5E12	9-17	12	6	3	3
5	PRNP176	1.5E13	9-17	6	6	3	3
6	PRNP176	4.5E13	9-17	6	6	3	3
7	PRNP176	4.5E13	12-17	12	6	3	3
8	PRNP177	5E12	9-17	6	6	3	3
9	PRNP177	5E12	12-17	12	6	3	3
10	PRNP177	1.5E13	9-17	6	6	3	3
11	PRNP177	4.5E13	9-17	6	6	3	3
12	PRNP177	4.5E13	9-17	12	6	3	3

Study readouts: The ZF-CHARM payload was designed to silence *PRNP* expression through targeted methylation and transcriptional repression of the *PRNP* locus. Primary readouts for the study included measurement of *PRNP* mRNA in brain and liver and prion protein (PrP) levels in brain. Brain samples were also collected for exploratory measurement of *PRNP* methylation. Body weight was monitored over the course of the study as a preliminary evaluation of test article safety and tolerability across dose levels.

3 ANALYSIS PLAN

The H3X study analysis plan was predefined and determined prior to study initiation.

Body weights: Body weight will be recorded for each animal on study prior to dosing to determine test article dosage, and at least once per week after dosing. Terminal body weights will be recorded for animals prior to necropsy.

Tissue collection: During necropsy, tissue samples will be collected for ELISA, RT-qPCR, and methylation analyses. The left hemisphere of the brain will be collected, frozen on dry ice, and stored at -80C for ELISA and RT-qPCR analyses. The right hemisphere of the brain will be embedded in Tissue-Tek OCT Compound (Sakura), frozen on dry ice, and stored at -80C for methylation analysis. The heart and two portions of one lobe of the liver will be collected, frozen on dry ice, and stored at -80C for RT-qPCR analyses.

ELISA: ELISA analysis will be used to measure levels of PrP present in brain tissue samples collected from study subjects[1]. The left hemisphere of the brain will be homogenized in CHAPS buffer. A portion of brain homogenate will be reserved for RNA isolation for RT-qPCR analysis. PrP abundance will be measured in dilutions of brain homogenate, comparing to recombinant PrP standards. Brain PrP abundance in dosed subjects will be normalized to levels measured in uninjected control mice to assess the magnitude of PrP reduction.

RT-qPCR: RT-qPCR will be used to measure transgene expression and the extent of human *PRNP* mRNA knockdown in brain and liver tissue samples. RNA will be isolated from brain homogenate reserved from ELISA analysis and from frozen liver samples using the QIAGEN RNEasy Lipid RNA Isolation Kit with on-column DNase digestion. RT-qPCR will leverage primers specific to human *PRNP*, the WPRE portion of the transgene RNA, and mouse *Tbp* as a reference. Control samples without reverse transcription will be included in analyses to measure transgene expression. *PRNP* RNA levels in dosed subjects will be normalized to levels measured in uninjected control mice to assess the magnitude of *PRNP* knockdown.

4 IN-LIFE PROCEDURES SUMMARY

H3X was executed as a non-GLP study at the Broad Institute (Cambridge, MA) according to established protocols approved by the institute's IACUC committee and standard operation procedures. The Broad Institute is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

Animals were dosed with test articles via retro-orbital intravenous injection according to the planned study enrollment. All animals were dosed successfully, and no issues were noted during dosing.

Over the duration of the study, animals were monitored for health issues, and body weights were recorded weekly. Three unscheduled deaths occurred over the course of the study. One animal (Animal ID 166879.2, Group 7) was euthanized due to reaching a humane endpoint of 20% body weight loss from the previous week (actual body weight loss was 25%); no tissues were collected from this animal. One animal (Animal ID 166884.2, Group 9) was euthanized due to reaching a humane endpoint due to corneal abrasion; brain, liver, and heart tissue were collected from this animal. One animal (Animal ID 166883.1, Group 6) was found dead; no tissues were collected from this animal. All other animals survived to their respective necropsy timepoints.

5 TERMINAL PROCEDURES SUMMARY

Animals were euthanized 6 or 12 weeks after dosing according to their group enrollment. Terminal body weights were recorded for sacrificed animals. During necropsy, tissue samples were collected for ELISA, RT-qPCR, and methylation analyses. The number of individual samples collected for each tissue and analysis is provided in Table 3. The left hemisphere of the brain was collected, frozen on dry ice, and stored at -80C for ELISA and RT-qPCR analyses. The right hemisphere of the brain was embedded in Tissue-Tek OCT Compound (Sakura), frozen on dry ice, and stored at -80C for methylation analysis. The right lobe of the liver and heart were collected, frozen on dry ice, and stored at -80C for RT-qPCR

analyses. All tissues were successfully collected for study analyses from animals reaching terminal necropsy.

Table 3. Tissues collected for study analyses.

Tissue	Number of samples collected per animal	Total samples collected	Purpose
Brain, left hemisphere	1	70	ELISA, RT-qPCR
Brain, right hemisphere	1	70	Reserved for future analysis
Liver, right lobe	2	140	RT-qPCR
Heart	1	70	Reserved for exploratory RT-qPCR analysis

6 POST-LIFE ANALYSIS AND RESULTS SUMMARY

Animals excluded from analysis: A total of 4 animals were not included in study analyses. No tissues were collected from animals 166879.2 (Group 7) and 166883.1 (Group 6) as detailed above, precluding tissue analyses. Animal 166884.2 (Group 9) was not included in study analysis due to early necropsy at 5 weeks (planned necropsy at 12 weeks) upon reaching a humane endpoint. Animal 170020.5 (Group 1) was excluded from analysis as it was determined to have the incorrect genotype. Final study enrollment for analysis is detailed in Table 4.

Table 4. Final enrollment in study groups

Group	Test Article ID	Dose level (vg/kg)	Age at dosing (weeks)	In-life duration (weeks)	Total mice	Female mice	Male mice
1	N/A (untreated)	0	9-17	6	5	2	3
2	N/A (untreated)	0	13-17	12	6	3	3
3	PRNP176	5E12	9-17	6	6	3	3
4	PRNP176	5E12	9-17	12	6	3	3
5	PRNP176	1.5E13	9-17	6	6	3	3
6	PRNP176	4.5E13	9-17	6	5	3	2
7	PRNP176	4.5E13	12-17	12	5	3	2
8	PRNP177	5E12	9-17	6	6	3	3
9	PRNP177	5E12	12-17	12	5	2	3
10	PRNP177	1.5E13	9-17	6	6	3	3
11	PRNP177	4.5E13	9-17	6	6	3	3
12	PRNP177	4.5E13	9-17	12	6	3	3

Body weight analysis: Body weights recorded weekly over the course of the study were analyzed to identify any test article-dependent effects on body weight. Mean weekly body weights for all dose groups are shown in Figure 1. The variable ages of subjects made it difficult to draw meaningful conclusions on body weight changes across groups; the observed body weight impacts are presented for descriptive purposes. Across the 12-week study duration, untreated control mice exhibited expected weight gain (10-20% increase relative to starting weight), while low-dose PRNP176 and PRNP177 treatment groups demonstrated modest weight gain (5-15% increase relative to starting weight). Mid-dose groups exhibited minimal weight gain at 6 weeks post-dosing (0-5% increase relative to starting weight), with the caveat that these groups were not taken out to 12 weeks post-dosing. Low-dose groups had gained ~5% body weight and high-dose groups had lost ~0-5% body weight relative to starting weight at 6 weeks post-dosing. High-dose groups showed reduced body weight over the study period (0-10% decrease relative to starting weight), suggesting a dose-dependent impact on body weight.

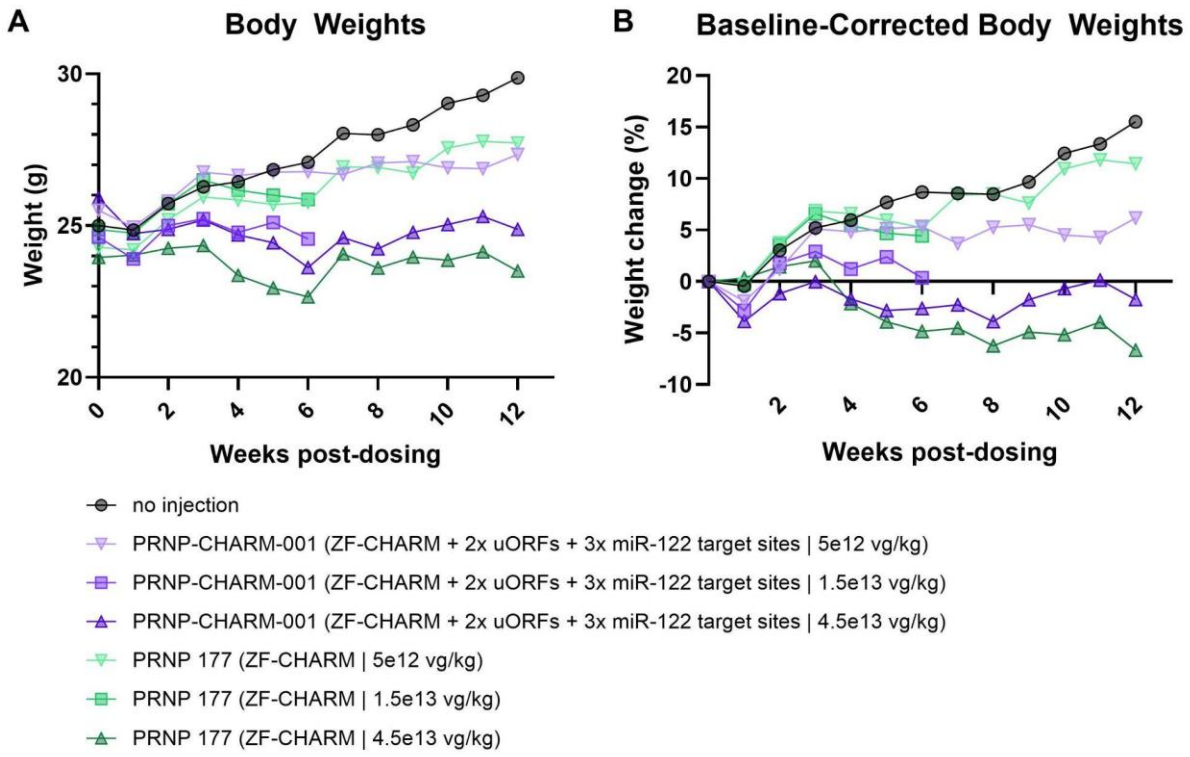


Figure 1. Body weights measured across the study for mice dosed with test articles. *hTFRC* KI;*hPRNP* mice were dosed with either PRNP-CHARM-001 (PRNP176) or PRNP177 at one of three doses (5E12 vg/kg, 1.5E13 vg/kg, or 4.5E13 vg/kg). (**A**) The graph shows body weight measurements across the 12-week in-life period (**B**) The graph shows baseline-corrected weekly body weights (Formula: 100*(Value–Baseline/Baseline)).

ELISA analysis: ELISA analysis was used to measure levels of PrP present in brain tissue samples collected from study subjects, and the results of this analysis are shown in Figure 2. PrP levels in treated mice are normalized to the uninjected controls taken down at the same timepoint. Treatment of mice with either PRNP176 or PRNP177 led to a dose-dependent decrease in brain PrP levels. Delivery of PRNP176 resulted in residual PrP levels in the brain of 25% in low-dose, 9% in mid-dose, and 5% in high-dose mice. PRNP177 treatment resulted in residual PrP levels in the brain of 25% in low-dose, 10% in mid-dose, and 5% in high-dose mice. For the low and high dose groups where PrP levels were evaluated at both 6 and 12 weeks post dosing, similar levels of knockdown were observed at 6 and 12 weeks, suggesting CHARM-based reduction of PrP is sustainable at least to 12 weeks after dosing (PRNP176: 22% residual PrP in low dose and 4% in high dose; PRNP177: 30% residual PrP in low dose and 5% in high dose).

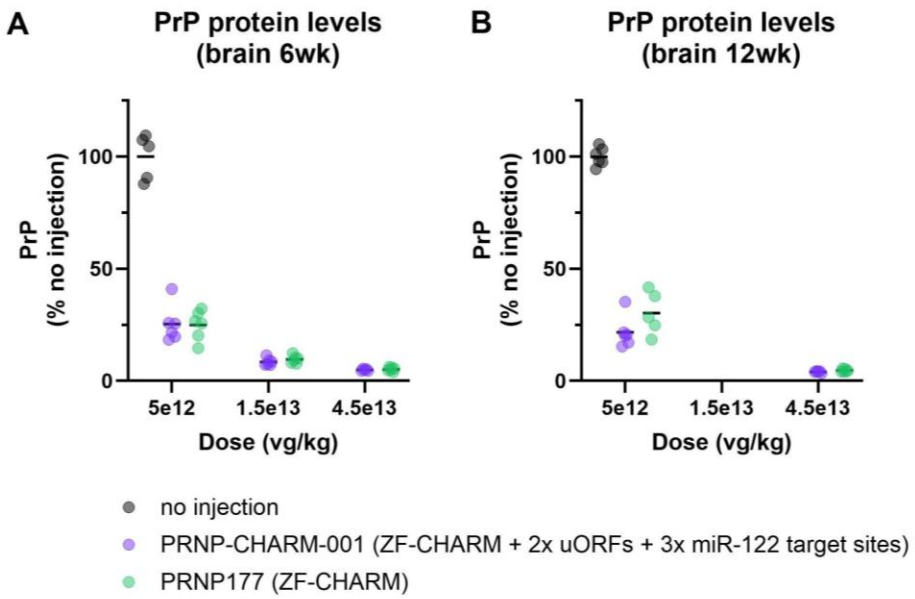


Figure 2. PrP lowering in the brain. *hTFRC* KI;*hPRNP* mice were dosed with either PRNP-CHARM-001 (PRNP176) or PRNP177 at one of three doses (5E12 vg/kg, 1.5E13 vg/kg, or 4.5E13 vg/kg). The graphs report whole brain hemisphere PrP protein levels (ELISA) at 6 weeks post-dose (**A**) or 12 weeks (**B**) post-dose.

RT-qPCR analysis: RT-qPCR was used to measure the extent of human *PRNP* RNA reduction in brain and liver tissue samples, and the results of this analysis are shown in Figures 3 and 4. *PRNP* levels in treated mice are normalized to *PRNP* levels in uninjected control mice taken down at the same timepoint. Treatment of mice with either PRNP176 or PRNP177 led to a dose-dependent decrease in brain *PRNP* mRNA levels, and similar levels of brain *PRNP* mRNA knockdown were observed for both PRNP176 and PRNP177. Treatment with PRNP176 resulted in residual brain *PRNP* mRNA levels of 29% in low-dose mice, 9% in mid-dose mice, and 4% in high-dose mice at 6 weeks post-dosing. Treatment with PRNP177 resulted in residual brain *PRNP* mRNA levels of 26% in low-dose, 10% in mid-dose, and 4% in high-dose mice at 6 weeks post-dosing. For the low and high dose groups where *PRNP* mRNA levels were evaluated at both 6 and 12 weeks post dosing, similar levels of knockdown were observed at 6 and 12 weeks, suggesting CHARM-based reduction of *PRNP* mRNA is sustainable at least to 12 weeks after dosing (PRNP176: 21% residual brain *PRNP* mRNA in low-dose mice and 9% in high-dose mice; PRNP177: 29% residual brain *PRNP* mRNA in low-dose mice and 8% in high-dose mice). In liver tissue, *PRNP* mRNA reduction was observed for both test articles. However, animals treated with PRNP176 demonstrated reduced knockdown at low (PRNP176 51% residual liver *PRNP* mRNA vs PRNP177 17%) and mid doses (PRNP176 30% residual liver *PRNP* mRNA vs PRNP177 4%) in liver relative to those treated with PRNP177 transgene, consistent with effective liver detargeting mediated by inclusion of miR-122 target sites in PRNP176. WPRE analysis in brain is ongoing.

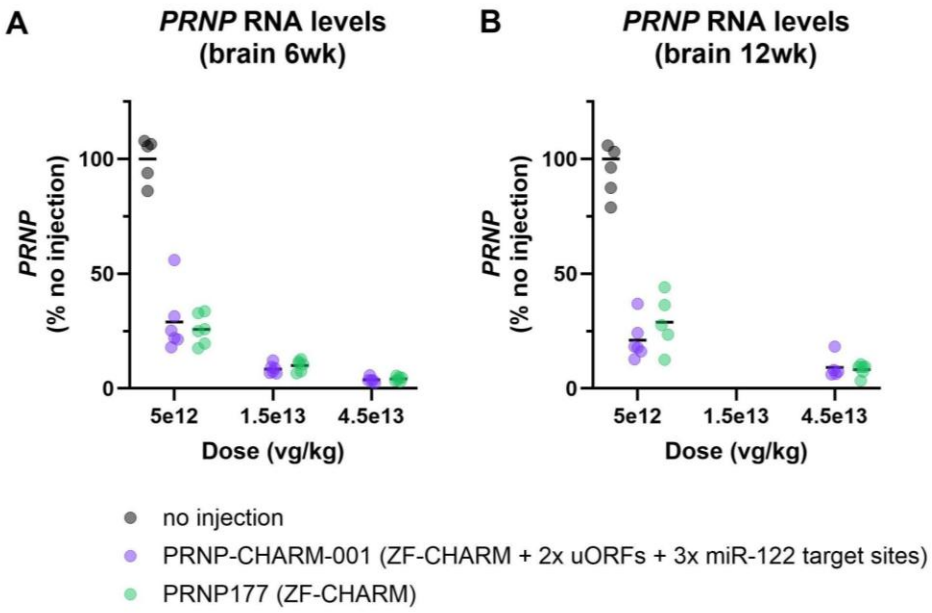


Figure 3. RT-qPCR analysis measuring *PRNP* silencing in the brain. *hTFRC* KI;*hPRNP* mice were dosed with either PRNP-CHARM-001 (PRNP176) or PRNP177 at one of three doses (5E12 vg/kg, 1.5E13 vg/kg, or 4.5E13 vg/kg). The graphs report whole brain hemisphere *PRNP* RNA level assessed by RT-qPCR at 6 weeks post-dose (A) or 12 weeks (B) post-dose.

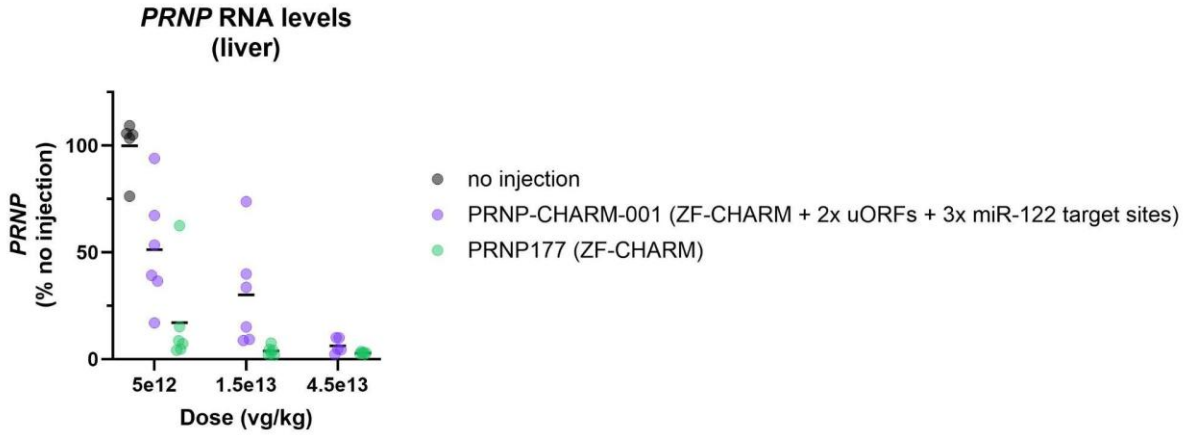


Figure 4. RT-qPCR analysis measuring *PRNP* silencing in the liver. *hTFRC* KI;*hPRNP* mice were injected with 5e12 vg/kg, 1.5e13 vg/kg, or 4.5e13 vg/kg TfR1 CapX at 9-17 weeks of age. Liver was analyzed for *PRNP* RNA by RT-qPCR (housekeeping gene TBP) at 6 weeks post-dose.

Methylation analysis: As of 2026-Jun-05, exploratory methylation analysis of study samples was not performed. Brain tissue samples collected for methylation analysis were stored at -80C and can be evaluated if necessary.

7 CONCLUSIONS

H3X was conducted to assess the potency of two candidate ZF-CHARM genome designs packaged into TfR1 CapX: PRNP176 (ultimately chosen for PRNP-CHARM-001) and the comparator PRNP177 following systemic administration in a translationally relevant *hTFRC* KI;*hPRNP* mice. The *hTFRC* KI;*hPRNP* mouse model allows evaluation of both the ZF-CHARM transgene and AAV capsid intended for clinical use. Key readouts for the study included measurement of *PRNP* mRNA in brain and liver, prion protein (PrP) levels in brain, and body weight over the course of the study.

Both PRNP176 and PRNP177 achieved robust and dose-dependent reductions of *PRNP* mRNA and PrP in brain tissue. The *PRNP* silencing was durable, persisting through the 12-week timepoint. In liver tissue, *PRNP* mRNA lowering was observed for both transgenes. However, PRNP176 demonstrated reduced lowering at low and mid doses in liver relative to PRNP177, confirming the liver detargeting mediated by inclusion of miR-122 target sites in PRNP176. A dose-dependent impact on weight gain over the course of the study was observed, where low-dose groups showed slight reductions in weight gain and high-dose groups showed little to no weight gain of the study period. A more detailed assessment of the impact of test article dosing on body weight using more closely age-matched mice will be obtained in the ongoing non GLP pilot toxicology study and the planned GLP toxicology study.

Overall, these data support selection of PRNP176 as the preferred lead PRNP-CHARM-001 candidate for further development, providing a favorable balance of CNS potency and peripheral tissue selectivity.

REFERENCES

1. Mortberg, M. A. et al. Regional variability and genotypic and pharmacodynamic effects on PrP concentration in the CNS. JCI Insight 7, e156532 (2022).

Appendix VIII: INTERIM STUDY REPORT: BRAV3

1 EXECUTIVE SUMMARY

The objective of BRAV3 is to evaluate the potency of PRNP-CHARM-001 following systemic administration at multiple doses in a translationally relevant *hTFRC* KI;*hPRNP* mouse model using TfR1 CapX. This non-GLP study is executed at the Broad Institute using test articles prepared at the Broad Institute and Viralgen, allowing for direct comparison of test articles produced by both groups. Moreover, this comparison allows more direct translation of efficacious dose levels using the same manufacturer that produced material for the non-GLP and GLP safety and distribution studies and for clinical production of PRNP-CHARM-001.

The mouse model used allows evaluation of both the *PRNP*-targeted ZF-CHARM transgene and AAV capsid intended for clinical use (see Appendix I for more information on the mouse model). Key readouts for the study will include measurement of *PRNP* mRNA in brain and liver, prion protein (PrP) levels in brain, and body weight over the course of the study.

hTFRC KI;*hPRNP* mice were dosed intravenously with test articles at 1.67E12, 5E12, 1.5E13, and 4.5E13 vg/kg and were monitored until necropsy at 7 weeks post-dosing. As of 2026-Jun-05, in-life has been completed, and post-life analyses are in progress.

2 STUDY SCOPE

BRAV3 was designed to evaluate the potency of PRNP-CHARM-001 following systemic administration at multiple doses in a translationally relevant *hTFRC* KI; *hPRNP* mouse model using TfR1 CapX. BRAV3 compares the potency of the PRNP-CHARM-001 test article as prepared by the Broad Institute and Viralgen (San Sebastian, Spain), the CDMO selected for clinical scale production of PRNP-CHARM-001, across multiple dose levels. This comparison allows more direct translation of efficacious dose levels using the same manufacturer that produced material for the non-GLP and GLP safety and distribution studies and for clinical production of PRNP-CHARM-001. BRAV3 also includes dosing at 1.67E12 vg/kg, enabling assessment of whether this dose level can provide potent *PRNP* silencing, which would further support its inclusion as a dose level in the GLP safety and distribution study.

Test articles: Test articles were manufactured at the Broad Institute (Cambridge, MA) and Viralgen (San Sebastian, Spain). Test article produced at the Broad Institute was formulated in phosphate-buffered saline supplemented with Pluronic F-68 to a final concentration of 0.001% (volume/volume). Test article produced at Viralgen was formulated in 20 mM Tris, 200 mM sodium chloride, 1 mM magnesium chloride, 0.005% poloxamer 188, pH 8.0. Prior to dosing, test articles were subjected to an analytical panel consisting of ddPCR-based titering and SDS-PAGE to assess vector purity. Test articles met all suitability criteria for use in the study. A summary of the test articles evaluated is presented in Table 1.

Table 1. Test articles evaluated in BRAV3 study

TA ID	Manufacturer	AAV Capsid	Transgene	Purpose
PRNP176	Broad Institute	TfR1 CapX	EFS-2XuORF-PRNP-CHARM-3XmiR-122-WPRE-SV40pA	Evaluates Broad Institute preparation of PRNP-CHARM-001
R-792	Viralgen	TfR1 CapX	EFS-2XuORF-PRNP-CHARM-3XmiR-122-WPRE-SV40pA	Evaluates Viralgen preparation of PRNP-CHARM-001

Study groups: Study groups were set to evaluate test article efficacy at 3-4 dose levels, 1.67E12, 5E12, 1.5E13, and 4.5E13 vg/kg, relative to cohorts of untreated control mice. Study groups were set to evaluate test article efficacy across an in-life duration of 7 weeks post-dosing. A range of ages and variable numbers of female and male mice were considered acceptable for study enrollment in the study to allow for available mice to be utilized. A summary of the planned study groups, dose levels, and target group sizes is presented in Table 2.

Table 2. Planned study groups, dose levels, and target group sizes

Group	TA ID	Dose level (vg/kg)	Age at dosing (weeks)	In-life duration (weeks)	Total mice	Female mice	Male mice
1	N/A (untreated)	0	8-15	7	5	3	2
2	PRNP176	5E12	8-15	7	5	4	1
3	PRNP176	1.5E13	8-15	7	5	4	1
4	R-792	1.67E12	8-15	7	5	3	2
5	R-792	5E12	8-15	7	5	3	2
6	R-792	1.5E13	8-15	7	5	3	2
7	R-792	4.5E13	8-15	7	5	3	2

Study readouts: The ZF-CHARM payload was designed to silence *PRNP* expression through targeted methylation of the *PRNP* locus. Primary efficacy readouts for the study will include measurement of *PRNP* mRNA in brain and liver and prion protein (PrP) levels in brain. Brain samples will be collected and reserved for exploratory measurement of *PRNP* methylation. Body weight will be monitored over the course of the study as a preliminary evaluation of test article safety and tolerability across dose levels.

3 ANALYSIS PLAN

The BRAV3 study analysis plan was predefined and determined prior to study initiation.

Body weights: Body weight will be recorded for each animal on study prior to dosing to determine test article dosage, and at least once per week after dosing. Terminal body weights will be recorded for animals prior to necropsy.

Tissue collection: Four days prior to necropsy, blood will be collected via submandibular vein, frozen on dry ice, and stored at -80C, if needed for viral distribution analyses. During necropsy, tissue samples will be collected for ELISA, RT-qPCR, qPCR, and exploratory methylation analyses. The left hemisphere of the brain will be collected, frozen on dry ice, and stored at -80C. The left hemisphere will then be cryopulverized and aliquoted for use in ELISA, RT-qPCR and vector distribution assays. The right hemisphere of the brain will be embedded in Tissue-Tek OCT Compound (Sakura), frozen on dry ice, and stored at -80C for methylation analysis. Two portions of one lobe of the liver will be collected, frozen on dry ice, and stored at -80C for RT-qPCR analyses. Heart, spleen, spinal cord, and gonadal tissues will be collected, frozen on dry ice, and stored at -80C, if needed for vector distribution or RT-qPCR assays.

ELISA: ELISA analysis will be used to measure levels of PrP present in brain tissue samples collected from study subjects[1]. An aliquot of pulverized brain tissue will be homogenized in CHAPS buffer. PrP abundance will be measured in dilutions of brain homogenate, comparing to recombinant PrP standards. Brain PrP abundance in dosed subjects will be normalized to levels measured in uninjected control mice to assess the magnitude of PrP reduction.

RT-qPCR: RT-qPCR will be used to measure transgene expression and the extent of human *PRNP* mRNA knockdown in brain and liver tissue samples. RNA will be isolated from pulverized brain hemisphere tissue and from frozen tissue samples using the QIAGEN RNEasy mini Kit (QIAGEN catalog number 74104) with on-column DNase digestion. RT-qPCR will leverage primers specific to human *PRNP*, the WPRE portion of the transgene RNA, and mouse *Tbp* as a reference. Control samples without reverse transcription will be included in analyses to measure transgene expression. *PRNP* RNA levels in dosed subjects will be normalized to levels measured in uninjected control mice to assess the magnitude of *PRNP* knockdown.

Vector distribution qPCR: qPCR will be used to measure vector genome abundance relative to mouse genome content in brain samples, with the possibility of assessing additional organs, if deemed necessary. DNA will be isolated from pulverized brain tissue or from frozen tissue samples using the QIAGEN DNeasy 96 Blood & Tissue Kit (QIAGEN catalog number 69581). Vector genome content will be measured using primers specific to the WPRE portion of the transgene, and mouse genome content will be measured using primers specific to *Gapdh*. Standard curves of known quantities of vector genomes and *Gapdh* target DNA will be used to convert Ct values for WPRE and *Gapdh* to vector genome (vg) copy number and diploid genome (dg) copy number, respectively.

Methylation analysis: Exploratory methylation analysis of the PRNP promoter via locus-enriched mapping of nucleotide methylation (LEMONmethyl-seq)[2] may be performed on the right brain hemisphere, if deemed necessary.

4 IN-LIFE PROCEDURES SUMMARY

The study is executed as a non-GLP study at the Broad Institute (Cambridge, MA) as study number CMR-4386 according to established protocols approved by the institute's IACUC committee and standard operation procedures. The Broad Institute is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

Animals were dosed with test articles via retro-orbital intravenous injection according to the planned study enrollment. All animals were dosed successfully, and no issues were noted during dosing.

Four days prior to terminal necropsy, blood was collected from the submandibular vein, frozen on dry ice, and stored at -80C for qPCR analysis of vector distribution.

As of 2026-Jun-05, in-life for BRAV3 has been completed. All animals survived to the planned necropsy timepoint.

5 TERMINAL PROCEDURES SUMMARY

Animals were euthanized 7 weeks after dosing according to their group enrollment. Terminal body weights were recorded 1 day prior to euthanasia for sacrificed animals. During necropsy, tissue samples were collected for ELISA, RT-qPCR, qPCR, and exploratory methylation analyses. The number of individual samples collected for each tissue and analysis is provided in Table 3. The left hemisphere of the brain was collected, frozen on dry ice, and stored at -80C for ELISA, qPCR, and RT-qPCR analyses. The right hemisphere of the brain was embedded in Tissue-Tek OCT Compound (Sakura), frozen on dry ice, and stored at -80C for methylation analysis. The right lobe of the liver was collected, frozen on dry ice, and stored at -80C for RT-qPCR analyses. The left lobe of the liver, spleen, heart, spinal cord, and gonads were collected, frozen on dry ice, and stored at -80C. All tissues were successfully collected for study analyses from animals reaching terminal necropsy.

Table 3. Tissues collected for study analyses.

Tissue	Number of samples collected per animal	Total samples collected	Purpose
Brain, left hemisphere	1	35	ELISA, RT-qPCR, qPCR
Brain, right hemisphere	1	35	Reserved for methylation analysis
Liver, right lobe	1	35	RT-qPCR
Liver, left lobe	1	35	Reserved for RT-qPCR or qPCR
Spleen	1	35	Reserved for RT-qPCR or qPCR
Heart	1	35	Reserved for RT-qPCR or qPCR
Spinal Cord	1	35	Reserved for RT-qPCR or qPCR
Gonads	1	35	Reserved for RT-qPCR or qPCR
Blood	1	35	Reserved for RT-qPCR or qPCR

6 POST-LIFE ANALYSIS AND RESULTS SUMMARY

As of 2026-Jun-05, post-life analyses for BRAV3 are currently in progress.

Body weight analysis: As of 2026-Jun-05, body weight analysis is in progress.

ELISA analysis: As of 2026-Jun-05, ELISA analysis of study samples is in progress.

RT-qPCR analysis: As of 2026-Jun-05, RT-qPCR analysis of study samples is in progress.

Vector distribution qPCR analysis: As of 2026-Jun-05, vector distribution qPCR analysis of brain study samples is in progress.

Methylation analysis: As of 2026-Jun-05, methylation analysis of study samples has not been performed. Samples have been collected and are reserved for potential exploratory methylation analysis.

7 CONCLUSIONS

The objective of BRAV3 is to evaluate the potency of PRNP-CHARM-001 following systemic administration at multiple doses in a translationally relevant *hTFRC* KI; *hPRNP* mouse model using TfR1 CapX. This study uses test articles prepared at the Broad Institute

and Viralgen, allowing for direct comparison of test articles produced by both groups. Key readouts for the study will include measurement of *PRNP* mRNA in brain and liver, prion protein (PrP) levels in brain, and body weight over the course of the study.

8 REFERENCES

1. Mortberg, M. A. et al. Regional variability and genotypic and pharmacodynamic effects on PrP concentration in the CNS. JCI Insight 7, e156532 (2022).

2. Christenson, A. E. *et al.* LEMONmethyl-seq: Targeted long-read DNA methylation profiling reveals dynamics of CRISPR epigenome editing and endogenous DNA methylation patterns. *bioRxiv* 2026.02.24.707761 (2026) doi:10.64898/2026.02.24.707761.

Protocol Amendment 7

Study Title	AAV-PRNP-CHARM-001: A Single-Dose Intravenous (Bolus) Injection Toxicity and Biodistribution Study in B-hTfR1 Mice Followed by a 4-Week or 13-Week Observation Period
Study Director	Diana Shefchek, MS
Test Facility	Labcorp Early Development Laboratories Inc. [redacted] Madison, Wisconsin 53704 United States of America
Labcorp Study Number	8566068
Labcorp Client Code	10020073
Sponsor	Broad Institute 415 Main Street Cambridge, Massachusetts 02142 United States of America
Page Number	1 of 44

1. APPROVALS, CHANGES, AND JUSTIFICATIONS

Protocol	**Date of Study Director Signature: 20-Jan-2026**
	Date of Sponsor Approval: 20-Jan-2026
Changes to the approved Protocol shall be made in the form of an Amendment, which will be signed and dated by the Study Director following authorization from the Sponsor or Sponsor Representative.	

Amendment and Section(s)	**Reason for Change:**
Amendment 1	**Date of Study Director Signature: 02-Feb-2026**
	Date of Sponsor Approval: 02-Feb-2026
GENERAL STUDY INFORMATION / Group Assignment and Dose Levels	To include a column for subgroups, as a Group 4 Subgroup 2 animal has been added for the purpose of exploratory tissue collections.
TEST ARTICLE AND VEHICLE CONTROL ARTICLE/DILUENT / Vehicle Control Article/Diluent	To correct the batch number of the Vehicle Control Article/Diluent in accordance with Sponsor-supplied paperwork.
TEST ARTICLE AND VEHICLE CONTROL ARTICLE/DILUENT / Formulation Preparation / Test Article Formulation Preparation	To update the procedures to include directives for serial dilutions in Groups 2, 3, and 4 (previously this was a single dilution). In the postdose formulation handling section, to clarify that the division of residual test article formulations to be divided into three approximately equal aliquots is on a per-concentration basis.
TEST SYSTEM / Mice / Animal Specifications and Acclimation	To update the number of males on study from 32 to 33, due to the addition of Subgroup 2.
TEST SYSTEM / Environmental Conditions, Diet, and Water	To remove positive pressure environmental conditions, as this is not required for this study design
TEST SYSTEM / Animal Identification and Selection	To include a column for subgroups, as a Group 4 Subgroup 2 animal has been added for the purpose of exploratory tissue collections. Additionally, to include the designated animal number for this additional animal.
INLIFE PROCEDURES / Food Consumption	To direct that only Subgroup 1 animals will have food consumption data collected in the dosing phase.
CLINICAL LABORATORY PROCEDURES / Hematology	To direct that only Subgroup 1 animals will have hematology data collected in the dosing phase.
CLINICAL LABORATORY PROCEDURES / Clinical Chemistry	To direct that only Subgroup 1 animals will have clinical chemistry data collected in the dosing phase.
MOCK NECROPSY PROCEDURES / Termination and Necropsy – Predose Phase Mock Necropsy	To remove the statement "Ultraclean procedures will be utilized" from the method of termination, as ultraclean directives already exist in the frozen tissue collection section. To remove the recording of organ weights for the mock necropsy due to scheduling constraints.
MOCK NECROPSY PROCEDURES / Special Collection Procedures / Mock Necropsy Frozen Tissue Collection	To remove the directive that frozen tissues will be collected after samples are collected for histology, as histology will not be conducted for these tissues. To include the following tissues in the tissue collection list, as these are standard for mouse collections: brain, frontal cortex; brain, hippocampus; brain, midbrain; brain, striatum; and brain, thalamus. Additionally, to update "Tail" to "Tail, proximal" in accordance with updated Labcorp SOPs. To remove the recording of tissue weights for the mock necropsy due to scheduling constraints. To remove collection procedure directives for the cerebellum, brainstem, and cerebrum due to the inclusion of additional brain

Amendment and Section(s)	Reason for Change:
	sections. Brain sectioning will be conducted in accordance with Labcorp SOPs.
MOCK NECROPSY PROCEDURES / Mock Necropsy Organ Weights and Tissues	To remove the directives for the collection of tissue weights, as tissue weights will not be collected due to scheduling constraints. To include a direct that for males, recuts of the mammary gland are not required, per Labcorp SOPs.
TERMINAL PROCEDURES / Termination and Necropsy - Unscheduled	To direct that organ weights will be recorded at unscheduled sacrifice(s) and death(s), per Sponsor request. To direct that tissue preservation and fixation is not required for Subgroup 2, as only frozen tissue collection for biodistribution will be conducted.
TERMINAL PROCEDURES / Termination and Necropsy - Interim Sacrifice	To direct that the interim sacrifice for Subgroup 1 will consist only of up to 4 animals/sex/group. To direct that the interim sacrifice for Subgroup 2 will consist of all surviving animal(s), per Sponsor request. To remove the statement "Ultraclean procedures will be utilized" from the method of termination, as ultraclean directives already exist in the frozen tissue collection section. To include separate directives for the Subgroup 2 animal for terminal body weight, blood sampling, organ weights, and tissues preservation and fixation.
TERMINAL PROCEDURES / Termination and Necropsy - Terminal Sacrifice	To clarify that only Subgroup 1 animals will be euthanized at the Terminal Sacrifice. To remove the statement "Ultraclean procedures will be utilized" from the method of termination, as ultraclean directives already exist in the frozen tissue collection section.
TERMINAL PROCEDURES / Special Collection Procedures / Bone Marrow Smear	To direct that only Subgroup 1 animals will have bone marrow smears collected.
TERMINAL PROCEDURES / Frozen Tissue Collection and Analysis for Biodistribution (Vector DNA Expression)	To direct that Subgroup 1 will have collections at the Interim, Terminal, and Unscheduled necropsy intervals, per Sponsor request. Subgroup 2 will have collections at the Interim and Unscheduled necropsy intervals, per Sponsor request. To include the following tissues in the tissue collection list, as these are standard for mouse collections: brain, frontal cortex; brain, hippocampus; brain, midbrain; brain, striatum; and brain, thalamus. Additionally, to specify "tail, proximal" for the injection site collection, in accordance with updated Labcorp SOPs. To remove collection procedure directives for the cerebellum, brainstem, and cerebrum due to the inclusion of additional brain sections. Brain sectioning will be conducted in accordance with Labcorp SOPs. To include a "Analysis (Labcorp-MSN)" section, and to direct that the tissues from the Subgroup 2 animal to be shipped by Labcorp-MSN to the Sponsor for exploratory analysis. To clarify which analysis directives apply only to Subgroup 1 animals. To update the list of tissues for analysis to include only brain, brainstem; brain, cerebellum; and brain, cerebrum samples from Groups 1 and 4 and liver samples from all groups, per Sponsor request. Additionally, to update the list of samples to be retained for potential future analysis to include remaining brain sections.

Amendment and Section(s)	Reason for Change:
TERMINAL PROCEDURES / Organ Weights and Tissues	To remove the directives for the collection of a weight of adrenal glands, lungs, and spinal cord, as this is not standard nor appropriate for mouse studies. To include a direct that for males, recuts of the mammary gland are not required, per Labcorp SOPs.
TERMINAL PROCEDURES / Histology / Embedding	To specify that only tissues from Subgroup 1 animals will undergo embedding and that embedded tissues will be held at the test facility.
DATA EVALUATION AND STATISTICAL ANALYSIS	To specify that only data from toxicity animals (Subgroup 1) will be evaluated, as the Subgroup 2 animal will only have tissues collected for exploratory analyses.
SHIPPING / Frozen Tissue Collection and Analysis for Biodistribution (Vector DNA Expression) - Subgroup 1 Sample Shipping	To update the section title to clarify that these shipping directives apply only to tissues from Subgroup 1 animals. To update the list of tissues to be shipped (previously "brain and liver samples"), and to specify that these directives apply only to tissues from Subgroup 1 animals.
SHIPPING / Frozen Tissue Collection and Analysis for Biodistribution (Vector DNA Expression) - Subgroup 2 Sample Shipping	To include shipping directives for the tissues from the Subgroup 2 animal to be shipped to the Sponsor, per Sponsor request.
Amendment 2	**Date of Study Director Signature: 12-Feb-2026**
	Date of Sponsor Approval: 12-Feb-2026
STUDY CONTACTS / Test Facility	To include the assigned Contributing Scientist for Clinical Pathology, previously "Added by Amendment."
STUDY CONTACTS / Sponsor Representatives	An Alternate Study Monitor was included to provide an additional contact for the Sponsor for animal updates or study related discussions.
GENERAL STUDY INFORMATION / Group Assignment and Dose Levels	To update the table footnote to direct that the interim sacrifice will be on Days 29 or 30 (previously Day 29 only) and the terminal sacrifice will be on Days 92 or 93 (previously Day 92 only), due to the number of animals and the complexity of the necropsy.
GENERAL STUDY INFORMATION / Proposed Study Timetable	Due to the complexity of the necropsy on this study and the number of animals, each scheduled necropsy will be conducted over two days. Thus an Interim Sacrifice Date 2 (Day 30) and Terminal Sacrifice Date 2 (Day 93) were included in the timetable.
INLIFE PROCEDURES / Body Weight	To include twice weekly body weight collections beginning on Day 22 of the dosing phase, due to the potential for body weight loss observed with similar compounds in this strain of animal.
CLINICAL LABORATORY PROCEDURES / Hematology	To update the hematology collection frequency, as the necropsy will occur over two days, and the hematology collections were adjusted to occur on the day of scheduled necropsy. Male collections will fall on Days 29 and 92 and female collections will fall on Days 30 and 93 (previously both sexes had interim necropsy on Day 29 and terminal necropsy on Day 92).
CLINICAL LABORATORY PROCEDURES / Clinical Chemistry	To update the clinical chemistry collection frequency, as the necropsy will occur over two days, and the clinical chemistry collections were adjusted to occur on the day of scheduled necropsy. Male collections will fall on Days 29 and 92 and female collections will fall on Days 30 and 93 (previously both sexes had interim necropsy on Day 29 and terminal necropsy on Day 92).
MOCK NECROPSY PROCEDURES / Special Collection Procedures / Mock Necropsy Frozen Tissue Collection	To include exploratory analysis directives for the brain tissues for the mock necropsy. Additionally, to update the sample disposition directives accordingly.
TERMINAL PROCEDURES / Termination and Necropsy - Interim Sacrifice	To split the interim sacrifice between Days 29 and 30, due to the complexity of the scheduled collections at necropsy. Males will be

Amendment and Section(s)	Reason for Change:
	sacrificed on Day 29 and females will be sacrificed on Day 30 (previously both sexes were sacrificed on Day 29).
TERMINAL PROCEDURES / Termination and Necropsy - Terminal Sacrifice	To split the terminal sacrifice between Days 92 and 93, due to complexity of the scheduled collections at necropsy. Males will be sacrificed on Day 92 and females will be sacrificed on Day 93 (previously both sexes were sacrificed on Day 92).
TERMINAL PROCEDURES / Frozen Tissue Collection and Analysis for Biodistribution (Vector DNA Expression)	To remove the following tissues from the biodistribution tissue collection list due to the size of the tissues and possible variability in collection of small tissue sizes, as evaluated at the time of the mock necropsy procedures: Brain, frontal cortex; Brain, hippocampus; Brain, midbrain; Brain, striatum; and Brain, thalamus. To update the following tissues to be collected from the left hemisphere (rather than the right hemisphere), to present the lowest possible risk for necropsy procedures to damage the brain sections designated for histopathology: Brain, brainstem; brain, cerebellum; and brain, cerebrum.
SHIPPING / Frozen Tissue Collection and Analysis for Biodistribution (Vector DNA Expression) - Mock Necropsy Sample Shipping	To include shipment directives for the brain samples from the mock necropsy, as these tissues will undergo exploratory analysis.
Amendment 3	**Date of Study Director Signature: 04-Mar-2026**
	Date of Sponsor Approval: 04-Mar-2026
TERMINAL PROCEDURES / Frozen Tissue Collection and Analysis for Biodistribution (Vector DNA Expression)	In the Procedure section, to direct that brain samples will be collected as a single sample, as the whole sample will be used for analysis and subdivision is not necessary. Additionally, to clarify that the collection of two samples applies to all other tissues listed in this section. In the Analysis (Labcorp-GRN) section, to remove directive to hold remaining brain sections at Labcorp-Madison for potential future analysis, as all brain sections will be shipped to Labcorp-Greenfield to be analyzed.
Amendment 4	**Date of Study Director Signature: 06-Apr-2026**
	Date of Sponsor Approval: 03-Apr-2026
Whole Study	To update the Study Director ahead of a planned leave of absence (previously Diana Shefchek).
STUDY CONTACTS / Test Facility	To update the Study Director ahead of a planned leave of absence (previously Diana Shefchek).
Amendment 5	**Date of Study Director Signature: 15-Apr-2026**
	Date of Sponsor Approval: 15-Apr-2026
Whole Study	To update the Study Director, previously Erin Slosarek, following the return of the prior Study Director from a planned leave of absence.
STUDY CONTACTS / Test Facility	To update the Study Director, previously Erin Slosarek, following the return of the prior Study Director from a planned leave of absence.
CLINICAL LABORATORY PROCEDURES / Hematology	To update the blood volume to be collected for the terminal sacrifice and for unscheduled sacrifices beginning on Day 44 of the dosing phase (previously 350 µL), so as to provide a sufficient quality of blood for reanalysis, if needed.
CLINICAL LABORATORY PROCEDURES / Clinical Chemistry	To update the blood volume to be collected for the terminal sacrifice and for unscheduled sacrifices beginning on Day 44 of the dosing

Amendment and Section(s)	Reason for Change:
	phase (previously 350 to 500 µL), so as to provide a sufficient quality of blood for reanalysis, if needed.
Amendment 6	**Date of Study Director Signature: 13-May-2026**
	Date of Sponsor Approval: 11-May-2026
GENERAL STUDY INFORMATION / Proposed Study Timetable	To correct "Terminal Sacrifice Date 1 (Day 93)" to "Terminal Sacrifice Date 2 (Day 93)," as this is the second terminal sacrifice date. To align the Inlife End Date with Terminal Sacrifice Date 2 (Day 93), as this date should reflect the final date of inlife activities on the study (previously listed as 06 May 2026).
TERMINAL PROCEDURES / Frozen Tissue Collection and Analysis for Biodistribution (Vector DNA Expression)	To include the assigned method number for the analysis at Labcorp-Greenfield (previously "Method number to be added by Amendment").
Amendment 7	**Date of Study Director Signature: 28-May-2026**
	Date of Sponsor Approval: 27-May-2026
STUDY CONTACTS / Test Site for Microscopic Examination	To include the assigned Test Site for Microscopic Examination, including the test site address, assigned Contributing Scientist for Anatomic Pathology, and test site reference number, as selected tissues will be microscopically examined on the study.
GENERAL STUDY INFORMATION / Proposed Study Timetable	To update the following dates to "Added by Amendment," as they will be reassigned following the addition of microscopic examination to the study: Draft Phase/Contributor Report Date, Unaudited Draft Report Date, Experimental Completion Date, and Study Completion Date.
TERMINAL PROCEDURES / Histology / Microtomy	To direct the microtomy of selected tissues from Groups 1 and 4 from the interim necropsy as these tissues will be microscopically examined on study at the request of the Sponsor.
TERMINAL PROCEDURES / Microscopic Examination and Anatomic Pathology	To direct the microscopic evaluation of of selected tissues from Groups 1 and 4 from the interim necropsy at the request of the Sponsor to examine these selected tissues.
SHIPPING / Microscopic Examination and Anatomic Pathology	To include shipping directives for the tissue slides for microscopic examination and the return shipment of slides back to the Test Facility, as microscopic examination will be conducted at a test site.

Note: When applicable, additions are indicated by **bold and underlined** text and deletions are indicated by ~~double strikethrough~~ text directly to the affected sections of the document. Section and/or page numbering (including linked references) may be renumbered in the Protocol or Table of Contents to account for additions or removal of sections, as applicable; these changes will not be detailed in this summary of changes.

2. TABLE OF CONTENTS

1. APPROVALS, CHANGES, AND JUSTIFICATIONS 2

2. TABLE OF CONTENTS....7

3. STUDY CONTACTS 9
3.1 Test Facility....9
3.2 Sponsor Representatives 9
3.3 Test Site for Biodistribution (qPCR) Analysis....9
3.4 Test Site for Microscopic Examination....10

4. GENERAL STUDY INFORMATION....11
4.1 Objective 11
4.2 Group Assignment and Dose Levels....11
4.3 Justifications....11
4.3.1 Dose Administration Rationale 11
4.3.2 Dose Justification 11
4.3.3 Species Selection....11
4.4 Proposed Study Timetable 12
4.5 Regulatory Compliance....12
4.6 Regulatory Test Guidelines....12
4.7 Animal Welfare, Care, and Use Statement and Veterinary Care/Palliative and Prophylactic Measures 12
4.8 Major Computer Systems....13

5. TEST ARTICLE AND VEHICLE CONTROL ARTICLE/DILUENT 14
5.1 Test Article....14
5.2 Vehicle Control Article/Diluent 15
5.3 Formulation Preparation....16
5.3.1 Test Article Formulation Preparation....16
5.3.2 Vehicle Control Article Preparation....17

6. TEST SYSTEM 17
6.1 Mice....17
6.1.1 Animal Specifications and Acclimation....17
6.2 Environmental Conditions, Diet, and Water 18
6.3 Animal Identification and Selection....19

7. INLIFE PROCEDURES....20
7.1 Dose Administration Details 20
7.2 Clinical Observations 20
7.2.1 Health Monitoring....20
7.2.2 Detailed Clinical Observation 21
7.2.3 Unscheduled Observations....21
7.3 Body Weight 21
7.4 Food Consumption 22

8. BLOOD FOR POTENTIAL BIODISTRIBUTION ANALYSIS 22
8.1 Blood for Potential Biodistribution Analysis....22

9. CLINICAL LABORATORY PROCEDURES 23
9.1 Hematology 23
9.2 Clinical Chemistry....24

9.3 Planned Tests....25
9.3.1 Hematology....25
9.3.2 Clinical Chemistry....25
9.3.2.1 Tier 1....25
9.3.2.2 Tier 2....25
9.4 Clinical Pathology Evaluation and Reporting....26

10. MOCK NECROPSY PROCEDURES....26
10.1 Termination and Necropsy – Predose Phase Mock Necropsy....26
10.2 Special Collection Procedures....27
10.2.1 Mock Necropsy Bone Marrow Smear....27
10.2.2 Mock Necropsy Frozen Tissue Collection....27
10.3 Mock Necropsy Organ Weights and Tissues....28

11. TERMINAL PROCEDURES....30
11.1 Fate of Animals....30
11.2 Termination and Necropsy - Unscheduled....30
11.3 Termination and Necropsy - Interim Sacrifice....31
11.4 Termination and Necropsy - Terminal Sacrifice....32
11.5 Special Collection Procedures....33
11.5.1 Bone Marrow Smear....33
11.6 Frozen Tissue Collection and Analysis for Biodistribution (Vector DNA Expression)....33
11.7 Organ Weights and Tissues....35
11.8 Histology....37
11.8.1 Embedding....37
11.8.2 Microtomy....37
11.9 Microscopic Examination and Anatomic Pathology....37

12. SHIPPING....38
12.1 Test Article Formulation Preparation - Shipping for Archival....38
12.2 Vehicle Control Article Preparation - Shipping for Archival....38
12.3 Frozen Tissue Collection and Analysis for Biodistribution (Vector DNA Expression) - Subgroup 1 Sample Shipping....39
12.4 Frozen Tissue Collection and Analysis for Biodistribution (Vector DNA Expression) - Subgroup 2 Sample Shipping....39
12.5 Frozen Tissue Collection and Analysis for Biodistribution (Vector DNA Expression) - Mock Necropsy Sample Shipping....40
12.6 Microscopic Examination and Anatomic Pathology....40

13. DATA EVALUATION AND STATISTICAL ANALYSIS....41

14. REPORTING....42

15. ARCHIVE STATEMENT....43

16. SIGNATURES....44

3. STUDY CONTACTS

3.1 Test Facility

Study Director	Diana Shefchek, MS Mobile: +[redacted] Office: +[redacted] E-mail: [redacted]
Study Coordinator	Jill Seablom, MS Office: +[redacted] E-mail: [redacted]
Reporting Associate	Stephanie Johnston, BS Office: +[redacted] E-mail: [redacted]
Contributing Scientist for Clinical Pathology	Klaudia Z. Polak, VMD, MS, Diplomate, ACVP (Clinical Pathology) Labcorp Early Development Laboratories Inc. [redacted] Greenfield, Indiana 46140 United States of America Office: +[redacted] E-mail: [redacted]

3.2 Sponsor Representatives

Study Monitor	Alissa Coffey, PhD Mobile: +[redacted] E-mail: [redacted]
Alternate Study Monitor	Sonia Vallabh, PhD Mobile: +[redacted] E-mail: [redacted]

3.3 Test Site for Biodistribution (qPCR) Analysis

Test Site Address	Labcorp Early Development Laboratories, Inc. [redacted] Greenfield, Indiana 46140 United States of America
Contributing Scientist for Biodistribution (qPCR) Analysis	Sarah Innis, PhD Labcorp Early Development Laboratories Inc. [redacted] Greenfield, Indiana 46140

	United States of America Office: +[redacted] E-mail: [redacted]
Project Manager for Biodistribution	Matthew Skidmore, MSc, BSc (Hons) Labcorp Early Development Laboratories Ltd. [redacted] North Yorkshire HG3 1PY United Kingdom Office: +[redacted] E-mail: [redacted]
Test Site Reference Number	8566068

3.4 Test Site for Microscopic Examination

Test Site Address	**Labcorp Early Development Laboratories Inc.** **[redacted]** **Bedford, Massachusetts 01730** **United States of America**
Contributing Scientist for Anatomic Pathology	**Ancuta Apreutese, DVM, DESV, PhD, Diplomate, ACVP** **Labcorp Early Development Laboratories, Inc.** **Office: +[redacted]** **E-mail: [redacted]**
Test Site Reference Number	**8566068**

4. GENERAL STUDY INFORMATION

4.1 Objective

The objective of this study is to evaluate the toxicity and determine the biodistribution of the AAV-delivered epigenic editor, AAV-PRNP-CHARM-001, when administered as a single dose via intravenous (bolus) injection to B-hTfR1 mice. After dosing, animals will be observed postdose for at least 28 or 91 days.

4.2 Group Assignment and Dose Levels

Group	Subgroup	Dose Level (vg/kg)	Dose Concentration (vg/mL)	No. of Animals	
				Males	Females
1 (Control)	1 (Tox)	0	0	8	8
2 (Low)	1 (Tox)	5.0×10^{12}	1.0×10^{12}	8	8
3 (Intermediate)	1 (Tox)	1.5×10^{13}	3.0×10^{12}	8	8
4 (High)	1 (Tox)	4.5×10^{13}	9.0×10^{12}	8	8
	2 (Exploratory)	4.5×10^{13}	9.0×10^{12}	1	0

Group 1 will be administered vehicle control article only.
Animals will be dosed at a volume of 5 mL/kg.
Up to 4 animals/sex/group will be designated for interim sacrifice on Days 29 or 30, and up to 4 animals/sex/group will be designated for terminal sacrifice on Days 92 or 93 (based on survival).

4.3 Justifications

4.3.1 Dose Administration Rationale

The intravenous (bolus) injection route of administration has been chosen because it is the intended human therapeutic route.

4.3.2 Dose Justification

The dose levels were selected by the Sponsor based on available information and to align with clinically meaningful dose levels (clinical ranges often range from 2.5×10^{12} to $> 1 \times 10^{14}$ vg/kg). These dose levels of other batches of representative test article have not been associated with any adverse events. Multiple dose levels will be evaluated on this study to establish dose response.

4.3.3 Species Selection

Mice historically have been used in safety evaluation studies and are recommended and accepted by appropriate regulatory agencies.

The B-hTfR1 mouse (C57BL/6-*Tfrc*$^{tm1[TFRC]}$) used in this study has human TfR1 (hTfR1) exons 4 through 19 knocked into the mouse locus and expresses a chimeric transferrin receptor with mouse intracellular domains (exons 1 through 3) and human extracellular domains (exons 4 through 19), all under the control of the endogenous mouse promoter. Published data (Huang, Q. et al. An AAV capsid reprogrammed to bind human Transferrin Receptor mediates brain-wide gene delivery. Science 384, 1220-1227 (2024). DOI:10.1126/science.adm8386) show that the B-hTfR1 mouse model can effectively demonstrate enhanced distribution in the central nervous system of hTfR1-binding capsids due to the expression of human TfR1 at

physiologically relevant levels. The B-hTfR1 mouse is therefore used to evaluate the biodistribution and tolerability of hTfR1-binding capsids in this study.

4.4 Proposed Study Timetable

Experimental Starting Date	21 January 2026
Mock Necropsy Date	03 February 2026
Inlife Start Date	04 February 2026
Interim Sacrifice Date 1 (Day 29)	04 March 2026
Interim Sacrifice Date 2 (Day 30)	05 March 2026
Terminal Sacrifice Date 1 (Day 92)	06 May 2026
Terminal Sacrifice Date 2 (Day 93)	07 May 2026
Inlife End Date	07 May 2026
Draft Phase/Contributor Report Date	~~17 June 2026~~**Added by Amendment**
Unaudited Draft Report Date	~~06 July 2026~~**Added by Amendment**
Experimental Completion Date	~~18 January 2027~~**Added by Amendment**
Study Completion Date	~~18 January 2027~~**Added by Amendment**

4.5 Regulatory Compliance

This study is a nonregulatory study for which a claim of Good Laboratory Practices (GLP) compliance will not be made. This study will be conducted in accordance with the applicable standard operating procedures (SOPs). This study has no specific requirement for Quality Assurance (QA) involvement.

4.6 Regulatory Test Guidelines

The study design is based on the principles of the following guideline, which is current at the time of Protocol issuance.

- International Council on Harmonisation (ICH) S12, Nonclinical Biodistribution Considerations for Gene Therapy Products (ICH, March 2023)

4.7 Animal Welfare, Care, and Use Statement and Veterinary Care/Palliative and Prophylactic Measures

All procedures in this Protocol are in compliance with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Office of Laboratory Animal Welfare. In the opinion of the Sponsor and Study Director, the study does not unnecessarily duplicate any previous work, and no other model can fulfill the study requirements.

Medical treatment necessary to minimize suffering and distress, including euthanasia, is the sole responsibility of the attending laboratory animal Veterinarian.

Palliative and prophylactic procedures, as applicable, may be based upon consensus agreement between the Study Director and attending laboratory animal Veterinarian. The Study Director and Sponsor/designee (if possible) will be included in discussions of palliative and prophylactic procedures (nonlife threatening conditions, including suspension of dosing and removal of animals from study) recommended by the attending Veterinarian. Final authority for decision making will be with the laboratory animal Veterinarian.

Labcorp Veterinarians may order further diagnostic investigations or treatments without prior notification of the Sponsor. Where unexpected deaths occur and no/minimal terminal procedures are required by the Protocol, diagnostic terminal procedures may be instigated to determine the cause of death.

4.8 Major Computer Systems

The major validated computer systems to be used at Labcorp during the conduct of this study may include, but will not be limited to, the following. All version numbers of the applications are maintained by Labcorp. Systems used by the test site(s) will be included in the respective contributing report(s), as appropriate.

Application Name	**Application Function**
Metasys	Monitors and controls environmental conditions and water flow within the facility (e.g., animal rooms)
REES Environmental Monitoring	Monitors and documents facility storage conditions (e.g., refrigerators, freezers, and constant temperature rooms)
Path/Tox System Pristima, supplied by Xybion Medical Systems Corporation	Captures direct online in-life toxicology, pharmacy, and clinical and anatomic pathology data and study maintenance information and randomizes animals; transfers data within Pristima and uploads data from external sources for translation/assembly into SEND dataset format
Tox Reporting	Transfers data from Pristima for reporting purposes
Study Designer	System for generation of study-related documents
DocuSign	Electronic signature of study-related documents
Documentum	Document management system for generation of study-related documents and electronic signatures
Electronic Notes (eNotes)	Electronic communication system
Veeva - Unified Quality Vault	Quality issues management system

Toxicology Software Foundation/Data Acquisition (TSF/DA)	Captures histology sample tracking information
SAS	Performs statistical analysis

5. TEST ARTICLE AND VEHICLE CONTROL ARTICLE/DILUENT

5.1 Test Article

Identification	AAV-PRNP-CHARM-001 The formulated test article will be provided at an actual concentration (titer value) of 4.4 x 10^{13} vg/mL (vector genomes per milliliter).
Class	Gene Therapy
Lot/Batch Number	R-792
Stability	Each lot of test article used on study will be characterized by the Sponsor, and a certificate of analysis (analytical report) will be supplied. The Sponsor is responsible for the stability of the test article and will provide documentation for inclusion in the Final Report.
Storage Conditions	In a freezer, set to maintain -60 to -80°C, and protected from light
Handling Requirements for Bulk Materials	Any transfer of material from original test article container(s) will be performed using aseptic technique, except for containers designated for non-aseptic use (e.g., a container designated for chemist use during forumlation analysis). Fresh vials of test article will be used at each preparation. The test article will be thawed at room temperature, while protected from light, for 15 to 30 minutes and subsequent dilutions will be used within 4 hours of the completion of thawing. The thaw completion times will be documented. Prior to use, each vial of bulk test article will be gently inverted at least 5 times.
Disposition of Bulk Test Article Thawed for Formulation Preparation	Each partially used vial/thawed unopened vial (as applicable) will not be returned to frozen storage. Each vial will be split into three approximately equal aliquots (if possible) in low binding plastic containers, following dose formulation completion. Aliquots will be stored on dry ice, while protected from light, until transferred to a freezer, set to maintain -60 to 80°C, until shipped.

	Aliquots and any empty bulk test article vials will be held at Labcorp to be shipped to the Sponsor-designated archive facility.
Safety	The Sponsor will provide relevant occupational safety information known about the articles used (e.g., Safety Data Sheet [SDS], safety instructions, test article identity, etc.).

5.2 Vehicle Control Article/Diluent

Identification	Diluent (Buffered Solution with Salts and Surfactants) Provided formulated by the Sponsor
Alternative Identification	7.5mM Tris (hydroxymethyl)aminomethane, 12.5mM Tris (hydroxymethyl)aminomethane hydrochloride, 1mM Magnesium chloride hexahydrate, 0.2M Sodium Chloride, 0.005% P188, pH 8
Lot/Batch Number	2026-01-07
Storage Conditions	In a freezer, set to maintain -60 to -80°C, and protected from light
Handling Requirements for Vehicle Control Article / Diluent	Any transfer of material from original vehicle control article/diluent container(s) will be performed using aseptic technique, except for containers designated for non-aseptic use (e.g., a container designated for chemist use during dose analysis). Fresh vials of vehicle control article/diluent will be used at each preparation. Vehicle control article/diluent will be thawed room temperature, while protected from light, for 15 to 30 minutes. The thaw completion times will be documented. Prior to use, each vial of bulk vehicle control article/diluent will be gently inverted at least 5 times.
Disposition of Bulk Vehicle Control Article/Diluent Thawed for Formulation Preparation	Each partially used vial/thawed unopened vial (as applicable) will not be returned to frozen storage. Each vial will be split into three approximately equal aliquots (if possible) in low binding plastic containers, following dose formulation completion. Aliquots will be stored on dry ice, while protected from light, until transferred to a freezer, set to maintain -60 to -80°C, until shipped. Aliquots and any empty vehicle control article/diluent vials will be held at Labcorp to be shipped to the Sponsor-designated archive facility.

Safety	The Sponsor will provide relevant occupational safety information known about the articles used (e.g., Safety Data Sheet [SDS], safety instructions, test article identity, etc.).

5.3 Formulation Preparation

5.3.1 Test Article Formulation Preparation

Article	**Frequency**
AAV-PRNP-CHARM-001	At least once on the day of dosing for use within 4 hours of completion of thawing
Procedures	
Procedures	Test article formulations will be prepared according to a formulation procedure developed by Labcorp. Sponsor supplied formulation procedures will be used as guidance, if provided. Test article dose formulations will be prepared in plastic containers (low protein-binding, as possible). For Group 4: Test article will be diluted volume:volume (as necessary) with diluent using aseptic technique; calculations will be based on the actual concentration of the respective test article. For Group 3: The Group 4 solution will be diluted volume:volume with diluent using aseptic technique; calculations will be based on the nominal concentration of the Group 4 solution (9.0×10^{12} vg/mL). For Group 2: The Group 3 solution will be diluted volume:volume with diluent using aseptic technique; calculations will be based on the nominal concentration of the Group 3 solution (3.0×10^{12} vg/mL). Each dilution will be mixed by gently inverting at least 10 times without introducing bubbles. The lot number and the vial designation from each Sponsor-supplied vial used to prepare formulations, will be documented.
Correction Factor	Not required. Dose concentration will be based on the test article as supplied.
Stability of Formulation	Formulations will be used within 4 hours of the completion of thawing.
Storage Conditions	Room temperature and protected from light

Postdose Formulation Handling	Any residual test article formulations will be divided into three approximately equal aliquots (if possible) per concentration in plastic low binding tubes following dose completion. Aliquots will be stored on dry ice, while protected from light, until transferred to a freezer, set to maintain -60 to 80°C, until shipped.
Disposition	Remaining Test Article dose formulation aliquots will be held at Labcorp to be shipped to the Sponsor designated archive facility at the end of the study.

5.3.2 Vehicle Control Article Preparation

Article	**Frequency**
Diluent (Buffered Solution with Salts and Surfactants)	At least once on the day of dosing
Procedures	
Procedures	Vehicle control article will be used as supplied and dispensed according to the dispensation procedure developed by Labcorp using aseptic technique. Sponsor supplied procedures will be used as guidance if provided.
Storage Conditions	Room temperature and protected from light
Postdose Formulation Handling	Any residual vehicle control article/diluent dose formulations will be divided into three approximately equal aliquots (if possible) in low binding tubes following dose completion. Aliquots will be stored on dry ice, while protected from light, until transferred to a freezer, set to maintain -60 to 80°C, until shipped.
Disposition	Remaining vehicle control article/diluent dose formulation aliquots will be held at Labcorp to be shipped to the Sponsor designated archive facility at the end of the study.

6. TEST SYSTEM

6.1 Mice

6.1.1 Animal Specifications and Acclimation

Species	Mouse
Strain and Supplier	B-hTfR1 from Biocytogen, Waltham, Massachusetts

	Animals will be sourced by the Sponsor.
Targeted Weight at Initiation of Dosing	15 to 40 g Animals outside of this range may be used at the discretion of the Study Director. The actual range will be documented and included in the report.
Targeted Age at Initiation of Dosing	7 to 9 weeks Animals outside of this range may be used at the discretion of the Study Director. The actual range will be documented and included in the report.
Number of Animals for Acclimation	35 males and 35 females
Number of Animals on Study	33 males and 32 females
Acclimation	At least 14 days

6.2 Environmental Conditions, Diet, and Water

Animal Housing	Females will be group-housed by sex and group (up to two animals/cage) in microisolator caging (e.g., Innocage® by Innovive) with sterile bedding. Males will be single-housed in in microisolator caging (e.g., Innocage® by Innovive) with sterile bedding. Females may be individually housed in microisolator caging (e.g., Innocage® by Innovive) for study-related procedures, behavioral or health reasons, or due to cage mate death (animals may be re-housed as appropriate). The cages will be changed at least once weekly throughout the study.
Bedding	Sterile, ALPHA-dri® bedding The type of bedding may be changed for behavioral or health reasons. The bedding is routinely analyzed by a certified laboratory for contaminants. The results are on file at Labcorp.
Water	Water will be supplied in sterile water bottles received prefilled by the supplier. Analytical data is provided by the vendor and results are on file at Labcorp. New water bottles will be supplied at least once weekly.

Diet	*Ad libitum* access, unless otherwise specified, to Certified Rodent Diet #2014C (Envigo RMS, Inc.). Animals may be fed the meal-form of this diet if indicated by health conditions. The diet is routinely analyzed by the manufacturer for nutritional components and environmental contaminants. Results of specified nutrient and contaminant analyses are on file at Labcorp.
Environment	Environmental controls for the animal room will be set to maintain a temperature range of 20 to 26°C, a relative humidity of 30 to 70%, a minimum of eight air changes/hour, and a 12-hour light/12-hour dark cycle. The light/dark cycle may be interrupted for study-related activities.
Dietary and Environmental Enrichment	Animals will be given various cage enrichment devices and dietary enrichment (that do not require analyses) as environmental enrichment.

6.3 Animal Identification and Selection

Means of Identification	Animals will be identified via implantable microchip identification devices, individual cage cards, an ear/neck tag, or a tail mark, as applicable. Cages will be identified with study information, including study number and animal numbers.
Animal Selection	Animals will be assigned sequentially to study groups based on pretest animal numbers, unless they are eliminated from consideration for study selection based on data collected during acclimation (predose phase). Prior to study initiation, all animals in a given sex will be confirmed to be within ±20% of the mean body weight of each sex. Additionally, group means and standard deviations will be reviewed prior to study initiation, and animals may be redistributed to balance body weights across groups.
Proposed Animal Numbers	Animals will be individually identified as follows.

Group	Subgroup	Study Animal Numbers	
		Males	Females
1	1	M0001-M0008	M0401-M0408
2	1	M0101-M0108	M0501-M0508
3	1	M0201-M0208	M0601-M0608
4	1	M0301-M0308	M0701-M0708
	2	M0309	NA

NA = Not applicable.
Note: Animal replacements will be documented in the raw data and discussed in the Final Report.

7. INLIFE PROCEDURES

7.1 Dose Administration Details

Animals	
All animals	
Procedures	
Formulation Handling	Dose formulations will be used within 4 hours of the completion of thawing of the bulk test article / bulk vehicle control article.
Frequency / Duration	Dosing phase: Once on Day 1
Dose Volume(s)	5 mL/kg, based on the most recently recorded scheduled body weight
Dose Route	Intravenous (bolus) injection
Dose Site Identification	The dose site is as follows. Dose Site A: Tail vein, lateral
Postdose Formulation Handling	Any residual formulations will be divided into three approximately equal aliquots (if possible) in low-binding tubes following dose formulation completion. Aliquots will be stored on dry ice, while protected from light, until transferred to a freezer, set to maintain -60 to 80°C, until shipped.

7.2 Clinical Observations

7.2.1 Health Monitoring

Animals	**Frequency**
All animals	At least twice daily (a.m. and p.m.) in each phase At least once on day(s) of arrival/termination

Procedures	
Data Recording	Abnormal findings will be recorded as they are observed for mortality or abnormalities.

7.2.2 Detailed Clinical Observation

Animals	**Frequency**
All animals	Predose phase: At least once weekly
Interim sacrifice animals	Dosing phase: On Days 1, 8, 15, 22, and 29
Terminal sacrifice animals	Dosing phase: On Days 1, 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, 78, 85, and 92
Procedures	
Data Recording	Abnormal findings or an indication of normal will be recorded.

7.2.3 Unscheduled Observations

Animals	**Frequency**
All animals	As observed
Procedures	
Data Recording	Abnormal findings will be recorded.

7.3 Body Weight

Animals	**Frequency**
All animals	Predose phase: At least once weekly
Interim sacrifice animals	Dosing phase: Prior to dosing on Day 1 and on Days 8, 15, 22, 25, and 28
Terminal sacrifice animals	Dosing phase: Prior to dosing on Day 1 and on Days 8, 15, 22, 25, 29, 32, 36, 39, 43, 46, 50, 53, 57, 60, 64, 67, 71, 74, 78, 81, 85, 88, and 91
Procedures	
Data Recording	Individual body weight will be recorded in grams. Additional body weights may be recorded to monitor animal health.

7.4 Food Consumption

Animals	Frequency
Subgroup 1: Interim sacrifice animals	Dosing phase: From Days 1 to 8, 8 to 15, 15 to 22, and 22 to 28
Subgroup 1: Terminal sacrifice animals	Dosing phase: From Days 1 to 8, 8 to 15, 15 to 22, 22 to 29, 29 to 36, 36 to 43, 43 to 50, 50 to 57, 57 to 64, 64 to 71, 71 to 78, 78 to 85, and 85 to 91
Procedures	
Type	Quantitative unless food is wet, spilled, or contaminated. Food consumption during intervals documented as wet, spilled, and/or contaminated will be recorded but excluded for the affected animal(s) from summary (females) and individual (males) values.

8. BLOOD FOR POTENTIAL BIODISTRIBUTION ANALYSIS

8.1 Blood for Potential Biodistribution Analysis

Animals	Frequency and Time Points
Interim sacrifice animals	Dosing phase: Once on Day 22
Terminal sacrifice animals	Dosing phase: Once on Day 85
Unscheduled sacrifice(s)	If possible Blood collection priority order is as follows: hematology > clinical chemistry > biodistribution
Procedures	
Fasting Status	Not fasted
Collection Site	Submandibular puncture An alternate site may be used if necessary and the site of blood collection will be documented.
Target Sample Volume	0.2 mL
Anticoagulant	Potassium (K2) EDTA

Processing	Following harvesting, samples will be placed on dry ice until stored in a freezer.
Storage Conditions	In a freezer, set to maintain -60 to -80°C
Analysis and Reporting	The samples will be retained for possible future analysis.

9. CLINICAL LABORATORY PROCEDURES

9.1 Hematology

Animals	**Frequency**
Subgroup 1: First 2 males/group	Dosing phase: Once on Day 29
Subgroup 1: First 2 females/group	Dosing phase: Once on Day 30
Subgroup 1: Third 2 males/group	Dosing phase: Once on Day 92
Subgroup 1: Third 2 females/group	Dosing phase: Once on Day 93
Subgroup 1: Unscheduled sacrifice(s)	If possible Blood collection priority order is as follows: hematology > clinical chemistry > biodistribution
Procedures	
Fasting Status	Not fasted
Collection Site	Vena cava An alternate site may be used if necessary and the site of blood collection will be documented.
Target Sample Volume	Interim sacrifice: 350 µL Terminal sacrifice: As much as possible, up to 1 mL Unscheduled sacrifice: 350 µL through Day 43. Beginning on Day 44, as much as possible, up to 1 mL

	Blood volumes may be adjusted as needed for animal welfare concerns and/or availability of blood collection tubes. Blood volumes collected will be noted in the raw data.
Anticoagulant	Potassium EDTA
Terminal Collection Procedures	Prior to blood collection, animals will be anesthetized with sodium pentobarbital.

9.2 Clinical Chemistry

Animals	**Frequency**
Subgroup 1: Second 2 males/group	Dosing phase: Once on Day 29
Subgroup 1: Second 2 females/group	Dosing phase: Once on Day 30
Subgroup 1: Fourth 2 males/group	Dosing phase: Once on Day 92
Subgroup 1: Fourth 2 females/group	Dosing phase: Once on Day 93
Subgroup 1: Unscheduled euthanasia(s)	If possible Blood collection priority order is as follows: hematology > clinical chemistry > biodistribution
Procedures	
Fasting Status	Not fasted
Collection Site	Vena cava An alternate site may be used if necessary and the site of blood collection will be documented.
Target Sample Volume	Interim sacrifice: 350 to 500 µL Terminal sacrifice: As much as possible, up to 1 mL Unscheduled sacrifice: 350 to 500 µL through Day 43. Beginning on Day 44, as much as possible, up to 1 mL

	Blood volumes may be adjusted as needed for animal welfare concerns and/or availability of blood collection tubes. Blood volumes collected will be noted in the raw data.
Anticoagulant	None; serum separator tubes will be used.
Terminal Collection Procedures	Prior to blood collection, animals will be anesthetized with sodium pentobarbital.

9.3 Planned Tests

In the event a planned test cannot be completed, the reason for the missing value will be recorded.

9.3.1 Hematology

red blood cell (erythrocyte) count
hemoglobin
mean corpuscular volume
mean corpuscular hemoglobin concentration
red cell distribution width
absolute neutrophil count
absolute monocyte count
absolute basophil count
blood smear
white blood cell (leukocyte) count
hematocrit
mean corpuscular hemoglobin
platelet count
absolute reticulocyte count
absolute lymphocyte count
absolute eosinophil count
absolute large unstained cell count

9.3.2 Clinical Chemistry

9.3.2.1 Tier 1

glucose
total protein
globulin
total bilirubin
alkaline phosphatase
urea nitrogen
albumin
albumin:globulin ratio
alanine aminotransferase

9.3.2.2 Tier 2

total cholesterol
gamma glutamyltransferase
aspartate aminotransferase

9.4 Clinical Pathology Evaluation and Reporting

Procedures	
Sample Disposition	Following sample analysis, any residual clinical pathology samples will be maintained by Labcorp until being disposed of in accordance with local Labcorp SOPs.
Reporting	The Contributing Scientist for Clinical Pathology will provide a clinical pathology report from the data generated to the Study Director for inclusion in the Final Report.

10. MOCK NECROPSY PROCEDURES

10.1 Termination and Necropsy – Predose Phase Mock Necropsy

Animals	**Frequency**
1 extra animal/sex	Predose phase: Once
Procedures	
Fasting Status	Not fasted
Method of Termination	Animals will be anesthetized with sodium pentobarbital and exsanguinated.
Terminal Body Weight	Recorded
Blood Sampling	Not required
Macroscopic Observations	An examination of the external features of the carcass; external body orifices; abdominal, thoracic, cranial, pelvic, and oral cavities; organs; and tissues
Organ Weights	Not recorded
Tissue Preservation and Fixation	Preserved in appropriate fixative or recorded as missing

10.2 Special Collection Procedures

10.2.1 Mock Necropsy Bone Marrow Smear

Animals	**Frequency**
1 extra animal/sex	Predose phase: Once
Procedures	
Collection Site	Femur
Number of Slides/Animal	Two
Disposition	Samples will be discarded prior to report signature.

10.2.2 Mock Necropsy Frozen Tissue Collection

Animals	**Frequency**
1 extra animal/sex	Predose phase: Once
Procedures	
Tissues/Sites	Tissues will be collected using ultraclean procedures. Brain, brainstem, right hemisphere Brain, cerebellum Brain, cerebrum Brain, frontal cortex Brain, hippocampus Brain, midbrain Brain, striatum Brain, thalamus Spinal cord, cervical Spinal cord, thoracic Spinal cord, lumbar Liver, left lateral lobe Heart, apex Spleen Adrenal gland, left Kidney, right pole Lung, accessory lobe Ovary, left Testis, left Tail, proximal
Target Sample Size to Collect	Each sample will be approximately 5x5x5 mm (actual size not documented), unless otherwise indicated below.

	For adrenal, ovary, and testis, the entire tissue will be collected.
Weight	Not recorded
Procedure	One sample will be collected from each location, if possible, unless otherwise indicated below. The sample will be placed in a cryotube, flash-frozen in liquid nitrogen, and held on dry ice until transferred to a freezer. For the spinal cord (cervical, lumbar, and thoracic), each region will be divided approximately into two halves; the caudal half will be collected in fixative for histology, and the cranial half will be used for frozen tissue collections. Tissues will be collected using ultra-clean procedures, according to Labcorp SOPs, in order to minimize the risk for potential contamination.
Storage Container	Samples will be collected into nuclease-free low binding polypropylene separate tubes.
Storage Conditions	In a freezer, set to maintain -60 to -80°C
Sample Analysis	Brain samples will be shipped to the Contributing Scientist for Biodistribution (qPCR) Analysis for exploratory analysis. Any analysis of these samples will be exploratory, for method development purposes, and outside of the scope of the toxicity evaluation of this study; therefore, results generated as part of these analyses will not be provided to the Study Director or reported as part of this study.
Sample Disposition	Samples will be held for potential future analysis, unless otherwise noted.

10.3 Mock Necropsy Organ Weights and Tissues

Organ/tissue	W	C	E	SP	Additional Information
Adrenal (2)		F			Left will be collected for frozen sample collection and right will be collected in fixative
Animal identification		F			Implantable chip, if applicable
Aorta		F			
Bone, femur with bone marrow (to include femorotibial joint)		F			
Bone, sternum with bone marrow		F			

Organ/tissue	W	C	E	SP	Additional Information
Brain		F		X	
Cecum		F			
Cervix		F			
Colon		F			
Duodenum		F			
Epididymis (2)		F			
Esophagus		F			
Eye (2)		MD			
Gallbladder (drained)		F			
Ganglia, dorsal root, cervical (2)		F			
Ganglia, dorsal root, lumbar (2)		F			
Ganglia, dorsal root, thoracic (2)		F			
Gland, Harderian (2)		MD			
Gland, coagulating (2)		F			
Gland, pituitary		F			
Gross lesions		F			
Gut-associated lymphoid tissue (GALT) - (Peyer's Patch)		F			
Heart		F			
Ileum		F			
Jejunum		F			
Kidney (2)		F			
Liver		F		X	
Lung with large bronchi		F			
Lymph node, mandibular		F			
Lymph node, mesenteric		F			
Mammary gland		F			For males, no recuts are needed
Muscle, biceps femoris		F			
Nerve, optic (2)		MD			
Nerve, sciatic		F			
Ovary (2)		F		X	Left will be collected for frozen sample collection and right will be collected in fixative
Oviduct (2)		F			Left will be collected for frozen sample collection and right will be collected in fixative
Pancreas		F			
Prostate		F			
Rectum		F			
Salivary gland, mandibular (2)		F			
Seminal vesicle (2)		F			
Skin/subcutis		F			
Spinal cord, cervical		F			
Spinal cord, lumbar		F			
Spinal cord, thoracic		F			
Spleen		F			
Stomach		F			

Organ/tissue	W	C	E	SP	Additional Information
Testis (2)		MD		X	Left will be collected for frozen sample collection and right will be collected in fixative
Thymus		F			
Thyroid (2 lobes) with parathyroid		F			
Tongue		F			
Trachea		F			
Urinary Bladder		F			
Uterus		F			
Vagina		F			

(#) = Number of sections for microscopic evaluation; C = Tissue collection and fixation; E = Tissue processing and examination according to the Histology and Microscopic Evaluation sections; IV = Intravenous; SP = Special procedures (Frozen tissue collection); F = 10% neutral buffered formalin (NBF); MD = Modified Davidson's fixative, stored in 10% NBF; W = Organs weighed; X = Action required.

Procedures	
Sample Disposition	Samples will be held for potential future analysis.

11. TERMINAL PROCEDURES

11.1 Fate of Animals

Predose Phase Terminations	Undosed predose phase animals found dead or sacrificed via carbon dioxide inhalation (followed by a secondary method of euthanasia) will be discarded, without necropsy or any collections, unless directed by a Veterinarian and/or the Study Director.

11.2 Termination and Necropsy - Unscheduled

Animals	**Frequency**
Unscheduled sacrifice(s) and death(s)	As needed
Procedures	
Method of Termination	Animals to be sacrificed will be anesthetized with sodium pentobarbital and exsanguinated.
Terminal Body Weight	Recorded for sacrificed animals
Blood Sampling	Required, if possible, from sacrificed animals

	Blood collection priority order is as follows: hematology > clinical chemistry > biodistribution
Macroscopic Observations	An examination of the external features of the carcass; external body orifices; abdominal, thoracic, cranial, pelvic, and oral cavities; organs; and tissues A Pathologist will be available for consultation during necropsy.
Organ Weights	Recorded
Tissue Preservation and Fixation	Subgroup 1: Preserved in appropriate fixative or recorded as missing Subgroup 2: Not required (frozen tissue collection for biodistribution only)

11.3 Termination and Necropsy - Interim Sacrifice

Animals	**Frequency**
Subgroup 1: Up to 4 males/group	Dosing phase: On Day 29
Subgroup 1: Up to 4 females/group	Dosing phase: On Day 30
Subgroup 2: All surviving animal(s)	Dosing phase: On Day 29
Procedures	
Fasting Status	Not fasted
Method of Termination	Animals will be anesthetized with sodium pentobarbital and exsanguinated.
Terminal Body Weight	Subgroup 1: Recorded Subgroup 2: Not required
Blood Sampling	Subgroup 1: Required Subgroup 2: Not required
Macroscopic Observations	An examination of the external features of the carcass; external body orifices; abdominal, thoracic, cranial, pelvic, and oral cavities; organs; and tissues

	A Pathologist will be available for consultation during necropsy.
Organ Weights	Subgroup 1: Recorded Subgroup 2: Not required
Tissue Preservation and Fixation	Subgroup 1: Preserved in appropriate fixative or recorded as missing Subgroup 2: Not required

11.4 Termination and Necropsy - Terminal Sacrifice

Animals	**Frequency**
Subgroup 1: All surviving males	Dosing phase: On Day 92
Subgroup 1: All surviving females	Dosing phase: On Day 93
Procedures	
Fasting Status	Not fasted
Method of Termination	Animals will be anesthetized with sodium pentobarbital and exsanguinated.
Terminal Body Weight	Recorded
Blood Sampling	Required
Macroscopic Observations	An examination of the external features of the carcass; external body orifices; abdominal, thoracic, cranial, pelvic, and oral cavities; organs; and tissues A Pathologist will be available for consultation during necropsy.
Organ Weights	Recorded
Tissue Preservation and Fixation	Preserved in appropriate fixative or recorded as missing

11.5 Special Collection Procedures

11.5.1 Bone Marrow Smear

Animals	**Frequency**
Subgroup 1: All animals	Interim and Terminal necropsy intervals
Procedures	
Collection Site	Femur
Number of Slides/Animal	Two
Disposition	Deliver to Clinical Pathology
Evaluation	Held for possible future evaluation

11.6 Frozen Tissue Collection and Analysis for Biodistribution (Vector DNA Expression)

Animals	**Frequency**
All animals	Subgroup 1: Interim, Terminal, and Unscheduled necropsy intervals Subgroup 2: Interim and Unscheduled necropsy intervals
Procedures	
Tissues/Sites	When applicable, frozen tisues will be collected without compromising histology sections. Tissues will be collected using ultraclean procedures. Brain, brainstem, left hemisphere Brain, cerebellum, left hemisphere Brain, cerebrum, left hemisphere Spinal cord, cervical Spinal cord, lumbar Spinal cord, thoracic Liver, left lateral lobe Heart, apex Spleen Adrenal gland, left Kidney, right pole Lung, accessory lobe Ovary, left Testis, left Injection site (tail, proximal)

Target Sample Size to Collect	Each sample will be approximately 5x5x5 mm (actual size not documented), unless otherwise indicated below. For adrenal, ovary, and testis, the entire tissue will be collected.
Weight	Not required
Procedure	Brain samples will be collected as a single sample. For all other tissues, unless otherwise directed, two samples will be collected from each location, if possible. The entire sample will be placed in a cryotube, flash-frozen in liquid nitrogen, and held on dry ice until transferred to a freezer. For the spinal cord (cervical, lumbar, and thoracic), each region will be divided approximately into two halves; the caudal half will be collected in fixative for histology, and the cranial half will be used for frozen tissue collections. Tissues will be collected using ultra-clean procedures, according to Labcorp SOPs, in order to minimize the risk for potential contamination.
Storage Container	Each sample will be collected into separate nuclease-free low binding polypropylene tubes.
Storage Conditions	In a freezer, set to maintain -60 to -80°C
Analysis (Labcorp-MSN)	For Subgroup 2 animal: All tissues will be shipped to the Sponsor for potential exploratory analysis.
Analysis (Labcorp-GRN)	For Subgroup 1 animals: Brain, brainstem; brain, cerebellum; and brain, cerebrum samples from Groups 1 and 4 and liver samples from all groups will be analyzed by Labcorp-Greenfield Molecular Bioanalysis using a qualified qPCR method (Method JT-00001). In the event a test cannot be completed, the reason will be recorded. Ovary, testis, spinal cord, adrenal glands, kidney, lung, heart, spleen, and injection site will be held at Labcorp-Madison for potential future biodistribution analysis.
Storage Conditions at Test Site (Labcorp-GRN)	In a freezer, set to maintain -60 to -80°C

Reporting	The Contributing Scientist for Biodistribution (qPCR) Analysis will provide a biodistribution report from the data generated to the Study Director for inclusion in the Final Report.
Sample Disposition	Samples will be discarded prior to report finalization.

11.7 Organ Weights and Tissues

Organ/tissue	W	C	E	SP	Additional Information
Adrenal (2)		F	X	X	At scheduled sacrifices, left will be collected for frozen sample collection and right will be collected in fixative. For unscheduled animals, both left and right will be collected in fixative.
Animal identification		F	X		Implantable chip, if applicable
Aorta		F	X		
Bone, femur with bone marrow (to include femorotibial joint)		F	X		
Bone, sternum with bone marrow		F	X		
Brain	X	F	X	X	
Cecum		F	X		
Cervix	X	F	X		Weighed with uterus
Colon		F	X		
Duodenum		F	X		
Epididymis (2)	X	F	X		Weighed together
Esophagus		F	X		
Eye (2)		MD	X		
Gallbladder (drained)	X	F	X		Weighed with liver
Ganglia, dorsal root, cervical (2)		F	X		
Ganglia, dorsal root, lumbar (2)		F	X		
Ganglia, dorsal root, thoracic (2)		F	X		
Gland, Harderian (2)		MD	X		
Gland, coagulating (2)		F	X		
Gland, pituitary		F	X		
Gross lesions		F	X		
Gut-associated lymphoid tissue (GALT) - (Peyer's Patch)		F	X		
Heart	X	F	X	X	
Ileum		F	X		
Injection site(s)		F	X	X	
Jejunum		F	X		
Kidney (2)	X	F	X	X	Weighed together
Liver	X	F	X	X	Weighed with gallbladder
Lung with large bronchi		F	X	X	
Lymph node, mandibular		F	X		
Lymph node, mesenteric		F	X		
Mammary gland		F	X		For males, no recuts are needed

Organ/tissue	W	C	E	SP	Additional Information
Muscle, biceps femoris		F	X		
Nerve, optic (2)		MD	X		
Nerve, sciatic		F	X		
Ovary (2)	X	F	X	X	Weighed together; with oviduct. Scheduled sacrifices: left will be collected for frozen sample collection and right will be collected in fixative. Unscheduled animals: both left and right will be collected in fixative.
Oviduct (2)	X	F	X		Weighed together; with ovary. Scheduled sacrifices: left will be collected for frozen sample collection and right will be collected in fixative. Unscheduled animals: both left and right will be collected in fixative.
Pancreas		F	X		
Prostate	X	F	X		
Rectum		F	X		
Salivary gland, mandibular (2)		F	X		
Seminal vesicle (2)		F	X		
Skin/subcutis		F	X		
Spinal cord, cervical		F	X	X	
Spinal cord, lumbar		F	X	X	
Spinal cord, thoracic		F	X	X	
Spleen	X	F	X	X	
Stomach		F	X		
Testis (2)	X	MD	X	X	Weighed together; Scheduled sacrifices: left will be collected for frozen sample collection and right will be collected in fixative. Unscheduled animals: both left and right will be collected in fixative.
Thymus	X	F	X		
Thyroid (2 lobes) with parathyroid		F	X		
Tongue		F	X		
Trachea		F	X		
Urinary Bladder		F	X		
Uterus	X	F	X		Weighed with cervix
Vagina		F	X		

(#) = Number of sections for microscopic evaluation; C = Tissue collection and fixation; E = Tissue processing and examination according to the Histology and Microscopic Evaluation sections; IV = Intravenous; SP = Special procedures (Frozen tissue collections); F = 10% neutral buffered formalin (NBF); MD = Modified Davidson's fixative, stored in 10% NBF; W = Organs weighed; X = Action required.
Note: Tissues collected from animals replaced after dosing on Day 1 will be held in fixative.

11.8 Histology

11.8.1 Embedding

Animals	**Frequency**
Subgroup 1: All animals	Interim, Terminal, and Unscheduled necropsy intervals
Procedures	
Procedures	All available study tissues (unless noted as missing), will be trimmed and embedded in paraffin. Parrafin-embedded tissue blocks will be retained at the test facility until processed for examination (to be added by Amendment, if warranted), shipped to a Sponsor-designated location, or discarded prior to overall report finalization.

11.8.2 Microtomy

Animals	**Frequency**
Groups 1 and 4, Subgroup 1: All animals	**Terminal necropsy interval**
Procedures	
Procedures	**Brain; Liver; Muscle, biceps femoris; Spinal cord, cervical; Spinal cord, lumbar; and Spinal cord, thoracic (unless noted as missing) will be sectioned at a nominal 5 µm and stained with hematoxylin and eosin, unless stated otherwise.**

11.9 Microscopic Examination and Anatomic Pathology

Animals	**Frequency**
Groups 1 and 4, Subgroup 1: All animals	**Terminal necropsy interval**
Procedures	
Procedures	**Brain; Liver; Muscle, biceps femoris; Spinal cord, cervical; Spinal cord, lumbar; and Spinal cord, thoracic (unless noted as missing) will be examined microscopically by the Anatomic Pathologist. Missing tissues will be recorded.**
Reporting	**The Contributing Scientist for Anatomic Pathology will provide a contributory report from the data generated (unsecured, in Portable Document Format [.pdf], and compliant with Labcorp and Sponsor**

	[if applicable] PDF specifications) to the Study Director for inclusion in the Final Report.
Digital Scans	**Upon request from the Anatomic Pathologist or Sponsor and after approval of the Study Director, digital scans (virtual slides) may be prepared from selected microscopic slides for communicative/illustrative purposes only. These digital scans will not be archived (microscopic slides are archived as the original source of the pathology data for a respective study).**

12. SHIPPING

12.1 Test Article Formulation Preparation - Shipping for Archival

Frequency	On the following Monday, Tuesday, or Wednesday after the end of inlife.
Conditions	Dry ice
Shipping	Samples will be shipped by overnight carrier to the following address. Fiona Serack The Broad Institute 415 Main Street[redacted] Cambridge, Massachusetts 02142 United States of America Telephone: +[redacted] E-mail: [redacted] Before shipping, the recipient, Study Director, and Study Monitor will be notified by e-mail as to the date and method of sample shipments.

12.2 Vehicle Control Article Preparation - Shipping for Archival

Frequency	On the following Monday, Tuesday, or Wednesday after the end of inlife.
Conditions	Dry ice
Shipping	Samples will be shipped by overnight carrier to the following address. Fiona Serack The Broad Institute 415 Main Street[redacted] Cambridge, Massachusetts 02142

	United States of America Telephone: +[redacted] E-mail: [redacted] Before shipping, the recipient, Study Director, and Study Monitor will be notified by e-mail as to the date and method of sample shipments.

12.3 Frozen Tissue Collection and Analysis for Biodistribution (Vector DNA Expression) - Subgroup 1 Sample Shipping

Frequency	On the following Monday, Tuesday, or Wednesday after each collection interval
Conditions	Dry ice
Shipping	Brain, brainstem; brain, cerebellum; brain, cerebrum; and liver samples collected from Subgroup 1 animals will be shipped by overnight carrier to the following address. BioCMC Sample Management Attn: Sarah Innis Labcorp Early Development Laboratories Inc. BioCMC/BioD [redacted] Greenfield, Indiana 46140 United States of America Telephone: +[redacted] E-mail: [redacted] Before shipping, the recipient, Study Director, and Study Monitor will be notified by e-mail as to the date and method of sample shipments. An electronic manifest will be included with each sample shipment.

12.4 Frozen Tissue Collection and Analysis for Biodistribution (Vector DNA Expression) - Subgroup 2 Sample Shipping

Frequency	On the following Monday, Tuesday, or Wednesday after the collection interval
Conditions	Dry ice
Shipping	Samples collected from the Subgroup 2 animal will be shipped by overnight carrier to the following address.

	Broad Institute Attn: Fiona Serack 415 Main Street Cambridge, Massachusetts 02142 United States of America Telephone: +[redacted] E-mail: [redacted] Before shipping, the recipient, Study Director, and Study Monitor will be notified by e-mail as to the date and method of sample shipments. An electronic manifest will be included with each sample shipment.

12.5 Frozen Tissue Collection and Analysis for Biodistribution (Vector DNA Expression) - Mock Necropsy Sample Shipping

Frequency	On a Monday, Tuesday, or Wednesday after the collection interval
Conditions	Dry ice
Shipping	Brain samples collected at the mock necropsy will be shipped by overnight carrier to the following address. BioCMC Sample Management Attn: Sarah Innis Labcorp Early Development Laboratories Inc. BioCMC/BioD [redacted] Greenfield, Indiana 46140 United States of America Telephone: +[redacted] E-mail: [redacted] Before shipping, the recipient, Study Director, and Study Monitor will be notified by e-mail as to the date and method of sample shipments. An electronic manifest will be included with each sample shipment.

12.6 Microscopic Examination and Anatomic Pathology

Frequency	**Slides will be shipped from the location of histology processing to the location of the Anatomic Pathologist for evaluation.**
Conditions	**Ambient conditions**

Shipping	**Slides will be shipped by overnight carrier to the following address.** **Labcorp Early Development Laboratories Inc.** **Attn: Ancuta Apreutese** [redacted] **Bedford, Massachusetts 01730** **United States of America** **Telephone: +**[redacted] **E-mail:** [redacted] **Before shipping, the recipient, Study Director, and Study Monitor will be notified by e-mail as to the date and method of slide shipment.**
Return Shipping	**Slides and any applicable raw data will be returned to the test facility prior to finalization of the report for archival. Slides and any applicable raw data will be returned to the following address.** **Labcorp Early Development Laboratories Inc.** **Pre-Archive** [redacted] **Madison, Wisconsin 53704** **United States of America** **Telephone: +**[redacted] **E-mail: ED-Madison-Sample-Management-Pre-**[redacted] **Before shipping, the recipient, Study Director, and Study Coordinator will be notified by e-mail as to the date and method of shipments.**

13. DATA EVALUATION AND STATISTICAL ANALYSIS

General Statistics
Data for each sex will be analyzed separately. Only data collected on or after the first day of dosing will be analyzed statistically. Only data from toxicity animals (Subgroup 1) will be evaluated. Analysis of variance (ANOVA) and pairwise comparisons, as applicable, will be used to analyze the following: • Absolute body weight • Body weight change • Quantitative food consumption • Terminal body weight

• Absolute organ weight, organ:body weight percentage, and organ:brain weight percentage The pairwise comparisons of interest are: • Group 1 versus Groups 2, 3, and 4 Prior to performing the ANOVA, Levene's test will be performed to test for equality of variances between groups: • Where Levene's test is significant ($P \leq 0.05$), a rank transformation (to stabilize the variances) will be applied before the ANOVA is conducted (note: Levene's test will not be applied to the rank-transformed data). • Where Levene's test is not significant ($P > 0.05$), ANOVA will be conducted. For comparisons to a single control or combined identical controls: • If the group effect of the ANOVA is significant ($P \leq 0.05$), Dunnett's test will be used for pairwise comparisons between each treated and control group. • If the ANOVA is not significant ($P > 0.05$), the Dunnett's test results are not applicable to the evaluation and will not be reported or used to interpret study data. For other comparisons (i.e., not against a single control or combined identical controls): • If the group effect of the ANOVA is significant ($P \leq 0.05$), t-tests will be used for pairwise comparisons. • If the ANOVA is not significant ($P > 0.05$), the t-test results are not applicable to the evaluation and will not be reported or used to interpret study data. Where only two groups are available for analysis, a two-sample t-test will be performed. Data with values above/below the limit of quantitation will not be analyzed, and the tables will be footnoted accordingly. Where insufficient data are available for meaningful analysis, no analysis will be performed, and the tables will be footnoted accordingly. All statistical tests will be evaluated at the 5.0% probability level. Due to system limitations, additional statistical analyses may be run but will not be reported or used to interpret study data.

14. REPORTING

Template	Labcorp
Draft Reports	An electronic copy of the unaudited draft report will be submitted to the Sponsor and will include relevant study data and contributing reports, as available. Sponsor comments should be supplied within 6 months of issuance of the draft report. If no comments are received, the study will be finalized and the data will be archived. The archive period will commence at that

	time. Any further changes after this time may be addressed as an Amended Final Report and may result in additional costs.
Final Report	An electronic version of the signed Final Report (to include all text and summary tables and all phase and/or contributor reports, as applicable) will be sent to the Sponsor. The electronic Final Report will be a text searchable, hyperlinked, bookmarked Portable Document Format (PDF) document.
Tabulated Summary	A separate tabulated summary will be provided to the Sponsor.

15. ARCHIVE STATEMENT

The raw data, including documentation, test and vehicle control article samples (if applicable), study Protocol, Final Report, study correspondence, and specimens (excluding wet specimens obtained from blood, urine, feces, and biological fluids), resulting from this study will be retained in the test facility archives for at least 1 year from the date of report finalization. After this period, the Sponsor will be contacted to determine requirements for further retention or disposition of the archived materials (excluding facility records, nontransferable electronic data, and facility copies of the Protocol/Final Report, which will be retained by Labcorp in accordance with test facility SOPs). Where continued retention is requested, the archived materials may subsequently be transferred to alternative Labcorp archive locations. In this event, the Sponsor will be informed, and a chain of custody records will be maintained. Electronic records relating to the study will be held on the secure servers of Labcorp or designated cloud providers, with access controlled through the designated Labcorp Archivist.

Raw data, including documentation, test and vehicle control article samples (if applicable), and specimens (excluding wet specimens obtained from blood, urine, feces, and biological fluids), generated from phases performed by test sites will be archived in accordance with test site SOPs.

Raw data, including documentation and tissue, from biodistribution evaluation performed by Labcorp-Greenfield will be archived at Labcorp-Greenfield.

16. SIGNATURES

[redacted]

28 Ma[redacted]

Diana Shefchek, MS
Study Director
Labcorp Early Development Laboratories Inc.

Date

Appendix X: INTERIM STUDY REPORT: PRNP-CHARM-001 SAFETY AND BIODISTRIBUTION STUDY

1 EXECUTIVE SUMMARY

The objective of this non-GLP study was to evaluate the biodistribution and safety of the PRNP-CHARM-001 product at 1 and 3 months post-intravenous product administration in the humanized transferrin receptor knock-in mouse model (Biocytogen, *hTFRC* KI). The study was executed at Labcorp (Madison, WI) as a non-GLP study, using test article manufactured by Viralgen (San Sebastian, Spain). In-life was completed for this study, and post-life analyses are in progress. Available interim study readouts are provided in this report, while interpretation and reporting by Labcorp is in progress.

Animals were dosed intravenously with PRNP-CHARM-001 at three dose levels, 5E12, 1.5E13, and 4.5E13 vg/kg, relative to cohorts of vehicle-treated control mice; no issues were noted during dosing. During body weight monitoring, two animals from the 4.5E13 vg/kg dose group showed minor body weight loss, leading to supplementation of all groups with diet gel on Day 59 of the study. This supplementation impacted the food consumption readout, as consumption of diet gel could not be reliably monitored.

All animals survived to their respective necropsy timepoints, and tissues were collected successfully. Body weight analyses indicated reduced weight gain in animals treated with 4.5E13 vg/kg PRNP-CHARM-001 relative to vehicle-treated animals. The results of this study so far have informed the design of the GLP PRNP-CHARM-001 safety and distribution study, which will not use the 4.5E13 vg/kg dose level due to the body weight impacts observed in this study.

2 STUDY SCOPE

This non-GLP study evaluated the biodistribution and safety of the PRNP-CHARM-001 product at 1 and 3 months post-intravenous product administration in the humanized transferrin receptor knock-in mouse model (Biocytogen, *hTFRC* KI). The study was executed at Labcorp (Madison, WI), a GLP-compliant preclinical contract research organization (CRO) with expertise in conducting in vivo nonclinical studies, including for AAV-based gene therapies.

Test articles: Test article was manufactured at Viralgen (San Sebastian, Spain). Test article was formulated in 20 mM Tris, 200 mM sodium chloride, 1 mM magnesium chloride, 0.005% poloxamer 188, pH 8.0. Prior to dosing, test article was subjected to an analytical panel consisting of ddPCR-based titering and SDS-PAGE to assess vector purity. Test article met all suitability criteria for use in the study. A summary of the test article evaluated is presented in Table 1.

Table 1. Test article evaluated in study

Test Article ID	AAV Capsid	Transgene	Purpose
R-792	TfR1 CapX	EFS-2XuORF-PRNP-CHARM-3XmiR-122-WPRE-SV40pA	Evaluates safety outcomes associated with dosing PRNP-CHARM-001

Study groups: Study groups were set to evaluate test article safety at three dose levels, 5E12, 1.5E13, and 4.5E13 vg/kg, relative to cohorts of vehicle-treated control mice. Study groups were planned to include even numbers of female and male mice (4 each, 8 mice total per dose group per time point). An additional exploratory group (4-2) consisting of a single male mouse dosed at 4.5E13 vg/kg was included, where tissues will be processed for exploratory biodistribution analyses to be conducted at the Broad Institute. Study groups were set to evaluate test article safety across two in-life durations: 1 and 3 months post-dosing. A summary of the planned study groups, dose levels, and target group sizes is presented in Table 2.

Table 2. Planned study groups, dose levels, and target group sizes

Group	Test Article ID	Dose level (vg/kg)	Age at dosing (weeks)	In-life duration (months)	Total mice	Female mice	Male mice
1-1	Formulation buffer	0	7-9	1	8	4	4
1-1	Formulation buffer	0	7-9	3	8	4	4
2-1	R-792	5E12	7-9	1	8	4	4
2-1	R-792	5E12	7-9	3	8	4	4
3-1	R-792	1.5E13	7-9	1	8	4	4
3-1	R-792	1.5E13	7-9	3	8	4	4
4-1	R-792	4.5E13	7-9	1	8	4	4
4-1	R-792	4.5E13	7-9	3	8	4	4
4-2	R-792	4.5E13	7-9	1	1	0	1

Study readouts: Safety readouts include survival/mortality, clinical observations, body weight, food consumption, anatomic pathology, histopathology, hematology, and clinical chemistry. Vector biodistribution across tissues will be determined using qPCR.

3 ANALYSIS PLAN

The study analysis plan was predefined and determined prior to study initiation.

Survival/Mortality and clinical observations: The animals were observed twice daily for morbidity, mortality, and injury. Any animals in poor health will be identified for further monitoring.

Body weights: Body weight were recorded for each animal on study prior to dosing to determine test article dosage, and at least once per week after dosing. Specifically, animals were weighed on Days 8, 15, 22, 25, 28 (1-month cohort only for Day 28), 29, 32, 36, 39, 43,

46, 50, 53, 57, 60, 64, 67, 71, 74, 78, 81, 85, 88, and 91 (3-month cohort only for Days 29-91). Terminal body weights were recorded for animals prior to necropsy.

Food consumption: Food consumption was monitored over the course of each week in the study.

Blood collection: Blood samples were collected on Day 22 and Day 85 and stored for potential biodistribution analysis. Blood samples for evaluation of hematology (2M/2F per group per timepoint) and clinical chemistry (2M/2F per group per timepoint) were collected at necropsy.

Anatomic pathology: During necropsy, macroscopic evaluations were performed on external features of the carcass, external body orifices, abdominal, thoracic, cranial, pelvic, and oral cavities, organs, and tissues. Terminal body weight and organ weights were recorded.

Tissue collection: During necropsy, tissue samples were collected for biodistribution and fixed and embedded for histopathology analyses. Males were euthanized on Day 29 and 92, and females were euthanized on Day 30 and 93 according to their group enrollment. Necropsy was performed on scheduled animals; no animals were prematurely terminated or found dead. Brain was removed and bisected. Right hemisphere was fixed and embedded and left hemisphere was divided into brainstem, cerebellum, and cerebrum for biodistribution (3 samples). Segments of the spinal cord (cervical, thoracic, and lumbar) were separated into two halves: the caudal half was fixed and embedded and the cranial half was frozen for biodistribution. Portions of the liver, heart, spleen, kidney, lung, gonads, adrenal glands, and injection site were collected for embedding as well as frozen for biodistribution. Bone marrow smears were collected from the femur and are being held for potential future evaluation.

Hematology: Blood samples were analyzed for red blood cell count, hemoglobin, hematocrit, white blood cell count, basophils (absolute count and percent), eosinophils (absolute count and percent), large unstained cells (absolute count and percent), lymphocytes (absolute count and percent), monocytes (absolute count and percent), neutrophils (absolute count and percent), reticulocytes (absolute count and percent), red blood cell distribution width, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and mean corpuscular volume.

Clinical chemistry: Blood samples were analyzed for glucose, total protein, globulin, total bilirubin, alkaline phosphatase, urea nitrogen, albumin, albumin:globulin ratio, alanine aminotransferase, total cholesterol, aspartate aminotransferase, and gamma glutamyltransferase.

Histopathology: Histopathological analyses will focus initially on hematoxylin and eosin-stained brain, liver, muscle (biceps femoris), and spinal cord (cervical, lumbar, thoracic) sections from Groups 1 and 4. Additional fixed and embedded tissues will be held for potential future evaluations.

Vector distribution qPCR: DNA will be extracted from tissues and will be analyzed by using a qualified duplex quantitative polymerase chain reaction (qPCR) assay for the detection of the WPRE portion of the PRNP-CHARM-001 payload to measure viral genome abundance and mouse *Tert* to provide a measure of diploid genome content. A total of 4 samples will be analyzed per animal; tissues to be analyzed are brain (3 samples: brainstem, cerebellum, and cerebrum) and liver. Spinal cord (cervical, thoracic, lumbar), gonads (ovary and testis), adrenal glands, kidney, lung, heart, spleen, and injection site will be reserved for potential future biodistribution analysis.

4 IN-LIFE PROCEDURES SUMMARY

The study was executed as a non-GLP study at Labcorp (Madison, WI) as study number 8566068 according to established protocols approved by Labcorp's IACUC and standard operation procedures. Labcorp is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

Animals were dosed with test articles via tail vein intravenous injection according to the planned study enrollment. All animals were dosed successfully, and no issues were noted during dosing. Retains of formulated test article were reserved and stored at -80C for potential future analysis.

Over the duration of the study, animals were monitored for health issues, and body weights were recorded as scheduled. During the intervals between Days 43-50 and 50-57, two animals from Group 4 were observed to have minor body weight loss. Diet gel was supplemented in all cages on Day 59 to provide further nutritional support for animals with minor body weight loss and to control for the impact of diet gel supplementation on study readouts.

No unscheduled deaths occurred over the course of the study; all animals survived to their respective necropsy timepoints.

5 TERMINAL PROCEDURES SUMMARY

Animals were euthanized 29-30 or 92-93 days after dosing according to their group enrollment. Terminal body weights and organ weights were recorded for sacrificed animals, and macroscopic examination of the body was performed. Tissue samples were successfully collected from tissues for biodistribution and histopathology analyses.

6 POST-LIFE ANALYSIS AND RESULTS SUMMARY

Animals excluded from analysis: No animals were excluded from study analyses. Final study enrollment for analysis is detailed in Table 3.

Table 3. Final enrollment in study groups

Group	Test Article ID	Dose level (vg/kg)	Age at dosing (weeks)	In-life duration (months)	Total mice	Female mice	Male mice
1-1	Formulation buffer	0	7-9	1	8	4	4
1-1	Formulation buffer	0	7-9	3	8	4	4
2-1	R-792	5E12	7-9	1	8	4	4
2-1	R-792	5E12	7-9	3	8	4	4
3-1	R-792	1.5E13	7-9	1	8	4	4
3-1	R-792	1.5E13	7-9	3	8	4	4
4-1	R-792	4.5E13	7-9	1	8	4	4
4-1	R-792	4.5E13	7-9	3	8	4	4
4-2	R-792	4.5E13	7-9	1	1	0	1

Survival/Mortality: All animals survived to their scheduled necropsies.

Clinical observations: No PRNP-CHARM-001-related clinical observations were noted. Abnormal clinical observations included thinning pelage/hair loss, scabs, skin sores or missing whiskers. These findings were considered not related to PRNP-CHARM-001 as they were observed for a single animal, lacked a dose response, appeared rather infrequently, or are a common background finding for this species.

Body weight analysis: Body weights recorded over the course of the study were analyzed to identify any test article-dependent effects on body weight. Mean group body weights over the course of the study and body weight change relative to starting weight for all dose groups are shown in Figure 1. Accounting for initial baseline weight, no PRNP-CHARM-001-related effects on body weight or body weight change were observed for animals administered 5E12 or 1.5E13 vg/kg PRNP-CHARM-001. Accounting for initial baseline weight, treatment with 4.5E13 vg/kg PRNP-CHARM-001 led to reduced body weight gain relative to vehicle-treated animals.

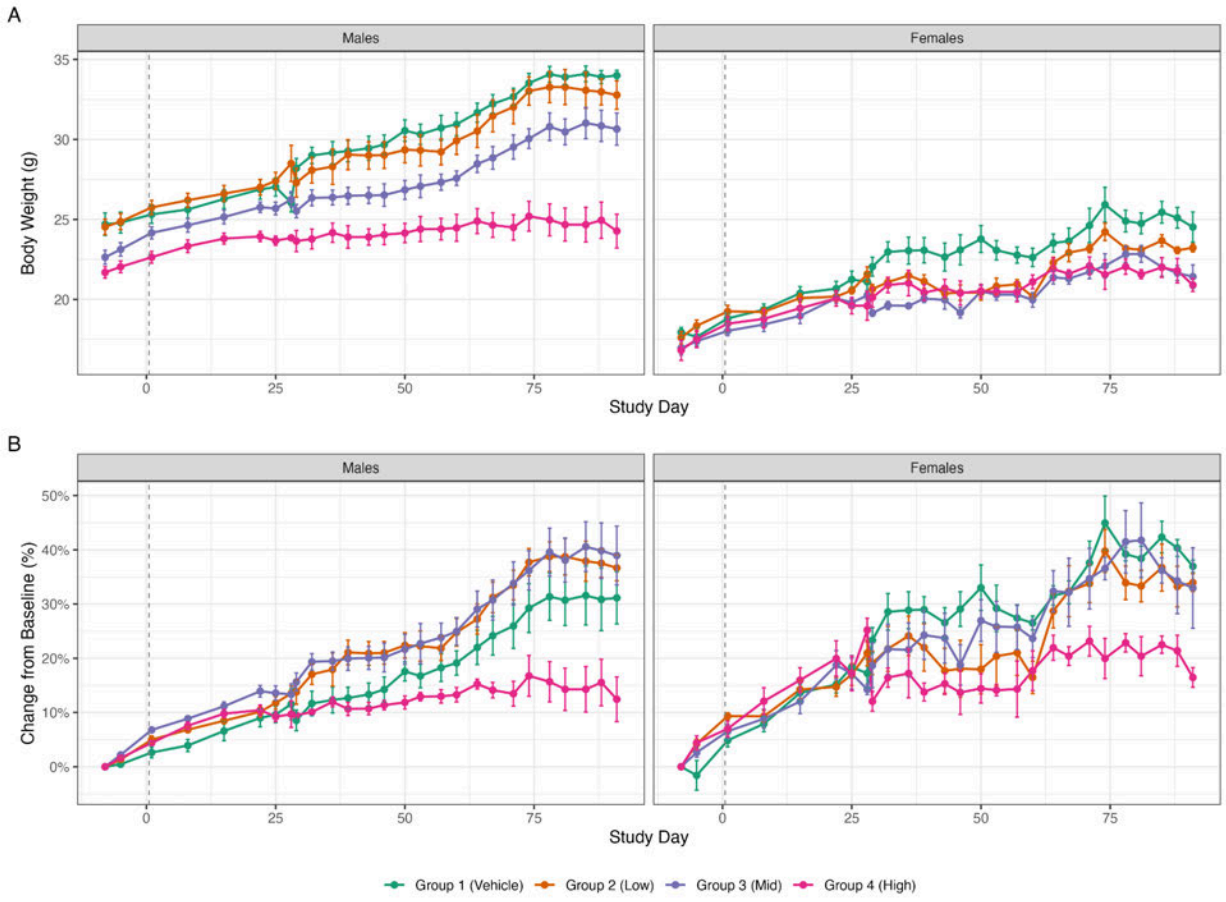


Figure 1. Body weight analysis. (**A**) Mean body weight for each group, separated by sex. The dotted line represents the day of dosing, and error bars represent standard deviation. (**B**) Mean body weight for each group, separated by sex, normalized to starting body weight prior to dosing. The dotted line represents the day of dosing, and error bars represent standard deviation.

Food consumption analysis: On Day 59 of the dosing phase, diet gel was offered to each cage of animals when continued minor body weight loss was observed in two females administered 4.5E13 vg/kg PRNP-CHARM-001. Following the placement of diet gel, food consumption values were reduced overall but since diet gel was placed in all cages, this reduction was consistent across all groups. Given the impact of diet gel supplementation on the consistency of the food consumption readout, we cannot make strong conclusions on the impact of PRNP-CHARM-001 treatment on food consumption for the full duration of the study.

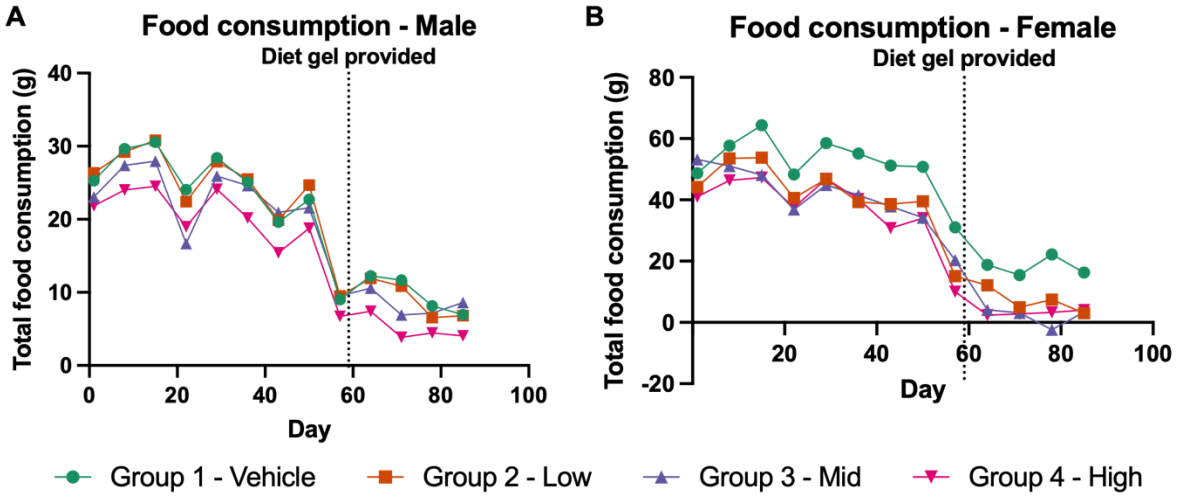


Figure 2. Food consumption analysis. Mean weekly food consumption is plotted for each group over the course of the study. Food consumption for males (**A**) represents individual mice (males are singly housed), while food consumption for (**B**) females represents values for two group-housed females. The dotted line at day 59 indicates the point at which cages were supplemented with diet gel.

Hematology analysis: As of 2026-Jun-05, hematology analysis by Labcorp is in progress. A subset of the raw hematology data is provided in Figure 3. Additional hematological data collected but not shown include measurements of basophils (absolute and percent), eosinophils (absolute and percent), large unstained cells (absolute and percent), percent lymphocytes, percent monocytes, percent neutrophils, percent reticulocytes, red blood cell distribution width, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and mean corpuscular volume. Data are plotted alongside reference hematological data for the *hTFRC* KI mouse strain available from Biocytogen (pink and blue shading for female and male reference data, respectively). The reference data represent mean values +/- 1 standard deviation for each hematological parameter, collected from eight 8-week-old mice of each sex. Efforts to collect reference hematological data from more closely age-matched *hTFRC* KI mice are ongoing.

No data were obtained from 1 male from Group 2 (1 month cohort), 1 male from Group 3 (1 month cohort), 1 male from Group 4 (1 month cohort), and 1 female from Group 4 (3 month cohort) due to insufficient sample volume to run or repeat measurements. Given the low number of samples, we cannot make strong conclusions on the impact of PRNP-CHARM-001 treatment on hematological parameters.

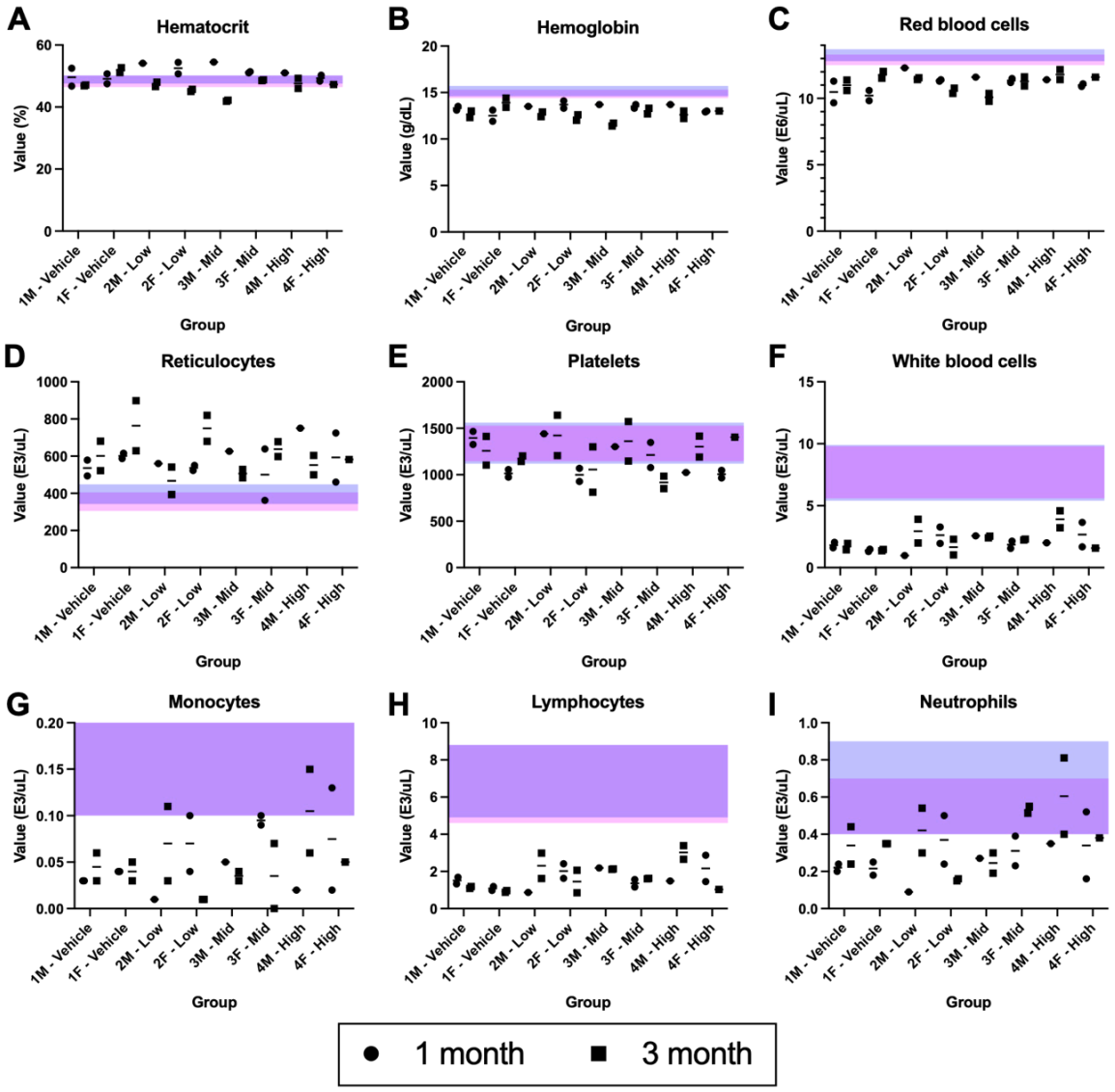


Figure 3. Hematology analysis. Individual animal data are plotted for 1-month (circles) and 3-month (squares) cohorts, where the mean value is indicated with a horizontal line. Pink and blue shading represents mean values +/- 1 standard deviation for female and male 8-week-old *hTFRC* KI mouse reference data (Biocytogen), respectively.

Clinical chemistry analysis: As of 2026-Jun-05, clinical chemistry analysis by Labcorp is in progress. A subset of the raw clinical chemistry data is provided in Figure 4. Additional clinical chemistry data collected but not shown include measurements of gamma glutamyl transferase and total bilirubin. Nearly all values for these two parameters were below the limit of detection. Efforts to collect reference clinical chemistry data from age-matched *hTFRC* KI mice are ongoing.

No data were obtained from 1 male from Group 2 (1 month cohort) due to insufficient sample volume to run or repeat measurements. Total cholesterol data from one male from Group 4 (1 month cohort) were not available due to insufficient sample volume to run

measurements. Given the low number of samples, we cannot make strong conclusions on the impact of PRNP-CHARM-001 treatment on clinical chemistry parameters.

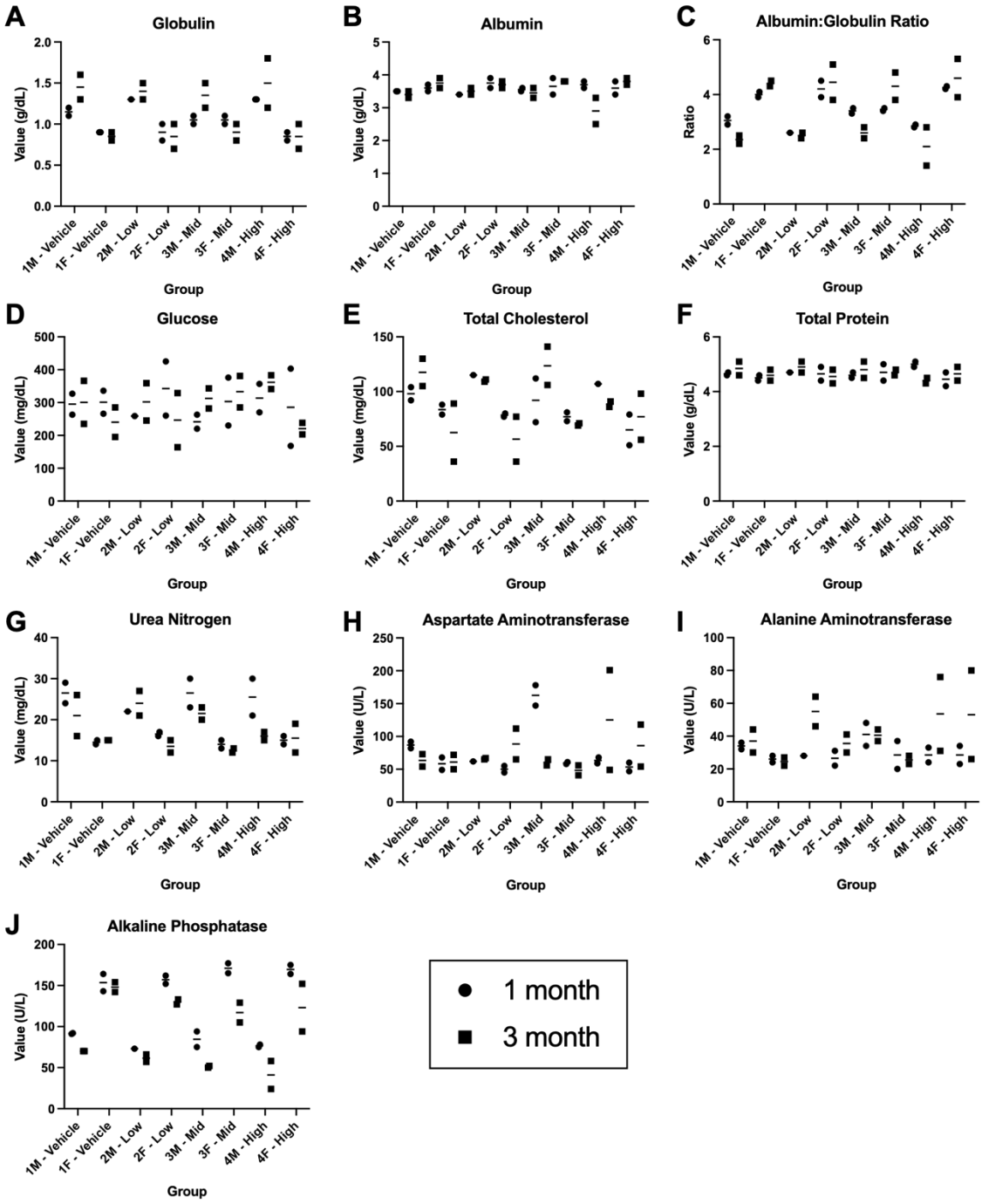


Figure 4. Clinical chemistry analysis. Individual animal data are plotted for 1-month (circles) and 3-month (squares) cohorts, where the mean value is indicated with a horizontal line.

Anatomic pathology analysis: As of 2026-Jun-05, anatomic pathology interpretation of organ weights, macroscopic observations, and microscopic observations of collected tissues is in progress.

Histopathology analysis: As of 2026-Jun-05, histopathology analysis of Group 1 and 4 brain, spinal cord, muscle, and liver samples is in progress.

Vector distribution qPCR analysis: As of 2026-Jun-05, vector distribution qPCR analysis of study samples is in progress.

7 CONCLUSIONS

Complete study conclusions are pending completion of post-life analyses. Based on study readouts examined thus far, all animals survived to their respective necropsy timepoints, indicating that PRNP-CHARM-001 treatment did not lead to early mortality. Body weight analyses indicated reduced weight gain in animals treated with 4.5E13 vg/kg PRNP-CHARM-001 relative to vehicle-treated animals. Food consumption analyses from this study have limited utility given the supplementation of diet gel at day 59 of the study, impacting the reliability of measuring the amount of chow consumed.

The results of this study thus far have informed the design of the GLP PRNP-CHARM-001 safety and distribution study. Additional mice will be included in each study group to increase the number of samples available for hematology and clinical chemistry analyses. Additionally, the GLP study will not use the 4.5E13 vg/kg dose level used in this study due to the body weight impacts observed.

Draft Protocol

Study Title	AAV-PRNP-CHARM-001: A Single-Dose Intravenous (Bolus) Injection Toxicity and Biodistribution Study in B-hTfR1 Mice Followed by a 4-Week or 13-Week Observation Period
Study Director	Diana Shefchek, MS
Test Facility	Labcorp Early Development Laboratories Inc. [redacted] Madison, Wisconsin 53704 United States of America
Labcorp Study Number	8569541
Labcorp Client Code	10020073
Sponsor	Broad Institute 415 Main Street Cambridge, Massachusetts 02142 United States of America
Page Number	1 of 40

1. APPROVALS, CHANGES, AND JUSTIFICATIONS

Protocol	**Date of Study Director Signature: DATE**
	Date of Sponsor Approval: DATE
Changes to the approved Protocol shall be made in the form of an Amendment, which will be signed and dated by the Study Director following authorization from the Sponsor or Sponsor Representative.	

2. TABLE OF CONTENTS

1. APPROVALS, CHANGES, AND JUSTIFICATIONS 2

2. TABLE OF CONTENTS 3

3. STUDY CONTACTS 5
- 3.1 Test Facility 5
- 3.2 Sponsor Representatives 5
- 3.3 Test Site for Formulation Analysis 5
- 3.4 Test Site for Biodistribution (qPCR) Analysis 6

4. GENERAL STUDY INFORMATION 7
- 4.1 Objective 7
- 4.2 Group Assignment and Dose Levels 7
- 4.3 Justifications 7
  - 4.3.1 Dose Administration Rationale 7
  - 4.3.2 Dose Justification 7
  - 4.3.3 Species Selection 7
- 4.4 Proposed Study Timetable 8
- 4.5 Regulatory Compliance 8
  - 4.5.1 Regulatory Compliance 8
  - 4.5.2 Exceptions 8
- 4.6 Regulatory Test Guidelines 9
- 4.7 Animal Welfare, Care, and Use Statement and Veterinary Care/Palliative and Prophylactic Measures 9
- 4.8 Quality Assurance 10
- 4.9 Major Computer Systems 10

5. TEST ARTICLE AND VEHICLE CONTROL ARTICLE 11
- 5.1 Test Article 11
- 5.2 Vehicle Control Article 12
- 5.3 Formulation Preparation 13
  - 5.3.1 Test Article Formulation Preparation 13
  - 5.3.2 Vehicle Control Article Formulation Preparation 14
- 5.4 Formulation Analysis 15
  - 5.4.1 Formulation Sample Collection 15
    - 5.4.1.1 Stability 15
    - 5.4.1.2 Homogeneity 16
    - 5.4.1.3 Concentration Verification 16
  - 5.4.2 Formulation Analysis 17

6. TEST SYSTEM 18
- 6.1 Mice 18
  - 6.1.1 Animal Specifications and Acclimation 18
- 6.2 Environmental Conditions, Diet, and Water 18
- 6.3 Animal Identification and Selection 19

7. INLIFE PROCEDURES 20
- 7.1 Dose Administration Details 20

7.2 Clinical Observations 21
7.2.1 Health Monitoring 21
7.2.2 Detailed Clinical Observation 21
7.2.3 Unscheduled Observations 21
7.3 Body Weight 21
7.4 Food Consumption 22

8. BLOOD FOR POTENTIAL BIODISTRIBUTION ANALYSIS 22
8.1 Blood for Potential Biodistribution Analysis 22

9. CLINICAL LABORATORY PROCEDURES 23
9.1 Hematology23
9.2 Clinical Chemistry 24
9.3 Planned Tests 25
9.3.1 Hematology 25
9.3.2 Clinical Chemistry 25
9.3.2.1 Tier 1 25
9.3.2.2 Tier 2 26
9.4 Clinical Pathology Evaluation and Reporting 26

10. TERMINAL PROCEDURES 26
10.1 Fate of Animals 26
10.2 Termination and Necropsy - Unscheduled 26
10.3 Termination and Necropsy - Interim Sacrifice 27
10.4 Termination and Necropsy - Terminal Sacrifice 28
10.5 Special Collection Procedures 28
10.5.1 Bone Marrow Smear 28
10.6 Frozen Tissue Collection for Potential Future Analysis 29
10.7 Frozen Tissue Collection and Analysis for Biodistribution (Vector DNA Expression) 30
10.8 Organ Weights and Tissues 32
10.9 Histology 34
10.9.1 Embedding 34
10.9.2 Microtomy34
10.10 Special Processing 35
10.10.1 Immunohistochemistry 35
10.11 Microscopic Examination and Anatomic Pathology 35

11. SHIPPING 36
11.1 Formulation Analysis 36
11.2 Frozen Tissue for Biodistribution (Vector DNA Expression) 37

12. DATA EVALUATION AND STATISTICAL ANALYSIS 37

13. REPORTING 38

14. ARCHIVE STATEMENT 39

15. SIGNATURES 40

3. STUDY CONTACTS

3.1 Test Facility

Study Director	Diana Shefchek, MS Mobile: +[redacted] Office: +[redacted] E-mail: [redacted]
Study Coordinator	Jill Seablom, MS Office: +[redacted] E-mail: [redacted]
Reporting Associate	Added by Amendment
Quality Assurance Contact	Tana Derringer, BS Office: +[redacted] E-mail: [redacted]
Contributing Scientist for Clinical Pathology	Added by Amendment
Contributing Scientist for Anatomic Pathology	Added by Amendment

3.2 Sponsor Representatives

Study Monitor	Sonia Vallabh, PhD Mobile: +[redacted] E-mail: [redacted]
Alternate Study Monitor	Alissa Coffey, PhD Mobile: +[redacted] E-mail: [redacted]

3.3 Test Site for Formulation Analysis

Test Site Address	Viralgen Please provide address
Principal Investigator for Formulation Analysis	Please provide
Quality Assurance Contact	Please provide
Test Site Reference Number	Please provide

3.4 Test Site for Biodistribution (qPCR) Analysis

Test Site Address	Labcorp Early Development Laboratories Inc. [redacted] Greenfield, Indiana 46140 United States of America
Principal Ivestigator for Biodistribution (qPCR) Analysis	Sarah Innis, PhD Office: +[redacted] E-mail: [redacted]
Project Manager for Biodistribution	Matthew Skidmore, MSc, BSc (Hons) Labcorp Early Development Laboratories Ltd. [redacted]North Yorkshire HG3 1PY England Office: +[redacted] E-mail: [redacted]
Quality Assurance Contact	Added by Amendment
Test Site Reference Number	8569541

4. GENERAL STUDY INFORMATION

4.1 Objective

The objective of this study is to evaluate the toxicity and determine the biodistribution of the AAV-delivered epigenic editor, AAV-PRNP-CHARM-001, when administered as a single dose via intravenous (bolus) injection to B-hTfR1 mice. After dosing, animals will be observed postdose for at least 28 or 91 days.

4.2 Group Assignment and Dose Levels

Group	Subgroup	Dose Level (vg/kg)	Dose Concentration (vg/mL)	No. of Animals	
				Males	Females
1 (Control)	1 (Tox)	0	0	16	16
2 (Low)	1 (Tox)	1.7×10^{12}	3.3×10^{11}	16	16
3 (Intermediate)	1 (Tox)	5.0×10^{12}	1.0×10^{12}	16	16
4 (High)	1 (Tox)	1.5×10^{13}	3.0×10^{12}	16	16

Group 1 will be administered vehicle control article only.
Animals will be dosed at a volume of 5 mL/kg.
Up to 8 animals/sex/group will be designated for interim sacrifice on Days 29 or 30 (based on survival), and up to 8 animals/sex/group will be designated for terminal sacrifice on Days 92 or 93 (based on survival).

4.3 Justifications

4.3.1 Dose Administration Rationale

The intravenous (bolus) injection route of administration has been chosen because it is the intended human therapeutic route.

4.3.2 Dose Justification

The dose levels were selected by the Sponsor based on available information and to align with clinically meaningful dose levels (clinical ranges often range from 2.5×10^{12} to $> 1 \times 10^{14}$ vg/kg). These dose levels of other batches of representative test article have not been associated with any adverse events. Multiple dose levels will be evaluated on this study to establish dose response.

4.3.3 Species Selection

Mice historically have been used in safety evaluation studies and are recommended and accepted by appropriate regulatory agencies.

The B-hTfR1 mouse (C57BL/6-*Tfrc*$^{tm1[TFRC]}$) used in this study has human TfR1 (hTfR1) exons 4 through 19 knocked into the mouse locus and expresses a chimeric transferrin receptor with mouse intracellular domains (exons 1 through 3) and human extracellular domains (exons 4 through 19), all under the control of the endogenous mouse promoter. Published data (Huang, Q. et al. An AAV capsid reprogrammed to bind human Transferrin Receptor mediates

brain-wide gene delivery. Science 384, 1220-1227 (2024). DOI:10.1126/science.adm8386) show that the B-hTfR1 mouse model can effectively demonstrate enhanced distribution in the central nervous system of hTfR1-binding capsids due to the expression of human TfR1 at physiologically relevant levels. The B-hTfR1 mouse is therefore used to evaluate the biodistribution and tolerability of hTfR1-binding capsids in this study.

4.4 Proposed Study Timetable

Experimental Starting Date	20 July 2026
Inlife Start Date	03 August 2026
Interim Sacrifice Date (Males)	31 August 2026
Interim Sacrifice Date (Females)	01 September 2026
Terminal Sacrifice Date (Males)	02 November 2026
Terminal Sacrifice Date (Females)	03 November 2026
Inlife End Date	03 November 2026
Draft Contributor Report Date	Added by Amendment
Unaudited Draft Report Date	Added by Amendment
Audited Draft Report Date	Added by Amendment
Experimental Completion Date	Added by Amendment
Study Completion Date	Added by Amendment

4.5 Regulatory Compliance

4.5.1 Regulatory Compliance

The study will be conducted in compliance with the site regulations as follows.

• United States Food and Drug Administration (FDA): Good Laboratory Practice for Nonclinical Laboratory Studies, Code of Federal Regulations, Title 21 Part 58, and any applicable Amendments

4.5.2 Exceptions

The following portion(s) of the study will not be conducted according to stated regulations.

• If conducted under GLP conditions in a GLP lab, no exception is needed.

• Test article and vehicle control article characterization, provided in the form of a Certificate of Analysis or Certificate of Testing, will be conducted in accordance with Good Manufacturing Practices by the Sponsor/Sponsor subcontractor.

• Test article and vehicle control article characterization, provided in the form of a Certificate of Analysis or Certificate of Testing, will be conducted by the Sponsor/Sponsor subcontractor in accordance with internal SOPs and will not comply with GLP regulations, but the analysis will be conducted in a laboratory with facilities, methods, and controls compliant with GLP.

• Test article and vehicle control article characterization, provided in the form of a Certificate of Analysis or Certificate of Testing, will be conducted in accordance with Good Manufacturing Practices by the Sponsor/Sponsor subcontractor.

• Test article and vehicle control article characterization, provided in the form of a Certificate of Analysis or Certificate of Testing, will be conducted by the Sponsor/Sponsor subcontractor in accordance with internal SOPs and will not comply with GLP regulations. The testing will be conducted in a laboratory with facilities, methods, and controls compliant with Good Manufacturing Practices.

• Test article and vehicle control article characterization and stability will be conducted by the Sponsor in accordance with internal written procedures. The analyses will be conducted by qualified personnel in a laboratory with established methods and controls.

4.6 Regulatory Test Guidelines

The study design is based on the principles of the following guidelines and requirements, which are current at the time of Protocol issuance.

• International Council on Harmonisation (ICH) M3 (R2), Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals (ICH, June 2009)

• International Council on Harmonisation (ICH) S3A, Note for Guidance on Toxicokinetics: The Assessment of Systemic Exposure in Toxicity Studies (ICH, October 1994)

• International Council on Harmonisation (ICH) S6 (R1), Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals (ICH, June 2011)

• International Council on Harmonisation (ICH) S12, Nonclinical Biodistribution Considerations for Gene Therapy Products (ICH, March 2023)

• United States Food and Drug Administration (FDA) Center for Biologics Evaluation and Research (CBER)/Office of Cellular, Tissue, and Gene Therapies (OCTGT), Preclinical Assessment of Investigational Cellular and Gene Therapy Products (CBER, November 2013)

4.7 Animal Welfare, Care, and Use Statement and Veterinary Care/Palliative and Prophylactic Measures

All procedures in this Protocol are in compliance with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Office of Laboratory Animal Welfare. In the opinion of the Sponsor and Study Director, the study does not unnecessarily duplicate any previous work, and no other model can fulfill the study requirements.

Medical treatment necessary to minimize suffering and distress, including euthanasia, is the sole responsibility of the attending laboratory animal Veterinarian.

Palliative and prophylactic procedures, as applicable, may be based upon consensus agreement between the Study Director and attending laboratory animal Veterinarian. The Study Director and Sponsor/designee (if possible) will be included in discussions of palliative and prophylactic procedures (nonlife threatening conditions, including suspension of dosing and removal of animals from study) recommended by the attending Veterinarian. Final authority for decision making will be with the laboratory animal Veterinarian.

Labcorp Veterinarians may order further diagnostic investigations or treatments without prior notification of the Sponsor. Where unexpected deaths occur and no/minimal terminal procedures are required by the Protocol, diagnostic terminal procedures may be instigated to determine the cause of death.

4.8 Quality Assurance

The Labcorp Quality Assurance Unit (QAU) will act as the lead QAU and will perform Protocol, study conduct, data, and Final Report reviews for activities performed at the test facility. Study conduct inspections will be completed as per testing facility SOP.

Test site QAUs (unless otherwise specified) will monitor study phases according to site specific SOPs. Inspection/audit reports will be forwarded to the Principal Investigator, test site management, Study Director, test facility management, and lead QAU. A Quality Assurance (QA) statement from each test site will be issued, as part of the contributing/phase report if applicable, for inclusion in the overall Final Report.

4.9 Major Computer Systems

The major validated computer systems to be used at Labcorp during the conduct of this study may include, but will not be limited to, the following. All version numbers of the applications are maintained by Labcorp. Systems used by the test site(s) will be included in the respective contributing report(s), as appropriate.

Application Name	**Application Function**
Metasys	Monitors and controls environmental conditions and water flow within the facility (e.g., animal rooms)
REES Environmental Monitoring	Monitors and documents facility storage conditions (e.g., refrigerators, freezers, and constant temperature rooms)
Path/Tox System Pristima, supplied by Xybion Medical Systems Corporation	Captures direct online in-life toxicology, pharmacy, and clinical and anatomic pathology data and study maintenance information and randomizes animals; transfers data within Pristima and uploads data from external sources for translation/assembly into SEND dataset format
Tox Reporting	Transfers data from Pristima for reporting purposes

Study Designer	System for generation of study-related documents
DocuSign	Electronic signature of study-related documents
Documentum	Document management system for generation of study-related documents and electronic signatures
Electronic Notes (eNotes)	Electronic communication system
Veeva - Unified Quality Vault	Quality issues management system
Toxicology Software Foundation/Data Acquisition (TSF/DA)	Captures histology sample tracking information
SAS	Performs statistical analysis

5. TEST ARTICLE AND VEHICLE CONTROL ARTICLE

5.1 Test Article

Identification	AAV-PRNP-CHARM-001 The formulated test article will be provided at an actual concentration (titer value) of 2.3 x 10^{13} vg/mL (vector genomes per milliliter).
Class	Gene Therapy
Lot/Batch Number	T-POLARIS-123
Purity	Please provide
Stability / Retest Date	21 August 2026 Each lot of test article used on study will be characterized by the Sponsor, and a certificate of analysis (analytical report) will be supplied. The Sponsor is responsible for the stability of the test article and will provide documentation for inclusion in the Final Report.
Storage Conditions	In a freezer, set to maintain -60 to -80°C, and protected from light
Handling Requirements for Bulk Materials	Any transfer of material from original test article container(s) will be performed using aseptic technique, except for containers designated for non-aseptic use (e.g., a container designated for chemist use during forumlation analysis).

	Fresh vials of test article will be used at each preparation. The test article will be thawed at room temperature, while protected from light, for 15 to 30 minutes and subsequent dilutions will be used within 4 hours of the completion of thawing of the bulk test article. The thaw completion times will be documented. Prior to use, each vial of bulk test article will be gently inverted at least 5 times.
Disposition of Bulk Test Article Thawed for Formulation Preparation	Each partially used vial/thawed unopened vial (as applicable) will not be returned to frozen storage. Each vial will be split into three approximately equal aliquots (if possible) in low binding plastic containers, following dose formulation completion. Aliquots will be stored on dry ice, while protected from light, until transferred to a freezer, set to maintain -60 to 80°C, until shipped. Aliquots and any empty bulk test article vials will be held at Labcorp to be shipped to the Sponsor-designated archive facility.
Reserve Archive Sample	A 1 mL reserve sample of each lot of the test article will be collected, maintained, and archived at the storage conditions.
Reserve Archive Sample Disposition	Archive in the Labcorp archives no later than 30 days following the completion of the dosing phase.
Safety	The Sponsor will provide relevant occupational safety information known about the articles used (e.g., Safety Data Sheet [SDS], safety instructions, test article identity, etc.).

5.2 Vehicle Control Article

Identification	Diluent (Buffered Solution with Salts and Surfactants) Provided formulated by the Sponsor
Alternative Identification	7.5mM Tris (hydroxymethyl)aminomethane, 12.5mM Tris (hydroxymethyl)aminomethane hydrochloride, 1mM Magnesium chloride hexahydrate, 0.2M Sodium Chloride, 0.005% P188, pH 8
Lot/Batch Number	FFB-T-017
Retest Date	Please provide
Storage Conditions	In a freezer, set to maintain -60 to -80°C, and protected from light
Handling Requirements for	Any transfer of material from original vehicle control article/diluent container(s) will be performed using aseptic technique, except for

Vehicle Control Article / Diluent	containers designated for non-aseptic use (e.g., a container designated for chemist use during dose analysis). Fresh vials of vehicle control article/diluent will be used at each preparation. Vehicle control article/diluent will be thawed room temperature, while protected from light, for 15 to 30 minutes. The thaw completion times will be documented. Prior to use, each vial of bulk vehicle control article/diluent will be gently inverted at least 5 times.
Reserve Archive Sample	At least 1 mL will be collected from each lot of vehicle control article components and archived at the storage conditions. A reserve sample of water supplied by in-house water systems (if applicable) is not required.
Reserve Archive Sample Disposition	Archive in the Labcorp archives no later than 30 days following the completion of the dosing phase.
Disposition of Bulk Vehicle Control Article/Diluent Thawed for Formulation Preparation	Each partially used vial/thawed unopened vial (as applicable) will not be returned to frozen storage. Each vial will be split into three approximately equal aliquots (if possible) in low binding plastic containers, following dose formulation completion. Aliquots will be stored on dry ice, while protected from light, until transferred to a freezer, set to maintain -60 to -80°C, until shipped. Aliquots and any empty vehicle control article/diluent vials will be held at Labcorp to be shipped to the Sponsor-designated archive facility.
Safety	The Sponsor will provide relevant occupational safety information known about the articles used (e.g., Safety Data Sheet [SDS], safety instructions, vehicle control article identity, etc.).

5.3 Formulation Preparation

5.3.1 Test Article Formulation Preparation

Article	**Frequency**
AAV-PRNP-CHARM-001	Once on the day of dosing for use within 4 hours of completion of thawing of the bulk test article
Procedures	

Procedures	Test article formulations will be prepared according to a formulation procedure developed by Labcorp. Sponsor supplied formulation procedures will be used as guidance, if provided. Test article dose formulations will be prepared in plastic containers (low protein-binding, as possible). For Group 4: Test article will be diluted volume:volume (as necessary) with diluent using aseptic technique; calculations will be based on the actual concentration of the respective test article. For Group 3: The Group 4 solution will be diluted volume:volume with diluent using aseptic technique; calculations will be based on the nominal concentration of the Group 4 solution (3.0×10^{12} vg/mL). For Group 2: The Group 3 solution will be diluted volume:volume with diluent using aseptic technique; calculations will be based on the nominal concentration of the Group 3 solution (1.0×10^{12} vg/mL). Each dilution will be mixed by gently inverting at least 10 times without introducing bubbles. The lot number and the vial designation from each Sponsor-supplied vial used to prepare formulations, will be documented.
Correction Factor	Not required. Dose concentration will be based on the test article as supplied.
Storage Conditions	Room temperature
Postdose Formulation Handling	After dosing, any residual test article formulations will be divided into three approximately equal aliquots (if possible) per concentration in plastic low binding tubes following dose completion. Aliquots will be stored on dry ice until transferred to a freezer.

5.3.2 Vehicle Control Article Formulation Preparation

Article	**Frequency**
Diluent (Buffered Solution with Salts and Surfactants)	Once on the day of dosing
Procedures	

Procedures	Vehicle control article will be used as supplied and dispensed according to the dispensation procedure developed by Labcorp using aseptic technique. Sponsor supplied procedures will be used as guidance if provided.
Storage Conditions	Room temperature
Postdose Formulation Handling	After dosing, any residual vehicle control article formulations will be divided into three approximately equal aliquots (if possible) in plastic low binding tubes following dose completion. Aliquots will be stored on dry ice until transferred to a freezer.

5.4 Formulation Analysis

5.4.1 Formulation Sample Collection

5.4.1.1 Stability

Article	**Frequency**
AAV-PRNP-CHARM-001	
Procedures	
Stability	The following sets of triplicate 1.00-mL weighed samples from 3.3 x 10^{11} to 3.0 x 10^{12} vg/mL will be collected on the day of formulation preparation.

Sets	Interval	Storage Conditions	Number of Samples
1	Time Zero (Initial)	NA	3 x 1.00 mL
1	At least 24 hours	15 to 25°C	3 x 1.00 mL
2	At least 8 and 15 days	15 to 25°C	3 x 1.00 mL
2	At least 8 and 15 days	2 to 8°C	3 x 1.00 mL
2	At least 8 and 15 days	In a sealed container at -10 to -30°C	3 x 1.00 mL
1	Backup	2 to 8°C	3 x 1.00 mL
1	Backup	In a sealed container at -10 to -30°C	3 x 1.00 mL

NA = Not applicable

5.4.1.2 Concentration Verification

Article	**Frequency**
AAV-PRNP-CHARM-001	Collect the following samples from formulations prepared for administration on Day 1 of the doing phase.

Diluent (Buffered Solution with Salts and Surfactants)	Additional samples may be collected at the request of the Study Director.

Group Number	Article	Dose Concentration	Middle	Total Samples
1	Diluent (Buffered Solution with Salts and Surfactants)	0 vg/mL	2 x 1.00 mL	2 x 1.00 mL
2	AAV-PRNP-CHARM-001	3.3 x 10^11 vg/mL	6 x 1.00 mL	6 x 1.00 mL
3	AAV-PRNP-CHARM-001	1.0 x 10^12 vg/mL	6 x 1.00 mL	6 x 1.00 mL
4	AAV-PRNP-CHARM-001	3.0 x 10^12 vg/mL	6 x 1.00 mL	6 x 1.00 mL

Note: When samples are collected for homogeneity at concentration verification intervals, the mean result from the homogeneity samples will be used for concentration verification. No additional samples from those formulations are required for concentration verification.

Procedures	
Storage Conditions	In a freezer, set to maintain -60 to -80°C

5.4.2 Formulation Analysis

Procedures	
Analytical Method	Dose formulations will be analyzed by Viralgen using a previously validated method (Method added by Amendment).
Storage at Test Site	In a freezer, set to maintain -60 to -80°C
Stability Analysis	One set will be analyzed at the indicated intervals. Backups will be stored as indicated.
Homogeneity Analysis	One set of duplicate samples (for each stratum) will be analyzed for AAV-PRNP-CHARM-001 content. The second set will be retained as backup.
Concentration Verification Analysis	One control group sample will be analyzed to confirm the absence of AAV-PRNP-CHARM-001. The second sample will be retained as backup. One set of triplicate samples will be analyzed for AAV-PRNP-CHARM-001 content. The second set will be retained as backup.

Reporting	The Principal Investigator for Formulation Analysis will be responsible for all delegated phase activities and will provide a formulation analysis report from the data generated to the Study Director for inclusion in the Final Report. The report also will include a signed GLP compliance statement and a quality assurance statement.

6. TEST SYSTEM

6.1 Mice

6.1.1 Animal Specifications and Acclimation

Species	Mouse
Strain and Supplier	B-hTfR1 from Biocytogen, Linjian New District, Haimen, Jiangsu, China Animals will be sourced by the Sponsor.
Targeted Weight at Initiation of Dosing	15 to 40 g Animals outside of this range may be used at the discretion of the Study Director. The actual range will be documented and included in the report.
Targeted Age at Initiation of Dosing	7 to 9 weeks Animals outside of this range may be used at the discretion of the Study Director. The actual range will be documented and included in the report.
Number of Animals for Acclimation	68 males and 68 females
Number of Animals on Study	64 males and 64 females
Acclimation	At least 21 days

6.2 Environmental Conditions, Diet, and Water

Animal Housing	Females will be group-housed by sex and group (up to two animals/cage) in microisolator caging (e.g., Innocage® by Innovive) with sterile bedding. Males will be single-housed in microisolator caging (e.g., Innocage® by Innovive) with sterile bedding. Females may be individually housed in microisolator caging (e.g., Innocage® by

	Innovive) for study-related procedures, behavioral or health reasons, or due to cage mate death (female animals may be re-housed as appropriate). The cages will be changed at least once weekly throughout the study.
Bedding	Sterile, ALPHA-dri® bedding The type of bedding may be changed for behavioral or health reasons. The bedding is routinely analyzed by a certified laboratory for contaminants. The results are on file at Labcorp.
Water	Water will be supplied in sterile water bottles received prefilled by the supplier. Analytical data is provided by the vendor and results are on file at Labcorp. New water bottles will be supplied at least once weekly.
Diet	*Ad libitum* access, unless otherwise specified, to Certified Rodent Diet #2014C (Envigo RMS, Inc.). Animals may be fed the meal-form of this diet if indicated by health conditions. The diet is routinely analyzed by the manufacturer for nutritional components and environmental contaminants. Results of specified nutrient and contaminant analyses are on file at Labcorp.
Environment	Environmental controls for the animal room will be set to maintain a temperature range of 20 to 26°C, a relative humidity of 30 to 70%, a minimum of eight air changes/hour, and a 12-hour light/12-hour dark cycle. The light/dark cycle may be interrupted for study-related activities.
Dietary and Environmental Enrichment	Animals will be given various cage enrichment devices and dietary enrichment (that do not require analyses) as environmental enrichment.

6.3 Animal Identification and Selection

Means of Identification	Animals will be identified via implantable microchip identification devices, individual cage cards, an ear/neck tag, or a tail mark, as applicable. Cages will be identified with study information, including study number and animal numbers.

Animal Selection	Animals may be eliminated from consideration for study selection based on data collected during acclimation (predose phase). Animals will be assigned to the study using a computerized procedure designed to achieve body weight balance with respect to group assignment. Prior to group assignment, animals may be excluded from the selection pool/sex to produce minimal variation. After group assignment, it will be established that homogeneity of variance has been achieved at the 5.0% probability level, as indicated by Levene's test for heterogeneity of variance. Additionally, the mean body weight for each group/sex will not be statistically different at the 5.0% probability level, as indicated by analysis of variance F probability.
Proposed Animal Numbers	Animals will be individually identified as follows.

		Study Animal Numbers	
Group	**Subgroup**	**Males**	**Females**
1	1	M0001-M0012	M0401-M0412
2	1	M0101-M0112	M0501-M0512
3	1	M0201-M0212	M0601-M0612
4	1	M0301-M0312	M0701-M0712

Note: Animal replacements will be documented in the raw data and discussed in the Final Report.

7. INLIFE PROCEDURES

7.1 Dose Administration Details

Animals	
All animals	
Procedures	
Formulation Handling	Dose formulations will be used within 4 hours of the completion of thawing of the bulk test article or bulk vehicle control article (as applicable).
Frequency / Duration	Dosing phase: Once on Day 1
Dose Volume(s)	5 mL/kg, based on the most recently recorded scheduled body weight
Dose Route	Intravenous (bolus) injection

Dose Site Identification	The dose site is as follows. Dose Site A: Tail vein, lateral
Postdose Formulation Handling	Any residual formulations will be divided into three approximately equal aliquots (if possible) per concentration in low-binding tubes following dosing completion. Aliquots will be stored on dry ice until transferred to a freezer, set to maintain -60 to 80°C.

7.2 Clinical Observations

7.2.1 Health Monitoring

Animals	**Frequency**
All animals	At least twice daily (a.m. and p.m.) in each phase At least once on day(s) of arrival/termination
Procedures	
Data Recording	Abnormal findings will be recorded as they are observed for mortality or abnormalities.

7.2.2 Detailed Clinical Observation

Animals	**Frequency**
All animals	Predose phase: At least once weekly
Interim sacrifice animals	Dosing phase: On Days 1, 8, 15, 22, and 29
Terminal sacrifice animals	Dosing phase: On Days 1, 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, 78, 85, and 92
Procedures	
Data Recording	Abnormal findings or an indication of normal will be recorded.

7.2.3 Unscheduled Observations

Animals	**Frequency**
All animals	As observed

Procedures	
Data Recording	Abnormal findings will be recorded.

7.3 Body Weight

Animals	**Frequency**
All animals	Predose phase: At least once weekly
Interim sacrifice animals	Dosing phase: Prior to dosing on Day 1 and on Days 8, 15, 22, 25, and 28
Teriminal sacrifice animals	Dosing phase: Prior to dosing on Day 1 and on Days 8, 15, 22, 25, 29, 32, 36, 39, 43, 46, 50, 53, 57, 60, 64, 67, 71, 74, 78, 81, 85, 88, and 91
Procedures	
Data Recording	Individual body weight will be recorded in grams. Additional body weights may be recorded to monitor animal health.

7.4 Food Consumption

Animals	**Frequency**
Interim sacrifice animals	Dosing phase: From Days 1 to 8, 8 to 15, 15 to 22, and 22 to 28
Terminal sacrifice animals	Dosing phase: From Days 1 to 8, 8 to 15, 15 to 22, 22 to 29, 29 to 36, 36 to 43, 43 to 50, 50 to 57, 57 to 64, 64 to 71, 71 to 78, 78 to 85, and 85 to 91
Procedures	
Type	Quantitative unless food is wet, spilled, or contaminated. Food consumption during intervals documented as wet, spilled, and/or contaminated will be recorded but excluded for the affected animal(s) from summary values.

8. BLOOD FOR POTENTIAL BIODISTRIBUTION ANALYSIS

8.1 Blood for Potential Biodistribution Analysis

Animals	**Frequency**

Interim sacrifice animals	Dosing phase: Once on Day 22
Terminal sacrifice animals	Dosing phase: Once on Day 85
Unscheduled sacrifice(s)	If possible Blood collection priority order is as follows: hematology > clinical chemistry > biodistribution
Procedures	
Fasting Status	Not fasted
Collection Site	Submandibular puncture An alternate site may be used, if necessary, and the site of blood collection will be documented.
Target Sample Volume	0.2 mL
Anticoagulant	Potassium (K2) EDTA
Processing	Following harvesting, samples will be placed on dry ice until stored in a freezer.
Storage Conditions	In a freezer, set to maintain -60 to -80°C
Reporting	The samples will be retained for possible future analysis.

9. CLINICAL LABORATORY PROCEDURES

9.1 Hematology

Animals	**Frequency**
First, second, and third males/group	Dosing phase: Once on Day 29
First, second, and third females/group	Dosing phase: Once on Day 30

Seventh, eighth, and ninth males/group	Dosing phase: Once on Day 92
Seventh, eighth, and ninth females/group	Dosing phase: Once on Day 93
Unscheduled sacrifice(s)	If possible Blood collection priority order is as follows: hematology > clinical chemistry > biodistribution
Procedures	
Fasting Status	Not fasted
Collection Site	Vena cava An alternate site may be used if necessary and the site of blood collection will be documented.
Target Sample Volume	As much as possible, up to 1 mL Blood volumes may be adjusted as needed for animal welfare concerns and/or availability of blood collection tubes. Blood volumes collected will be noted in the raw data.
Anticoagulant	Potassium EDTA
Terminal Collection Procedures	Prior to blood collection, animals will be anesthetized with sodium pentobarbital.

9.2 Clinical Chemistry

Animals	**Frequency**
Fourth, fifth, and sixth males/group	Dosing phase: Once on Day 29
Fourth, fifth, and sixth females/group	Dosing phase: Once on Day 30
Tenth, eleventh, and twelfth males/group	Dosing phase: Once on Day 92

Tenth, eleventh, and twelfth females/group	Dosing phase: Once on Day 93
Unscheduled euthanasia(s)	If possible Blood collection priority order is as follows: hematology > clinical chemistry > biodistribution
Procedures	
Fasting Status	Not fasted
Collection Site	Vena cava An alternate site may be used if necessary and the site of blood collection will be documented.
Target Sample Volume	As much as possible, up to 1 mL Blood volumes may be adjusted as needed for animal welfare concerns and/or availability of blood collection tubes. Blood volumes collected will be noted in the raw data.
Anticoagulant	None; serum separator tubes will be used.
Terminal Collection Procedures	Prior to blood collection, animals will be anesthetized with sodium pentobarbital.

9.3 Planned Tests

In the event a planned test cannot be completed, the reason for the missing value will be recorded.

9.3.1 Hematology

white blood cell (leukocyte) count
hematocrit
mean corpuscular hemoglobin
platelet count
absolute reticulocyte count
absolute lymphocyte count
absolute eosinophil count
absolute large unstained cell count
red blood cell (erythrocyte) count gated
hemoglobin
mean corpuscular volume
mean corpuscular hemoglobin concentration
red cell distribution width
absolute neutrophil count
absolute monocyte count
absolute basophil count
blood smear

1.1.1 Clinical Chemistry

9.3.1.1 Tier 1

glucose	urea nitrogen
total protein	albumin
globulin	albumin:globulin ratio
total bilirubin	alanine aminotransferase
alkaline phosphatase	

9.3.1.2 Tier 2

total cholesterol	aspartate aminotransferase
gamma glutamyltransferase	

9.4 Clinical Pathology Evaluation and Reporting

Procedures	
Sample Disposition	Following sample analysis, any residual clinical pathology samples will be maintained by Labcorp until being disposed of in accordance with local Labcorp SOPs and following Sponsor approval.
Reporting	The Contributing Scientist for Clinical Pathology will provide a clinical pathology report from the data generated to the Study Director for inclusion in the Final Report.

10. TERMINAL PROCEDURES

10.1 Fate of Animals

Predose Phase Terminations	Undosed predose phase animals found dead or sacrificed via carbon dioxide inhalation (followed by a secondary method of euthanasia) will be discarded, without necropsy or any collections, unless directed by a Veterinarian and/or the Study Director.

10.2 Termination and Necropsy - Unscheduled

Animals	**Frequency**

Unscheduled sacrifice(s) and death(s)	As needed
Procedures	
Method of Termination	Animals to be sacrificed will be anesthetized with sodium pentobarbital and exsanguinated.
Terminal Body Weight	Recorded for sacrificed animals
Blood Sampling	Required, if possible, from sacrificed animals Blood collection priority order is as follows: hematology > clinical chemistry > biodistribution
Macroscopic Observations	An examination of the external features of the carcass; external body orifices; abdominal, thoracic, cranial, pelvic, and oral cavities; organs; and tissues A Pathologist will be available for consultation during necropsy.
Organ Weights	Required
Tissue Preservation and Fixation	Preserved in appropriate fixative or recorded as missing

10.3 Termination and Necropsy - Interim Sacrifice

Animals	**Frequency**
Up to 6 males/group	Dosing phase: On Day 29
Up to 6 females/group	Dosing phase: On Day 30
Procedures	
Fasting Status	Not fasted
Method of Termination	Animals will be anesthetized with sodium pentobarbital and exsanguinated.
Terminal Body Weight	Recorded

Blood Sampling	Required
Macroscopic Observations	An examination of the external features of the carcass; external body orifices; abdominal, thoracic, cranial, pelvic, and oral cavities; organs; and tissues A Pathologist will be available for consultation during necropsy.
Organ Weights	Recorded
Tissue Preservation and Fixation	Preserved in appropriate fixative or recorded as missing

10.4 Termination and Necropsy - Terminal Sacrifice

Animals	**Frequency**
All surviving males	Dosing phase: On Day 92
All surviving females	Dosing phase: On Day 93
Procedures	
Fasting Status	Not fasted
Method of Termination	Animals will be anesthetized with sodium pentobarbital and exsanguinated.
Terminal Body Weight	Recorded
Blood Sampling	Required
Macroscopic Observations	An examination of the external features of the carcass; external body orifices; abdominal, thoracic, cranial, pelvic, and oral cavities; organs; and tissues A Pathologist will be available for consultation during necropsy.
Organ Weights	Recorded
Tissue Preservation and Fixation	Preserved in appropriate fixative or recorded as missing

10.5 Special Collection Procedures

10.5.1 Bone Marrow Smear

Animals	**Frequency**
All animals	Interim and Terminal necropsy intervals
Procedures	
Collection Site	Femur
Number of Slides/Animal	Two
Disposition	Deliver to Clinical Pathology
Evaluation	Held for possible future evaluation

10.6 Frozen Tissue Collection for Potential Future Analysis

Animals	**Frequency**
All animals	Interim and Terminal necropsy intervals
Procedures	
Tissues/Sites	When applicable, frozen tisues will be collected without compromising histology sections. Tissues will be collected using ultraclean procedures. Brain, brainstem, left hemisphere Brain, cerebellum, left hemisphere Brain, cerebrum, left hemisphere Spinal cord, cervical Spinal cord, lumbar Spinal cord, thoracic Liver, left lateral lobe Heart, apex Spleen Kidney, right pole Lung, accessory lobe Injection site (tail, proximal)
Target Sample Volume to Collect	Each sample will be approximately 5x5x5 mm (actual size not documented), unless otherwise indicated below.

Weight	Not required
Procedure	For all tissues, unless otherwise directed, two samples will be collected from each location for potential future analysis, if possible. The entire sample will be placed in a cryotube, flash-frozen in liquid nitrogen, and held on dry ice until transferred to a freezer. Brain samples will be collected as a single sample. For the spinal cord (cervical, lumbar, and thoracic), each region will be divided approximately into two halves; the caudal half will be collected in fixative for histology, and the cranial half will be used for frozen tissue collections. Tissues will be collected using ultra-clean procedures, according to Labcorp SOPs, in order to minimize the risk for potential contamination. Additional sections may be collected for biodistribution analysis and will be detailed in the "Frozen Tissue Collection and Analysis for Biodistribution (Vector DNA Expression)" section.
Storage Container	Each sample will be collected into separate nuclease-free low binding polypropylene tubes.
Storage Conditions	In a freezer, set to maintain -60 to -80°C
Analysis	Samples will be held for potential future analysis.

10.7 Frozen Tissue Collection and Analysis for Biodistribution (Vector DNA Expression)

Animals	**Frequency**
All animals	Interim and Terminal necropsy intervals
Procedures	
Tissues/Sites	When applicable, frozen tisues will be collected without compromising histology sections. Tissues will be collected using ultraclean procedures. Brain, brainstem, left hemisphere Brain, cerebellum, left hemisphere Brain, cerebrum, left hemisphere Spinal cord, cervical Spinal cord, lumbar Spinal cord, thoracic

	Dorsal root ganglia, lumbar Liver, left lateral lobe Heart, apex Spleen Adrenal gland, left Kidney, right pole Lung, accessory lobe Ovary, left Testis, left Injection site (tail, proximal)
Target Sample Volume to Collect	Each sample will be approximately 5x5x5 mm (actual size not documented), unless otherwise indicated below. For ovary, testis, and adrenal, the entire tissue will will be collected.
Weight	Not required
Procedure	For all tissues, unless otherwise directed, two samples will be collected from each location for biodistribution analysis, if possible. The entire sample will be placed in a cryotube, flash-frozen in liquid nitrogen, and held on dry ice until transferred to a freezer. Brain samples will be collected as a single sample. For the spinal cord (cervical, lumbar, and thoracic), each region will be divided approximately into two halves; the caudal half will be collected in fixative for histology, and the cranial half will be used for frozen tissue collections. For the ovary, testis, and adrenal, the entire tissue will be collected. Tissues will be collected using ultra-clean procedures, according to Labcorp SOPs, in order to minimize the risk for potential contamination. Additional sections may be collected for potential future analysis and will be detailed in the "Frozen Tissue Collection for Potential Future Analysis" section.
Storage Container	Each sample will be collected into separate nuclease-free low binding polypropylene tubes.
Storage Conditions	In a freezer, set to maintain -60 to -80°C

Analysis (Labcorp-GRN)	Brain tissues, spinal cord tissues, DRG tissue, heart tissue, spleen tissue, liver, lung, and blood from all groups will be analyzed by Labcorp-Greenfield Molecular Bioanalysis using a qualified qPCR method (Method number to be added by Amendment). In the event a test cannot be completed, the reason will be recorded. All other tissues will be held at Labcorp-Madison for potential future biodistribution analysis.
Storage Conditions at Test Site	In a freezer, set to maintain -60 to -80°C
Reporting	The Principal Ivestigator for Biodistribution (qPCR) Analysis will provide a biodistribution report from the data generated to the Study Director for inclusion in the Final Report. The report also will include a signed Good Laboratory Practices compliance statement and Quality Assurance statement.
Sample Disposition	Analyzed samples will be discarded, with written permission from the Sponsor, prior to report finalization. Unanalyzed samples will be held for potential future analysis.

10.8 Organ Weights and Tissues

Organ/tissue	W	C	E	SP	Additional Information
Adrenal (2)		F	X	X	
Animal identification		F		X	Implantable chip, if applicable
Aorta		F	X	X	
Bone, femur with bone marrow (to include femorotibial joint)		F	X	X	
Bone, sternum with bone marrow		F	X	X	
Brain	X	F	X	X	
Cecum		F	X	X	
Cervix	X	F	X	X	Weighed with uterus
Colon		F	X	X	
Dose site(s)		F	X	X	
Duodenum		F	X	X	
Epididymis (2)	X	F	X	X	Weighed together
Esophagus		F	X	X	
Eye (2)		MD	X	X	
Gallbladder (drained)	X	F	X	X	Weighed with liver
Gland, Harderian (2)		MD	X	X	
Gland, coagulating (2)		F	X	X	
Gland, pituitary		F	X	X	
Gross lesions		F	X	X	

Organ/tissue	W	C	E	SP	Additional Information
Gut-associated lymphoid tissue (GALT) - (Peyer's Patch)		F	X	X	
Heart	X	F	X	X	
Ileum		F	X	X	
Injection site(s)		F	X	X	
Jejunum		F	X	X	
Kidney (2)	X	F	X	X	Weighed together
Liver	X	F	X	X	Weighed with gallbladder
Lung with large bronchi		F	X	X	
Lymph node, mandibular		F	X	X	
Lymph node, mesenteric		F	X	X	
Mammary gland		F	X	X	For males, no recuts are needed
Muscle, biceps femoris		F	X	X	
Nerve, optic (2)		MD	X	X	
Nerve, sciatic		F	X	X	
Ovary (2)	X	F	X	X	Weighed together; with oviduct. Scheduled sacrifices: left will be collected for frozen tissues and right will be collected in fixative. Unscheduled animals: both will be collected in fixative.
Oviduct (2)	X	F	X	X	Weighed together; with ovary. Scheduled sacrifices: left will be collected for frozen tissues and right will be collected in fixative. Unscheduled animals: both will be collected in fixative.
Pancreas		F	X	X	
Prostate	X	F	X	X	
Rectum		F	X	X	
Salivary gland, mandibular (2)		F	X	X	
Seminal vesicle (2)	X	F	X	X	Weighed together
Skin/subcutis		F	X	X	
Spinal cord, cervical		F	X	X	
Spinal cord, lumbar		F	X	X	
Spinal cord, thoracic		F	X	X	
Spleen	X	F	X	X	
Stomach		F	X	X	
Testis (2)	X	MD	X	X	Weighed together; Scheduled sacrifices: left will be collected for frozen sample collection and right will be collected in fixative. Unscheduled animals: both left and right will be collected in fixative.
Thymus	X	F	X	X	

Organ/tissue	W	C	E	SP	Additional Information
Thyroid (2 lobes) with parathyroid		F	X	X	
Tongue		F	X	X	
Trachea		F	X	X	
Urinary Bladder		F	X	X	
Uterus	X	F	X	X	Weighed with cervix
Vagina		F	X	X	

(#) = Number of sections for microscopic evaluation; C = Tissue collection and fixation; E = Tissue processing and examination according to the Histology and Microscopic Evaluation sections; IV = Intravenous; SP = Special procedures; F = 10% neutral buffered formalin (NBF); MD = Modified Davidson's fixative, stored in 10% NBF; W = Organs weighed; X = Action required.

Note: Tissues collected from animals replaced after dosing on Day 1 will be held in fixative.

Note: Only dose sites dosed will be collected, and only the last site dosed will be processed to slide for microscopic evaluation.

Note: For all scheduled sacrifice animals, collect and maintain in 10% neutral-buffered formalin for at least two overnights but no more than three overnights before transferring to 70% alcohol. Tissues will then be processed to paraffin block within 9 days of being transferred to 70% alcohol for immunohistochemical evaluation.

10.9 Histology

10.9.1 Embedding

Animals	Frequency
Unscheduled sacrifice(s) and death(s)	Unscheduled necropsy interval
All animals	Interim and Terminal necropsy intervals
Procedures	
Procedures	All available study tissues (unless noted as missing), will be trimmed and embedded in paraffin.

10.9.2 Microtomy

Animals	Frequency
Unscheduled sacrifice(s) and death(s)	Unscheduled necropsy interval
Group 2	Interim and Terminal necropsy intervals
Groups 1, 3, and 4	Interim and Terminal necropsy intervals

Procedures	
Procedures	<u>Unscheduled sacrifice(s) and death(s)</u> All available embedded tissues will be sectioned at a nominal 5 µm and stained with hematoxylin and eosin, unless stated otherwise. <u>Groups 1, 3, and 4</u> All available embedded tissues will be sectioned at a nominal 5 µm and stained with hematoxylin and eosin, unless stated otherwise. <u>Group 2</u> Gross lesions only will be sectioned at a nominal 5 µm and stained with hematoxylin and eosin, unless stated otherwise. At the request of the Sponsor and added by Amendment, suspected target organs may be added.

1.2 Special Processing

10.9.3 Immunohistochemistry

Animals	**Frequency**
Unscheduled sacrifice(s) and death(s)	Unscheduled necropsy interval
Procedures	
Embedding Media	Paraffin
Tissues	All available study tissues
Immunolabeled for the Detection of:	GFAP and IBA-1
Procedures	Immunohistochemistry (IHC) procedures will be optimized prior to performing IHC on study slides. Additional slides may be made and used for method development or controls. Method development slides will not be included with the study data.
Examination	Immunolabeled tissue sections will be examined microscopically; findings will be included in the anatomic pathology report.

Reporting	The Contributing Scientist for Anatomic Pathology will provide a contributory report from the data generated (unsecured, in Portable Document Format [.pdf], and compliant with Labcorp and Sponsor [if applicable] PDF specifications) to the Study Director for inclusion in the Final Report.

10.10 Microscopic Examination and Anatomic Pathology

Animals	**Frequency**
Unscheduled sacrifice(s) and death(s)	Unscheduled necropsy interval
Group 2	Interim and Terminal necropsy intervals
Groups 1, 3, and 4	Interim and Terminal necropsy intervals
Procedures	
Procedures	Unscheduled sacrifice(s) and death(s) Tissues (if available) will be examined microscopically by the Anatomic Pathologist. Missing tissues will be recorded. Groups 1, 3, and 4 Brain (cerebrum, cerebellum, brainstem), spinal cord (cervical, thoracic, lumbar), DRGs, liver, heart, spleen, and skeletal muscle will be analyzed for vehicle, high, and mid dose animals at the terminal necropsy. Group 2 Gross lesions only will be examined microscopically by the Anatomic Pathologist. At the request of the Sponsor and added by Amendment, suspected target organs noted at the intermediate and/or high dose will be examined microscopically. Missing tissues will be recorded.
Reporting	The Anatomic Pathologist will provide an anatomic pathology report from the data generated to the Study Director for inclusion in the Final Report.
Digital Scans	Upon request from the Anatomic Pathologist or Sponsor and after approval of the Study Director, digital scans (virtual slides) may be prepared from selected microscopic slides for communicative/illustrative purposes only. These digital scans will not be archived (microscopic

	slides are archived as the original source of the pathology data for a respective study).

2. SHIPPING

2.1 Formulation Analysis

Frequency	On the following Monday, Tuesday, or Wednesday after the collection interval
Conditions	Dry ice
Shipping	Samples will be shipped by overnight carrier to the following address. Viralgen Attn: Please provide Address: Please provide Telephone: Please provide E-mail: Please provide Before shipping, the recipient, Study Director, and Study Monitor will be notified by e-mail as to the date and method of sample shipments.

2.2 Frozen Tissue for Biodistribution (Vector DNA Expression)

Frequency	On the following Monday, Tuesday, or Wednesday after each collection interval
Conditions	Dry ice
Shipping	Brain, spinal cord, and liver samples will be shipped by overnight carrier to the following address. BioCMC Sample Management Attn: Sarah Innis Labcorp Early Development Laboratories Inc. BioCMC/BioD [redacted] Greenfield, Indiana 46140 United States of America Telephone: +[redacted] E-mail: [redacted]

	Before shipping, the recipient, Study Director, and Study Monitor will be notified by e-mail as to the date and method of sample shipments. An electronic manifest will be included with each sample shipment.

11. DATA EVALUATION AND STATISTICAL ANALYSIS

General Statistics

Data for each sex will be analyzed separately. Only data collected on or after the first day of dosing will be analyzed statistically.

Analysis of variance (ANOVA) and pairwise comparisons, as applicable, will be used to analyze the following:

- Absolute body weight
- Body weight change
- Quantitative food consumption
- Continuous clinical pathology values
- Terminal body weight
- Absolute organ weight, organ:body weight percentage, and organ:brain weight percentage

The pairwise comparisons of interest are:

- Group 1 versus Groups 2, 3, and 4

Prior to performing the ANOVA, Levene's test will be performed to test for equality of variances between groups:

- Where Levene's test is significant ($P \leq 0.05$), a rank transformation (to stabilize the variances) will be applied before the ANOVA is conducted (note: Levene's test will not be applied to the rank-transformed data).
- Where Levene's test is not significant ($P > 0.05$), ANOVA will be conducted.

For comparisons to a single control or combined identical controls:

- If the group effect of the ANOVA is significant ($P \leq 0.05$), Dunnett's test will be used for pairwise comparisons between each treated and control group.
- If the ANOVA is not significant ($P > 0.05$), the Dunnett's test results are not applicable to the evaluation and will not be reported or used to interpret study data.

For other comparisons (i.e., not against a single control or combined identical controls):

- If the group effect of the ANOVA is significant ($P \leq 0.05$), t-tests will be used for pairwise comparisons.

• If the ANOVA is not significant (P > 0.05), the t-test results are not applicable to the evaluation and will not be reported or used to interpret study data. Where only two groups are available for analysis, a two-sample t-test will be performed. Data with values above/below the limit of quantitation will not be analyzed, and the tables will be footnoted accordingly. Where insufficient data are available for meaningful analysis, no analysis will be performed, and the tables will be footnoted accordingly. All statistical tests will be evaluated at the 5.0% probability level. Due to system limitations, additional statistical analyses may be run but will not be reported or used to interpret study data.

12. REPORTING

Template	Labcorp
Draft Reports	An electronic copy of the unaudited and audited draft report will be submitted to the Sponsor and will include relevant study data and contributing reports, as available. Sponsor comments should be supplied within 6 months of issuance of the draft report. If no comments are received, the study will be finalized and the data will be archived. The archive period will commence at that time. Any further changes after this time may be addressed as an Amended Final Report and may result in additional costs.
Final Report	An electronic version of the signed Final Report (to include all text and summary tables and all phase and/or contributor reports, as applicable) will be sent to the Sponsor. The electronic Final Report will be a text searchable, hyperlinked, bookmarked Portable Document Format (PDF) document.
Tabulated Summary	A separate tabulated summary will be provided to the Sponsor.

3. ARCHIVE STATEMENT

The raw data, including documentation, test and vehicle control article samples (if applicable), study Protocol, Final Report, study correspondence, and specimens (excluding wet specimens obtained from blood, urine, feces, and biological fluids), resulting from this study will be retained in the test facility archives for at least 1 year from the date of report finalization. After

this period, the Sponsor will be contacted to determine requirements for further retention or disposition of the archived materials (excluding facility records, nontransferable electronic data, and facility copies of the Protocol/Final Report, which will be retained by Labcorp in accordance with test facility SOPs). Where continued retention is requested, the archived materials may subsequently be transferred to alternative Labcorp archive locations. In this event, the Sponsor will be informed, and a chain of custody records will be maintained. Electronic records relating to the study will be held on the secure servers of Labcorp or designated cloud providers, with access controlled through the designated Labcorp Archivist.

Raw data, including documentation, test and vehicle control article samples (if applicable), and specimens (excluding wet specimens obtained from blood, urine, feces, and biological fluids), generated from phases performed by test sites will be archived in accordance with test site SOPs.

13. SIGNATURES

[redacted]

[redacted]

Diana Shefchek, MS
Study Director
Labcorp Early Development Laboratories Inc.

Date

Appendix XII: STUDY REPORT: PRNP-CHARM-001 IN VITRO OFF-TARGET ANALYSIS

1 EXECUTIVE SUMMARY

Development of an epigenetic editor such as PRNP-CHARM-001 requires careful evaluation of off-target editing outcomes. The Sponsor outlines below the systematic approach which has been taken, informed by Agency guidance documents, and their expertise in the field of epigenetic editing. It is the Sponsor's position as it is detailed below in this Appendix, that PRNP-CHARM-001's off-target profile is most thoroughly and efficiently evaluated by use of RNA-seq, and that the in vitro studies executed are appropriate and sufficient to serve as the *in vitro* characterization of the off-target profile of PRNP-CHARM-001. Critically, *PRNP* was the only gene showing consistent PRNP-CHARM-001 dose-responsive downregulation across all of the cell lines evaluated.

PRNP-CHARM-001 uses an epigenetic editing technology, CHARM, to recruit the endogenous DNA methyltransferase DNMT3A to the *PRNP* locus, facilitating DNA methylation and silencing of *PRNP* expression. In addition to epigenetic silencing, PRNP-CHARM-001 also includes a KRAB domain to mediate rapid transcriptional repression of the *PRNP* promoter. CHARM and KRAB are recruited to the *PRNP* promoter using a zinc finger DNA binding domain designed to bind a unique site proximal to the *PRNP* transcriptional start site, which is within a CpG island.

In alignment with recent regulatory guidance for gene editing products, "Safety Assessment of Genome Editing in Human Gene Therapy Products Using Next-Generation Sequencing", we executed multiple *in silico* and *in vitro* studies to assess potential off-target impacts of PRNP-CHARM-001 activity on gene expression. Off-target analyses consisted of two orthogonal approaches: 1) *in silico* identification of genes with sequences similar to the predicted PRNP-CHARM-001 zinc finger binding site; 2) RNA-seq performed on a panel of cell lines transduced with increasing amounts of PRNP-CHARM-001 to identify genes showing dose-responsive changes in gene expression similar to *PRNP*. RNA-seq was used as a readout of off-target CHARM activity as a reduction in gene expression is the direct consequence of both DNA methylation and KRAB-mediated repression, therefore providing an off-target readout which is most closely tied to the therapeutic mechanism of action of PRNP-CHARM-001. Moreover, identification of changes in gene expression resulting from PRNP-CHARM-001 activity provides information on which off-target genes could pose a safety risk if their expression is altered.

In silico and *in vitro* studies were executed under non-GLP conditions at the Broad Institute using test articles prepared at the Broad Institute. A panel of representative cell lines derived from human brain, liver and muscle tissues was selected. The cells were subjected to time-course transduction assays at a high multiplicity of infection (MOI) using the first-generation human transferrin receptor-binding capsid (BI-hTFR1), packaging either ZF-CHARM or an mScarlet fluorescent reporter. Responsivity to CHARM-mediated *PRNP* silencing, permissivity to BI-hTFR1 transduction, and cell viability were assessed via RT-qPCR, automated counting of mScarlet-positive cells, and LIVE/DEAD viability assays, respectively. Based on these assessments, we nominated a panel of cell lines for RNA-seq off-target analyses.

In silico analyses indicated that *PRNP* is the only gene with a perfect sequence match for the CHARM zinc finger proximal to a gene body, further supporting the specificity of PRNP-CHARM-001 for *PRNP* silencing. We performed RNA-seq on each cell line after transduction with a range of MOIs, identifying dose-responsive candidate off-target genes in each cell line.

Importantly, *PRNP* was the only gene showing consistent dose-responsive downregulation across the cell lines tested.

Because DNA methylation analysis does not detect KRAB-mediated off-target repression, we believe that RNA-seq is the most relevant and sensitive readout of PRNP-CHARM-001 off-targets. We propose, based on the risk assessment performed in this study, that the off-target activity profile of PRNP-CHARM-001 is reasonable to support our proposed clinical plan.

2 OVERVIEW OF APPROACH AND RATIONALE

Off-target evaluation will leverage two orthogonal approaches to generate the candidate list of off-target sites:

(i) *In silico* binding site prediction: The 19-bp CHARM zinc finger target sequence (5'-CTCGGTCGTGAGNAGAGGA-3') is searched against GRCh38 by Bowtie 1 (using high-stringency exact / near-exact matches) and BLASTN (using gapped, lower stringency matches). Hits from each search are intersected with GENCODE gene annotations ±10 kb to yield a sequence-similarity candidate list of genes.

(ii) Dose-responsive RNA-seq in relevant human cell lines: RNA-seq is used across a panel of cell lines and MOIs of CHARM transgene delivered by AAV in a dose-response screen to identify genes with dose-responsive gene expression profiles consistent with the CHARM mechanism of action. Generally, candidate off-target genes should show a similar pattern of down-regulation as a function of increasing MOI, as is shown by the PRNP-CHARM-001 target gene *PRNP*.

The combined candidate off-target list is the union of (i) and (ii).

Rationale for use of RNA-seq: We propose that a multi-dose, multi-tissue RNA-seq screen is necessary and sufficient to identify causal off-target safety concerns from an epigenetic editor gene therapy. DNA methylation profiling is uninformative for safety assessment, as methylation is one degree removed from impact on gene expression and can occur without impact on gene expression. Further, methylation profiling is mechanistically informative for only one of two mechanisms by which CHARM acts on the target locus, as it will not report on KRAB-mediated silencing. We outline our rationale and key considerations for our selection of RNA-seq below:

(i) **Regulatory rationale:** Section VI.B of the "Safety Assessment of Genome Editing in Human Gene Therapy Products Using Next-Generation Sequencing" draft guidance notes "the sponsor should consider the mechanism of action of their proposed editor when selecting a published modality-specific off-target editing analysis approach and justify their strategy." We propose that dose-responsive RNA-seq be accepted as the off-target assay for CHARM-based epigenetic editing, with *in silico* zinc finger binding site similarity prediction as the orthogonal nomination method.

The example in Section VI.C.2 notes that an epigenome-editor program "should be assessed with an NGS-based genome-wide DNA methylation analysis method" which is, in the same paragraph, qualified as one of multiple appropriate strategies "irrespective of whether an editor targets the genome, transcriptome, or epigenome." We are applying that principle to use the readout where the safety-relevant terminal effect manifests (transcription).

(ii) **Gene expression is the proximate cause of phenotype and is most relevant for hazard identification:** Mechanistically, an organism-level effect of PRNP-CHARM-001 silencing proceeds through the following steps: 1) CHARM zinc finger binding; 2) DNMT3A recruitment; 3) DNA methylation; 4) functional gene silencing through DNA methylation and KRAB-mediated gene repression; 5) cellular phenotype; 6) organism-level safety effect. DNA methylation is an upstream consequence of CHARM, where consequent effects on gene expression direct the phenotype. A safety-relevant off-target is by definition a gene whose expression changes, and CHARM-mediated DNA methylation that fails to alter transcription is not a hazard.

While functional aspects outside of gene transcription (enhancer state, chromatin domain organization, non-coding regulatory RNAs) exist, their translation into organismal safety is outside the scope of any FDA approved therapeutic. Hazard identification at the transcriptional layer captures the safety-relevant signal.

(iii) **DNA methylation can occur without functional consequence:** CpG islands are routinely partially methylated with no transcriptional effect. DNMT3A can bind and deposit methylation that ultimately is not stable. Inferring safety from "methylation changed at locus X" risks both: 1) False positives where methylation occurred, but no expression change (equating to no hazard); 2) False negatives where marginal DNA methylation changes might be statistically missed, leading to failure to nominate a real expression-level hazard that could be captured by RNA-seq.

(iv) **RNA-seq methodology is mature, validated, and interpretable:** Generally, the magnitude of knockdown of a gene has predictable and interpretable phenotypic consequences. In comparison, changes in DNA methylation at a particular CpG island have variable, context-dependent consequences and potentially no effect at all. The bridge from RNA-seq gene expression outcome to safety claim is shorter and more defensible than DNA methylation outcome to safety claim.

DESeq2 / edgeR pipelines underlie FDA-accepted preclinical and clinical submissions; per-gene negative binomial generalized linear model (NB-GLM) dispersion estimation has over 15 years of methodological literature. RNA-seq's analytical validity profile is better-characterized than methylation-sequencing analysis.

(v) **RNA-seq permits superior cost-effective statistical sensitivity:** When comparing measurements made by RNA-seq and DNA methylation profiling, RNA-seq interrogates ~17,000 expressed genes, while DNA methylation arrays use ~935,000 array probes. This results in more "tests" in methylation analyses, but most are unused; multiple-testing-corrected power is lower per-test. One Illumina NovaSeq lane covers a multi-tissue, multi-dose, multi-replicate screen, while comparable methylation-array depth is multiple separate arrays with separate batch correction. By these metrics, RNA-seq is significantly more cost-effective and generates fewer unused data points. The analytical pipeline for RNA-seq is less subject to technical noise than the complex batch effects and normalization required for large-scale methylation arrays, providing higher statistical power per dollar spent. In practice, the same resources will almost surely better identify off-targets with higher fidelity using RNA-seq, rather than having to work with fewer replicates on a noisier assay.

(vi) **Dose-responsive screening across multiple cell types strengthens off-target identification:** A multi-MOI screen identifies dose-dependent effects mechanistically,

not just statistically. A monotonic dose-response curve through dose points is far stronger evidence of a causal effect than a single-dose two-arm comparison. A cross-tissue consistency filter (e.g., as shown in Table 6) strengthens off-target claims where a gene's response is statistically robust across tissues that express it and DNMT3A.

Accounting for human genetic variation: Our off-target analysis approach does not account for human genetic variation beyond any genetic variation present in the cell lines evaluated. Given the rarity of prion disease, we do not anticipate that human genetic variation would significantly impact the set of candidate off-target genes given the comparatively small patient population and resulting lower amount of genetic variation present across patients. This position is in alignment with Section IX of the "Safety Assessment of Genome Editing in Human Gene Therapy Products Using Next-Generation Sequencing" draft guidance, which states "Studies assessing off-target editing activity by accounting for human genetics variations may not be necessary with an original IND submission in some cases such as when the proposed indication is an ultra-rare disease or when it is intended for the treatment of a single patient."

3 STUDY SCOPE

Cell line justification: A summary of the cell lines evaluated for in vitro off-target analyses is presented in Table 1. Immortalized human cell lines were chosen from tissues reflective of the intended clinical target (central nervous system) as well as potential off-target tissues (liver, muscle). Immortalized human cell lines were selected over primary cells because:

(i) the purpose of this assay is hazard identification and nomination, not hazard characterization;
(ii) donor-to-donor variation of primary cells could mask off-target effects and increase sequencing needs beyond what is feasible; immortalized cell lines increase nomination sensitivity by minimizing inter-replicate variability and by increasing the number of replicates that can be feasibly tested;
(iii) candidate off-target sites are more easily characterized in immortalized lines, which express the necessary CHARM machinery (e.g. DNMT3A) and can be transduced by AAV.

The suitability of cell lines for CHARM off-target RNA-seq analyses was assessed through RT-qPCR to measure *PRNP* silencing, imaging to measure mScarlet fluorescence as a readout of transduction efficiency, and cell viability assessment after transduction. Cell lines were deemed suitable for RNA-seq if they maintained viability similar to untransduced cells after 14 days post-transduction, and if at least 75% reduction of *PRNP* expression was observed by RT-qPCR after transduction with PRNP146 at an MOI of 100K.

Table 1. Summary of cell lines used for *in vitro* off-target analyses

Cell line	Tissue	Description	Source	Catalog number
hCMEC/D3	Brain	Immortalized human brain endothelial cells	Sigma	SCC066
SVG p12	Brain	Immortalized human fetal glial cells	ATCC	CRL-8621
LN-229	Brain	Human glioblastoma cells	ATCC	CRL-2611
THLE-2	Liver	Human epithelial cells from the left lobe of a donor	ATCC	CRL-2706
Immortalized skeletal muscle cells	Muscle	Immortalized human skeletal muscle cells	ABM	T0034
HEK293	Kidney	Human embryonic kidney cells	ATCC	CRL-1573

Test articles: Test articles were manufactured at the Broad Institute (Cambridge, MA), where the evaluated transgenes were packaged in the human TfR1-binding capsids BI-hTFR1 or TfR1 CapX. Test articles produced at the Broad Institute were formulated in phosphate-buffered saline (PBS) supplemented with Pluronic F-68 to a final concentration of 0.001% (volume/volume). Prior to in vitro transduction, test articles were subjected to an analytical panel consisting of ddPCR-based titering and SDS-PAGE to assess vector purity. Test articles met all suitability criteria for use in the study. A summary of the test articles evaluated is presented in Table 2.

Table 2. Test articles used for *in vitro* off-target analyses

Test Article ID	AAV Capsid	AAV genome-transgene	Purpose
DLVC1034	TfR1 CapX	ssAAV-CAG-NLS-mScarlet-P2A-Luciferase-WPRE	Transduction control for cell line suitability assessment
PRNP146	BI-hTFR1	ssAAV-EFS-AlphaGlobin5'UTR-PRNP-CHARM-WPRE-SV40pA	Early version of PRNP CHARM used in cell line suitability assessment
PRNP183	TfR1 CapX	ssAAV-Noncoding stuffer-WPRE-SV40pA	Control AAV lacking CHARM; controls for gene expression changes due to AAV transduction
PRNP173	TfR1 CapX	ssAAV-EFS-PRNP-CHARM-WPRE-SV40pA	Candidate PRNP CHARM transgene lacking uORFs and miR-122 binding sites
PRNP174	TfR1 CapX	EFS-2XuORF-PRNP-CHARM-3XmiR-122-WPRE-SV40pA	PRNP-CHARM-001 transgene used in toxicology studies and intended for clinical use

Study readouts: The suitability of cell lines for off-target RNA-seq analyses was confirmed through RT-qPCR to measure *PRNP* silencing, fluorescence imaging to measure mScarlet fluorescence as a readout of transduction efficiency, and cell viability assessment after transduction. As described above, we used RNA-seq to identify candidate CHARM off-target genes.

4 EXPERIMENTAL PROCEDURES SUMMARY

The study was executed as a non-GLP study at the Broad Institute (Cambridge, MA).

AAV transduction for cell line suitability assessment: We compared direct and reverse AAV transduction approaches on each cell line to confirm their suitability. For direct transduction, cells were seeded in 48-well plates at a density of 1×10^4 cells per well in a volume of 320 µL. The following day, 80 µL of diluted PRNP146, DLVC1034 (equivalent to 1×10^9 vg), or PBS was added to each well in triplicate, resulting in an MOI of 1×10^5. For reverse transduction, 80 µL of diluted PRNP146, DLVC1034 (equivalent to 1×10^9 vg), or complete media was added to each well in triplicate. Cells were then seeded on top of the vectors at a density of 1×10^4 cells per well by adding 320 µL of diluted cell solution, resulting in an MOI of 1×10^5. Plates were incubated at 37°C with 5% CO_2. Every 48 hours for 15 days, cells were rinsed with 200 µL of PBS, followed by a complete media change. Three 48-well plates were prepared per time point (D0, 3, 6, 9, 12 and 15) and assigned to different analyses: 1) RT-qPCR to measure *PRNP* silencing; 2) Image cytometry to measure mScarlet fluorescence as a readout of transduction efficiency; 3) Cell viability.

RT-qPCR for cell line suitability assessment: RT-qPCR assays were performed using the Luna Cell Ready One-Step RT-qPCR kit (NEB), with Taqman primers and probes targeting *PRNP* (ThermoFisher HS00175591_m1) and the housekeeping gene *TBP* (ThermoFisher HS00427620_m1).

Imaging of reporter transduction for cell line suitability assessment: To evaluate the permissivity of different cell lines to transduction with TfR1-binding capsids, the number of mScarlet-positive cells was quantified in all wells treated with DLVC1034 using the image cytometry tool on a Keyence fluorescence microscope.

Cell viability assay for cell line suitability assessment: Cell viability was assessed at each time point using a two-color LIVE/DEAD viability assay for mammalian cells (ThermoFisher Scientific, L3224). Cells were incubated for 20 minutes at room temperature in 200 µL of LIVE/DEAD solution and subsequently imaged using the image cytometry tool on a Keyence fluorescence microscope.

AAV transduction for RNA-seq: For each cell line, three AAV constructs were delivered at six multiplicities of infection (MOIs): 0, 5×10^2, 2×10^3, 8×10^3, 3.2×10^4, and 1.28×10^5 viral genomes per cell. The MOI = 0 condition received PBS only. Total RNA was extracted from cells 12 days post-transduction. Each sample plate carries three duplicated MOI = 0 wells as plate-to-plate anchors for batch correction. These batch correction samples are excluded from the downstream RNA-seq expression matrix but are used through read alignment for quality control.

Nucleic acid extraction for RNA-seq: At 12 days post-transduction, cells were rinsed with 200 uL PBS, resuspended in 150 uL DNA/RNA shield buffer (ZYMO RESEARCH), and shipped at room temperature to Plasmidsaurus (Eugene, OR) facility. Total RNA extraction and quality control were performed using Plasmidsaurus' proprietary automated workflow, yielding RNA solutions at concentrations exceeding 10 ng/uL.

RNA-seq library preparation and sequencing: RNA-seq libraries were prepared and sequenced by Plasmidsaurus (Eugene, OR) using the Differential Gene Expression (DGE) RNA-Seq protocol, a 3′-end tag sequencing assay with unique molecular identifiers (UMIs) embedded in the read header. The DGE protocol uses an oligo-dT primer with an N_8 UMI for first-strand synthesis; no gene-specific primers are used. Sequencing was performed on an Illumina NovaSeq X Plus using 93 bp single-end reads. Approximately three of four nominal replicates were sequenced per construct × MOI per plate, yielding ≥ 6 sequenced replicates after cross-plate replication for the 108-sample dose-response batches.

5 BIOINFORMATIC PIPELINE

The pipeline source, Snakefile, configuration, and per-rule conda environments are versioned in the project repository. The pipeline is invoked from the project root with the standard Snakemake driver (--use-conda --cores 32 --resources mem gb=109 on the production virtual machine (VM) described below).

Compute environment: The pipeline runs on a single Google Compute Engine VM with the following specification:

- Machine: 32 vCPU / 128 GB RAM (n2-standard-32-class).
- Storage: 1.5 TB Hyperdisk Balanced (boot + data), sufficient for all RNA-seq datasets decompressed in parallel.
- OS: Ubuntu 22.04 LTS.

Snakemake schedules concurrency on RAM (resources: mem gb) rather than thread count: STAR jobs are gated to three simultaneous (mem gb=30 each) and UMICollapse jobs to three simultaneous (mem gb=28 each), which fits within a 109 GB budget after a 16 GB OS pad is reserved. Per-rule thread counts are fastp = 4, STAR = 8, UMICollapse = 4, featureCounts = 16.

Bioinformatic tools used: The specific versions of bioinformatic tools, relevant citations, the purpose of each tool, and the specific commands used with each tool in the pipeline are provided in Table 3.

Table 3. Summary of bioinformatic tools used for off-target analyses

Tool	Version	Citation	Purpose	Command used
Snakemake	8.20	Mölder et al., 2021	Workflow orchestration	N/A
fastp	0.24.0	Chen et al., 2018	Read quality and adapter trimming	fastp -i {in} -o {out} --trim_poly_x --cut tail --cut_tail_mean_quality 20 --qualified_quality_phred 20 --length_required 50
STAR	2.7.11b	Dobin et al., 2013	Splice-aware RNA-seq alignment	STAR --runThreadN 8 --genomeDir {idx} --readFilesIn {fq.gz} --readFilesCommand zcat –outFilterIntronMotifs RemoveNoncanonical --outSAMtype BAM SortedByCoordinate --outReadsUnmapped Fastx
samtools	1.22.1	Danecek et al., 2021	Coordinate-sorted BAM file indexing	samtools index {bam}
UMICollapse	1.1.0	Liu, 2020	UMI-aware deduplication, invoked with --two-pass for the memory-efficient streaming algorithm (input is coordinate-sorted by STAR). The bioconda wrapper's default JVM heap (-Xmx 15G) is overridden to -Xmx 24G to accommodate	umicollapse bam -i {bam} -o {out} --two-pass --umi-sep _

			transgene-region multi-mapper pile-ups	
Subread/featureCounts	2.1.1	Liao et al., 2014	Per-gene RNA-seq quantification	featureCounts -a {gtf} -o {counts} -s 1 -O -M --primary -t exon -g gene id -T 16 {bams}
MultiQC	1.32	Ewels et al., 2016	Generates aggregated QC report	N/A
DESeq2	1.42	Love et al., 2014	Size-factor normalization, dispersion estimation, and likelihood-ratio testing for RNA-seq	N/A
Julia	1.12	Bezanson et al., 2017	Per-panel three-component mixture-model inference (Step 7), sigmoid curve fitting, batch-effect removal (limma-style removeBatchEffect via OLS on a log2(count + 1) ~ batch + construct + log10(MOI + 1) design), and figure generation.	N/A
CairoMakie	0.15	Danisch and Krumbiegel, 2021		
DataFrames	1.8	Bouchet and Kamiński, 2023		
Bowtie 1	1.3.1	Langmead et al., 2009	Ungapped enumeration of all alignments with ≤ 3 mismatches (high-stringency hit set)	bowtie -a -v 3 -f –sam GRCh38_noalt_as/GRCh38_noalt_as zf_query.fa > zf_hits.sam
Bowtie 2	2.5.5	Langmead and Salzberg, 2012	Gapped/heuristic short-read aligner, run as an *in silico* cross-check (loose-seed settings to recover Bowtie 1's hit set within a broader penumbra)	bowtie2 -f -a -L 10 -N 1 \ --no-1mm-upfront \ --score-min L,-20,0 \ -x GRCh38_noalt_as/GRCh38_noalt_as \ -U zf_query.fa \ -S zf_hits_bt2.sam

BLAST+	2.16.0	Camacho et al., 2009	Gapped local alignment of the zinc finger consensus binding site against an hg38 BLAST nucleotide database	blastn -task blastn-short \ -query zf_query.fa \ -db hg38_blastdb \ -word_size 7 -evalue 1000 \ -outfmt 6 \ -out zf_blast_hits.tsv
BLASTN	N/A	Altschul, et al., 1990	Run interactively against the NCBI nt collection with megablast off, word size 7, expect 10, gapped. The alignment download (RID VKMTNCB9014) is archived as ncbi_web_blastn_VKMTNCB9014.txt	N/A
bedtools	2.31.1	Quinlan and Hall, 2010	Per-hit nearest GENCODE gene annotation within ±10 kb of the gene body (applied identically to each tool's BED output)	closestBed -a {hits.bed} -b {genes_sorted.bed} -d \ | awk '$NF <= 10000' \ > {nearest_genes.tsv}

RNA-seq genome reference: For RNA-seq analyses, a hybrid reference is assembled from:

(i) GRCh38 primary assembly (GENCODE release 46), distributed by Ensembl/EBI.

(ii) Three transgene plasmid sequences (corresponding to PRNP183, PRNP173, and PRNP174 evaluated in the study) provided as full-plasmid FASTA in the project repository.

Transgene annotations include three overlapping features per transgene: the whole-plasmid transgene, the woodchuck hepatitis post-transcriptional regulatory element WPRE (594 bp, detected via the 5′ AATCAACCTCTGGATTACAAA and 3′ GCCGCCTCCCCGCCTCTC flanking sequences), and transgene coding sequence for the region upstream of the WPRE region. featureCounts is invoked with -O so reads overlapping multiple features contribute to each.

Analysis steps and acceptance criteria: The pipeline produces, in order, the outputs listed below, with per-step acceptance criteria applied during MultiQC review.

(i) **Step 1. Read QC and trimming (fastp).** Removes adapters and low-quality 3′ tails (--cut tail --cut tail mean quality 20), enforces minimum Phred ≥ 20 (1% per-base error rate) and minimum post- trim length ≥ 50 bp.

Acceptance: ≥ 90% of reads pass fastp QC; mean Q30 ≥ 90% (consistent with NovaSeq X Plus vendor-specification output).

(ii) **Step 2. Splice-aware alignment (STAR).** Aligns trimmed reads to the hybrid reference; non-canonical splice junctions are removed (--outFilterIntronMotifs RemoveNoncanonical).

Acceptance: ≥ 70% uniquely-mapped reads (STAR Log.final.out); ≤ 20% reads mapping to multiple loci. The multi-mapping threshold is relaxed from the standard mRNA-seq ≤10% rule because (a) ~93 bp 3′-tag-seq reads multi-map more often than typical 100–150 bp paired-end reads, and (b) the AAV transgene cassette intentionally shares WPRE, polyA, and ITR sequence across all three transgene contigs, producing multi-mapper traffic that featureCounts -O -M --primary consolidates under a single transgene gene ID downstream.

(iii) **Step 3. UMI deduplication (UMICollapse).** Collapses PCR duplicates by 8-mer UMI in the read header. Run with --two-pass so per-locus pileups are streamed rather than held fully in memory; the input is coordinate-sorted by STAR, which --two-pass requires.

Acceptance: Duplication rate ≤ 60% post-deduplication (rate > 60% flags low-complexity libraries for review).

(iv) **Step 4. Gene quantification (featureCounts).** Strand-specific (-s 1); each multi-mapping read is assigned to its STAR primary alignment only (-O -M --primary); overlapping transgene, WPRE, and coding sequence subfeatures all receive that count.

Acceptance: ≥ 50% of mapped reads assigned to features; ≥ 1×10^6 post-deduplication uniquely-mapped reads per sample (consistent with 3′-tag-seq DGE depth norms; raw delivery is typically 5–10 M reads, of which 1–3 M survive UMI deduplication given the lower per-read complexity of 3′-tag protocols).

(v) **Step 5. Sample-level QC review (MultiQC).** Aggregates per-rule metrics into a single HTML report.

Acceptance: Samples failing any criterion in Steps 1–4 are flagged and logged in the QC-failure table and excluded from downstream analysis unless the cause can be resolved.

(vi) **Step 6. Automated outlier detection.** A scripted post-MultiQC step computes two complementary outlier statistics on the per-sample count matrix and emits a single tab-delimited table (“qc outliers.tsv”). The two statistics are:

a. **Covariate-corrected studentized residual on PCs.** The log2(CPM+1) matrix is restricted to genes with mean log-CPM > 1 and decomposed by SVD; the top three principal components (PCs) are extracted. Known structural covariates (cell line, construct, log2 MOI, plate batch) are regressed out of each PC by ordinary least squares, leverages are computed from the hat matrix, and studentized residuals are produced. A sample is flagged if |stud. resid.| > 3 on PC1, PC2, or PC3.

b. **Median pairwise correlation within design cell.** The log-CPM Pearson correlation matrix is computed across all samples; for each sample, the median correlation against its same-(cell line, construct, MOI) peers is recorded. A sample is flagged if the median peer correlation falls below 0.85.

Flagged samples are excluded only when a documented cause exists (sequencing-run failure, transgene-caller mislabel, contamination, or other technical artifact); all

adjudication decisions, including retentions, are recorded in "qc outliers.tsv" with a free-text reason field.

Acceptance: The outlier-detection script runs to completion on every panel; the QC-outliers table accompanies every per-panel deliverable. A run is held for review if more than 5% of samples in any single (cell line, construct) panel are flagged simultaneously by both checks.

(vii) **Step 7. Differential expression and dose response.** Median-of-ratios size factors and gene-wise negative-binomial dispersions are computed by DESeq2 1.42 on the raw count matrix, per cell line. The dispersions and size factors feed a per-panel Bayesian three-component finite mixture model in which each gene is classified as null (no dose-response), on-target-shaped (effect follows the CHARM dose-response shape learned from *PRNP*), or AAV-sensitivity-shaped (effect follows a sensitivity shape learned from reference-construct dose-responsive genes). All mixture proportions and effect-size hyperparameters governing the prior on βg are estimated from the data by empirical Bayes; none are fixed by hand. Per-gene posterior inclusion probabilities (PIPs) and effect-size estimates $\hat{\beta}g$ are reported for both non-null components. We welcome Agency feedback on the full mathematical specification — priors, MAP optimization, Laplace approximation, and EM updates — which can be provided upon request.

Decision rule: Genes with PIPCHARM > 0.99 and $\hat{\beta}g < 0$ are nominated as on-target-shaped off-targets; genes with PIPsens > 0.5 are nominated as AAV-sensitivity off-targets. Per-gene results are tabulated, with columns for each PIP, MAP $\hat{\beta}g$, the panel-level log2 EC50 for the CHARM and sensitivity shapes, and the converged mixture weights.

Acceptance: Per-gene MAP optimization that fails to converge within 20 Newton iterations (or where the Hessian determinant drops below 10^{-12}) yields an N/A in the results table. EM hyperparameter trajectories are inspected for stability — convergence of (πnull, πCHARM, πsens) within 0.005 between iterations 4 and 5 is required before nominations are released downstream. Both checks are logged automatically and accompany every per-panel run.

(viii) **Step 8. Bioinformatic off-target prediction.** In parallel with the transcriptomic screen, the target site of the zinc finger DNA-binding domain of the CHARM construct (19 bp consensus query) was searched against the GRCh38 primary assembly by two complementary sequence-similarity methods:

a. Bowtie (short-read aligner mode) — exact and near-exact matches up to a Hamming-distance threshold (default ≤ 3 mismatches, no gaps), yielding the high-stringency hit set;
b. BLASTN against a GRCh38 nucleotide database, providing gapped alignments at lower stringency, yielding the broader sensitivity hit set.

Hit coordinates are intersected with GENCODE gene bodies ±10 kb to yield a "nearest gene" annotation per hit; the union of Bowtie- and BLASTN-nominated genes is the bioinformatic off-target candidate list. The candidate list is independent of expression data and is used as the *a priori* mechanistic prior on the transcriptomic nominations: genes nominated by the mixture model (Step 7 above) and also sequence-flagged here are higher-confidence off-target genes.

Acceptance: A nominated CHARM off-target gene that is (a) sequence-flagged and (b) expressed in baseline samples (≥ 5 counts) and (c) shows the CHARM dose-response

pattern across multiple tissues that express both the gene and *DNMT3A* is reported as a high-confidence candidate. A gene that is dose-responsive in only a single tissue while being expressed in multiple is reported but flagged: the CHARM mechanism of action requires both target-sequence binding and DNMT3A-mediated methylation, so a single-tissue effect in the absence of cross-tissue concordance is inconsistent with the proposed mechanism of action.

6 RESULTS SUMMARY

Cell line suitability assessment: Prior to initiating RNA-seq off-target analyses, we screened a panel of cell lines to confirm their suitability for assessing PRNP CHARM off-target effects. We used RT-qPCR to measure the extent of *PRNP* silencing in each cell line over a 15 day time course, comparing knockdown achieved with direct and reverse transduction. As shown in Figure 1, *PRNP* silencing was detectable by 3 days post-transduction, with the highest level of knockdown observed in SVG p12 cells, followed by skeletal muscle cells ("muscle"), hCMEC/D3, THLE-2, and LN-229 cells. Comparable knockdown was observed with either transduction approach for each cell line.

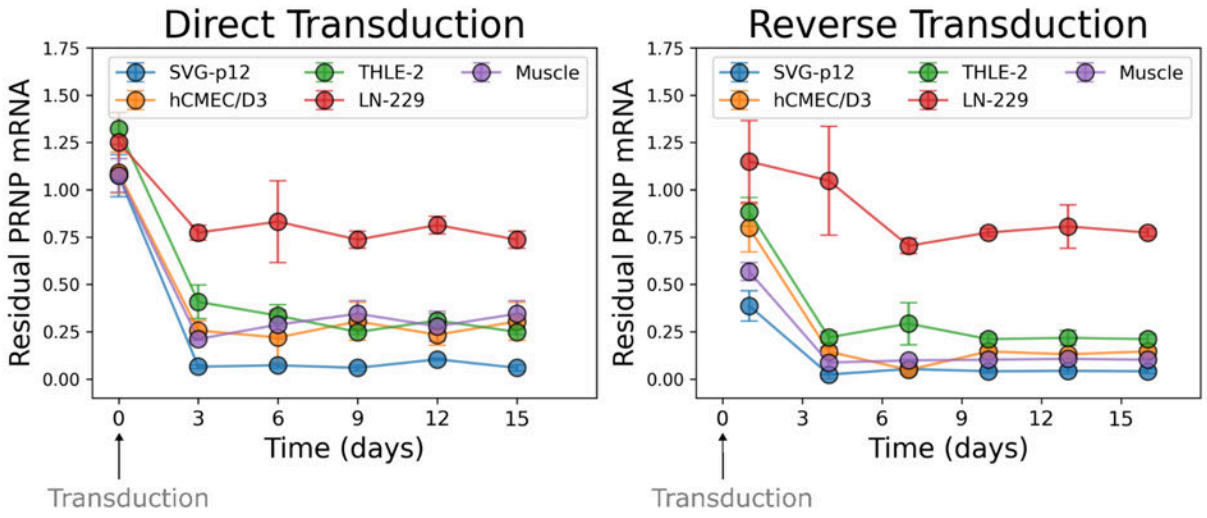


Figure 1. Residual *PRNP* levels measured by RT-qPCR after transduction as a function of time for the five cell lines investigated in this study. Panels compare results obtained with either direct or reverse transduction.

Transduction efficiency was evaluated 3 days post-transduction with DLVC1034, by quantifying mScarlet-positive cells and mScarlet fluorescence density using image cytometry, as shown in Figure 2. SVG p12 and hCMEC/D3 cell lines exhibited the highest number of transduced cells, with SVG p12 showing higher fluorescence density. THLE-2 and muscle cells exhibited fewer transduced cells, counterbalanced by high fluorescence density levels. Finally, in line with RT-qPCR data, LN-229 exhibited the lowest number of mScarlet-positive cells, combined to low fluorescence density levels.

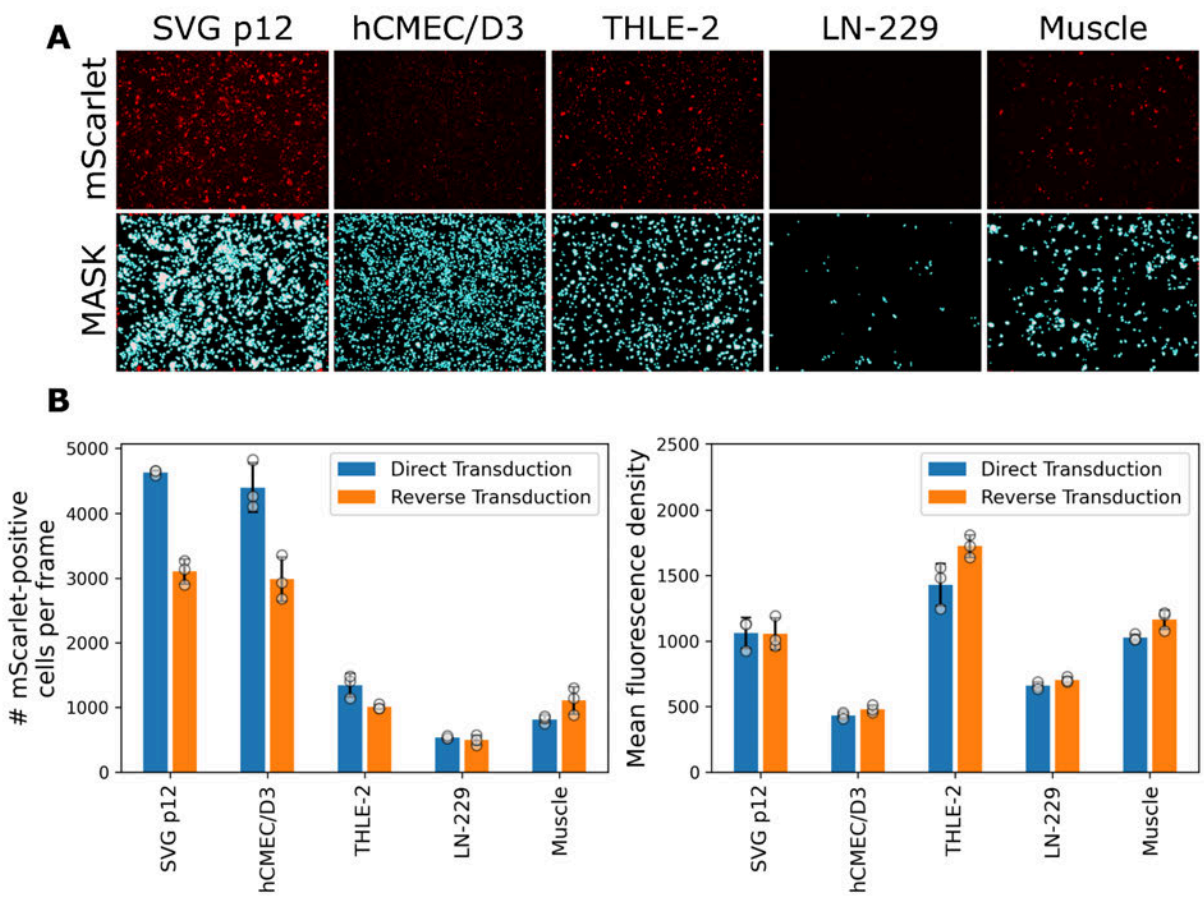


Figure 2. Permissivity of tested cell lines to BI-hTFR1 transduction. Cells were transduced with DLVC1034 at a MOI of 100k. The number of mScarlet-positive cells and fluorescence density were measured 3 days post-transduction. (**A**) Representative mScarlet fluorescence images of reverse-transduced cell lines, with corresponding binary masks used for analysis. (**B**) Quantification of mScarlet-positive cells and mScarlet fluorescence density across the cell line panel.

Cell viability was evaluated at each time point using imaging after staining with a live/dead assay kit, as shown in Figure 3. Due to the abundance of living cells forming a dense monolayer, direct counting in the wells was impractical (Figure 3, panel A). Therefore, the number of dead cells per frame was used as a proxy measurement to assess viability over time. The analysis showed minimal differences in dead cell counts between wells treated with virus (via direct or reverse transduction) and those treated with media only (Figure 3, panel B). These results indicated that transduction with TfR1-binding AAV vectors at an MOI of 100K does not adversely affect the viability of any of the five cell lines tested.

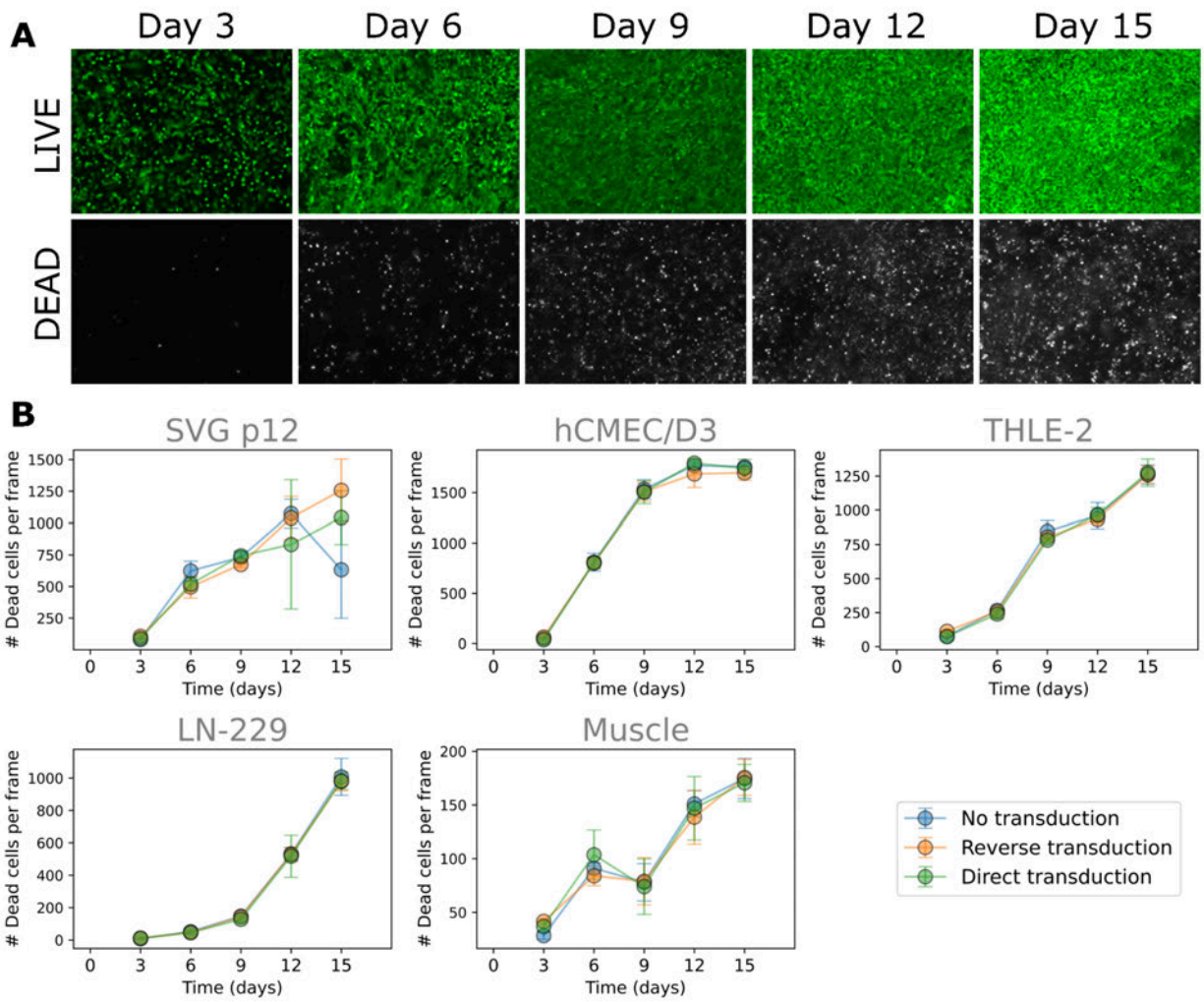


Figure 3. Cell Viability Assessment, (A) Representative Live/Dead fluorescence images of hCMEC/D3 cells (no virus control) at days 3-15 post-transduction. (B) Quantification of dead cells over time (MOI 100K) across all five cell lines (n =3 wells per condition). Because the high density of live cells made counting them impractical, the number of dead cells per well was used as a proxy for cell viability.

Based on transduction efficiency, *PRNP* silencing, and cell viability data, the cell lines summarized in Table 4 were deemed suitable for off-target safety assessment. LN-229 cells were not advanced for RNA-seq characterization due to the less efficient *PRNP* silencing observed. Though not evaluated in these cell suitability assessments, we also included HEK293 cells in our RNA-seq assessments due to the facility of using this cell line and its demonstrated utility in potency assessments of PRNP-CHARM-001 activity. While robust *PRNP* silencing was observed at day 3 post-transduction in most cell lines, day 12 was used for subsequent assays to allow sufficient time for onset of CHARM-mediated DNA methylation. Reverse transduction was selected for all future experiments, as it reduces workflow time by one day, improves MOI control (as cells are counted immediately before transduction), and yields more efficient *PRNP* silencing compared to direct transduction.

Table 4. Summary of cell lines assessed for RNA-seq suitability and advanced for RNA-seq analyses.

Cell line	Tissue	Description	Residual *PRNP* mRNA at day 12	Viability at day 12	Advanced to RNA-seq
hCMEC/D3	Brain	Immortalized human brain endothelial cells	13 ± 1%	Comparable to no virus control	Yes
SVG p12	Brain	Immortalized human fetal glial cells	4 ± 2%	Comparable to no virus control	Yes
LN-229	Brain	Human glioblastoma cells	81 ± 12%	Comparable to no virus control	No
THLE-2	Liver	Human epithelial cells from the left lobe of a donor	22 ± 4%	Comparable to no virus control	Yes
Immortalized skeletal muscle cells	Muscle	Immortalized human skeletal muscle cells	11 ± 0%	Comparable to no virus control	Yes
HEK293	Kidney	Human embryonic kidney cells	Not evaluated in this study	Not evaluated in this study	Yes

RNA-seq analysis: We used RNA-seq data across cell lines and MOIs to confirm expression of the endogenous DNA methyltransferase DNMT3A, which is required for CHARM silencing activity. A full list of the RNA-seq samples sequenced and their associated read depth, mapping rate, and any failures in meeting acceptance criteria for inclusion in analyses is provided in Appendix XIV. After filtering for samples meeting acceptance criteria, all cell lines and conditions examined were represented with at least 3 biological replicates. Using these data, we compared baseline *DNMT3A* expression at 0 MOI and 128K MOI when transduced with PRNP173, summarized in Table 5. Negligible, statistically insignificant changes in *DNMT3A* expression were observed, indicating CHARM activity does not significantly impact normal *DNMT3A* expression. Moreover, *DNMT3A* expression was detected across all cell lines and MOIs evaluated, shown in Figure 4 (top row). Overall, these data indicated that the cell lines examined expressed the endogenous machinery required for CHARM activity.

Table 5. ***DNMT3A* expression at MOI 0 vs. 128K across cell lines evaluated with RNA-seq.** CPM is counts per million mapped reads. Percent change is the 128K MOI vs. 0 MOI fold change for the active CHARM construct (PRNP173). Adjusted p-value is the DESeq2 Wald test of PRNP173 vs. reference PRNP183 at 128K MOI.

Cell line	*DNMT3A* CPM at 0 MOI	*DNMT3A* CPM at 128K MOI	Percent change	Adjusted p-value (DESeq2 Wald test)
SVG p12	237	232	-2%	0.781
hCMEC/D3	266	285	+7%	1.000
HEK293	112	119	+7%	1.000
Muscle	152	146	-4%	1.000
THLE-2	157	157	0%	1.000

We further analyzed RNA-seq data to measure the level of transgene expression across cell lines and MOIs, as well as the resulting extent of *PRNP* RNA reduction, as shown in Figure 4. Across most cell lines, we observed dose-dependent transgene expression which increases as a function of MOI (Figure 4, second row). This dose-dependent pattern of transgene expression was less apparent for hCMEC/D3 cells, though transgene expression was detectable in this cell line. Dose-dependent reduction of *PRNP* expression was observed across all cell lines treated with PRNP173 and PRNP174, but not the control test article PRNP183, consistent with increased CHARM transgene expression at higher MOI (Figure 4, third row). The relation between CHARM transgene expression and *PRNP* silencing was further examined, where increased expression of PRNP173 and PRNP174 transgenes (containing active CHARM) led to reduced *PRNP* expression (Figure 4, fourth row). These data confirmed our ability to use RNA-seq to measure CHARM-dependent effects on gene expression as a function of transgene dosage.

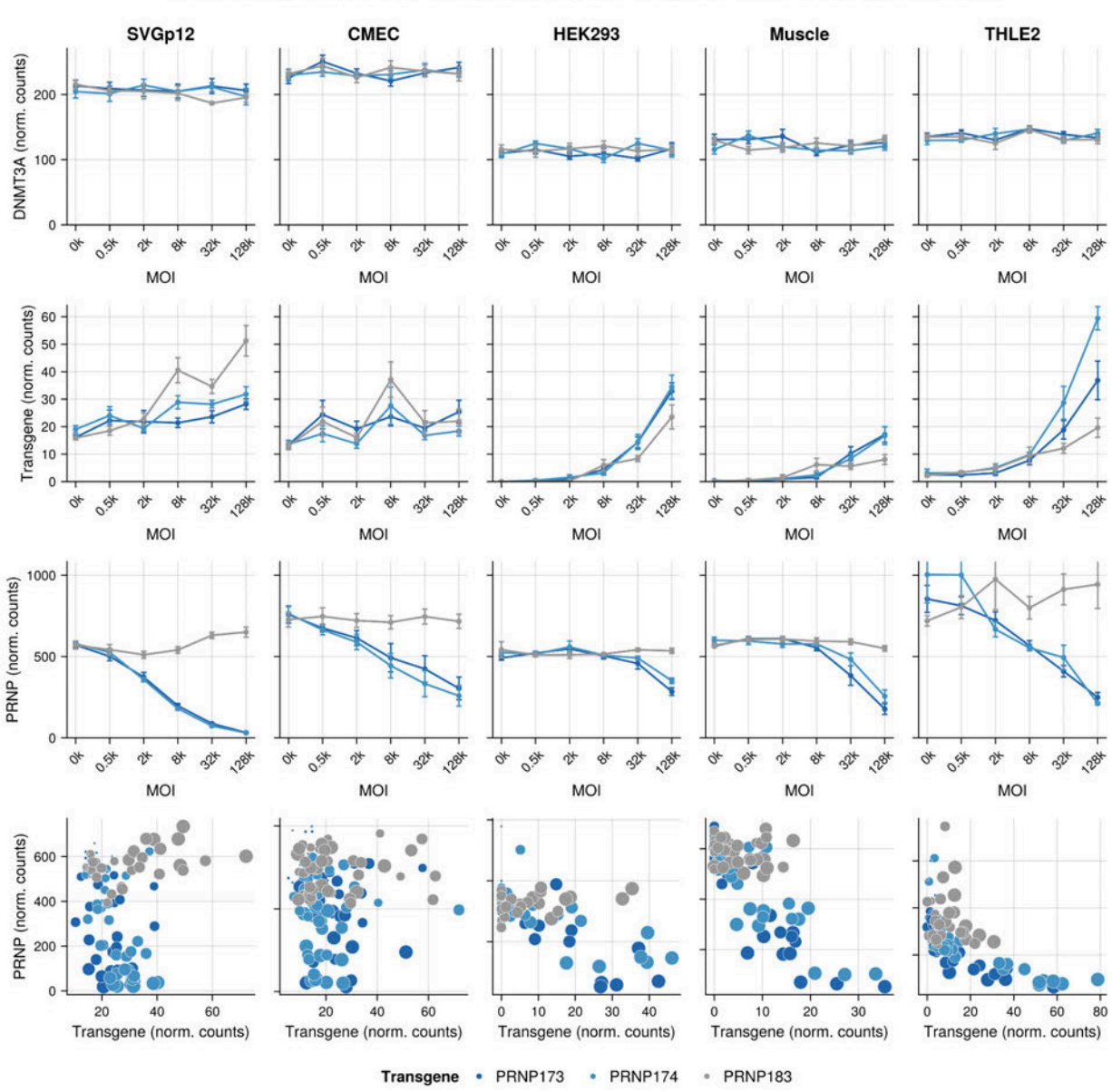


Figure 4. RNA-seq profiling of *DNMT3A*, transgene, and *PRNP* expression across cell lines and MOIs. Per-tissue dose response of *DNMT3A* (row 1), transgene (row 2), and *PRNP* (row 3), expressed as DESeq2-normalized counts after subtracting per-cell-line batch effects estimated by a limma-style log-linear correction. Single-batch cell lines (HEK293/GG83WH, SVGp12/PMJTDJ, THLE2/X26MQB) are passed through uncorrected. Row 4 shows a per-sample scatter of batch-corrected transgene expression (x) vs. batch-corrected *PRNP* expression (y), with marker area scaling with log(MOI + 1).

Using the RNA-seq analysis approach outlined above, we examined the RNA-seq data across cell lines and MOIs to identify other candidate off-target genes showing a similar pattern of dose-responsive gene expression down-regulation as compared to *PRNP*. The pattern of *PRNP* gene silencing is shown relative to all other genes measured in each cell line in Figure 5, and the full set of candidate off-target genes identified through our RNA-seq analysis approach is listed in Table 6. Importantly, we found that *PRNP* was the only gene whose gene expression response was statistically robust across the cell lines evaluated.

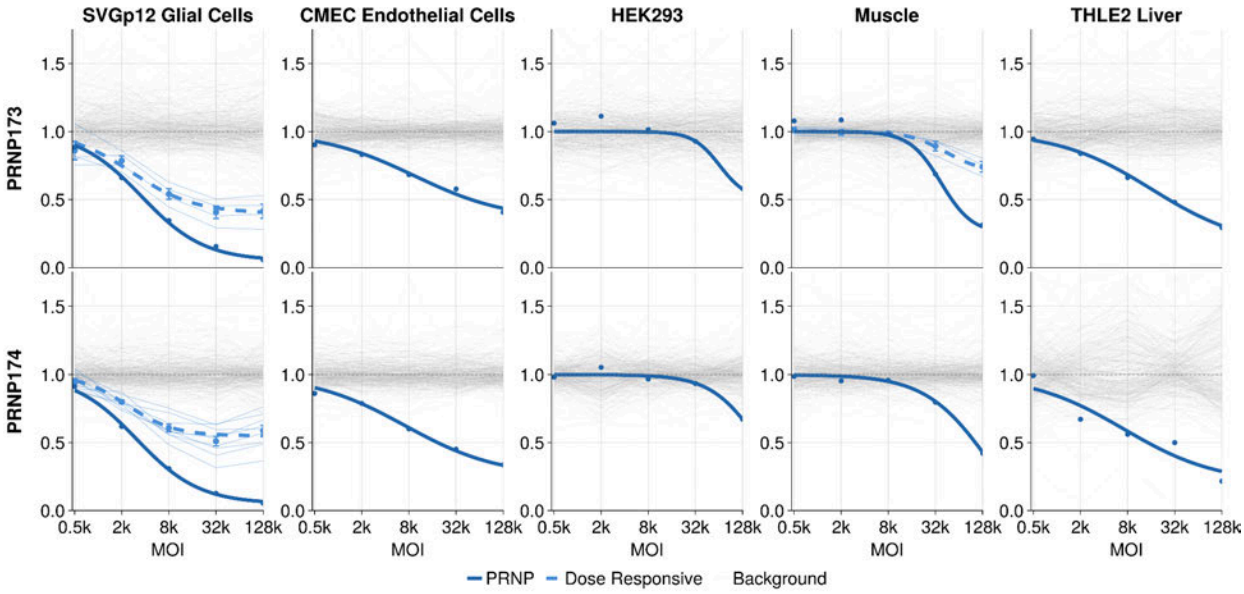


Figure 5. Cell line by transgene gene expression dose-response. For each cell line (columns) and active CHARM transgene (rows), per-gene self-baselined log-fold-change traces are shown (trajectory plot is active-arm only; the mixture-model nomination underlying the dashed line is stuffer-referenced). Solid blue: *PRNP*. Light blue dashed: mean of dose-responsive off-targets (three-component mixture, PIPCHARM > 0.99, $\hat{\beta}_g < 0$). Gray: representative background genes.

	SVGp12				CMEC				HEK293				Muscle				THLE2				
Gene	base	final	%	padj	base	final	%	padj	base	final	%	padj	base	final	%	padj	base	final	%	padj	**Consistent?**
PRNP	634	36	-94%	1.4×10^{-118}	975	329	-66%	1.000	501	291	-42%	3.0×10^{-9}	690	216	-69%	0.003	1143	247	-78%	4.7×10^{-21}	Y
CXCL6	423	119	-72%	5.3×10^{-16}	314	268	-15%	1.000	–	–	–	–	–	–	–	–	15995	12072	-25%	0.147	–
CXCL1	539	211	-61%	4.3×10^{-32}	856	711	-17%	1.000	–	–	–	–	11	13	+16%	1.000	12533	9907	-21%	0.141	–
TFPI2	274	125	-54%	4.2×10^{-11}	325	293	-10%	1.000	198	212	+7%	1.000	162	140	-13%	1.000	1542	1038	-33%	0.147	–
IL1B	93	49	-47%	9.2×10^{-10}	48	41	-15%	1.000	–	–	–	–	–	–	–	–	402	309	-23%	1.000	–
RARRES1	103	64	-38%	0.001	151	173	+15%	1.000	–	–	–	–	–	–	–	–	172	192	+12%	0.816	–
CXCL2	56	29	-47%	2.9×10^{-5}	155	133	-14%	1.000	–	–	–	–	–	–	–	–	1180	814	-31%	0.114	–
DHRS3	501	301	-40%	1.4×10^{-4}	219	242	+10%	1.000	232	270	+16%	1.000	266	239	-10%	1.000	296	351	+19%	0.143	–
LXN	61	43	-29%	0.999	125	135	+9%	1.000	24	13	-47%	1.000	91	92	+1%	1.000	85	69	-19%	0.778	–
DNAJC15	447	488	+9%	0.880	1123	980	-13%	1.000	53	45	-15%	1.000	88	59	-33%	0.011	1015	940	-7%	0.566	–
QPCT	245	187	-24%	0.486	220	226	+3%	1.000	581	596	+3%	1.000	337	318	-6%	1.000	728	619	-15%	0.378	–
LINC00958	–	–	–	–	–	–	–	–	28	21	-24%	1.000	681	512	-25%	0.040	–	–	–	–	–
PABIR2	270	290	+8%	0.732	298	295	-1%	1.000	488	534	+9%	1.000	798	642	-20%	0.160	491	342	-30%	0.130	–
THBS2	1334	655	-51%	0.046	–	–	–	–	81	75	-8%	1.000	121	106	-12%	1.000	60	44	-27%	1.000	–
PKIB	102	183	+79%	0.514	18	26	+43%	1.000	419	524	+25%	1.000	108	110	+1%	1.000	71	65	-7%	0.756	–
CPA4	1447	2220	+53%	0.574	47	51	+10%	1.000	23	34	+47%	1.000	29	28	-5%	1.000	164	185	+13%	1.000	–
CD200	44	25	-44%	0.842	102	100	-1%	1.000	–	–	–	–	–	–	–	–	121	90	-25%	0.243	–
AR	40	53	+34%	0.428	233	260	+12%	1.000	140	124	-11%	1.000	24	30	+25%	1.000	146	148	+1%	1.000	–
GATA6	314	202	-35%	0.983	15	21	+37%	1.000	454	477	+5%	1.000	374	401	+7%	1.000	305	268	-12%	0.361	–
LETM2	66	102	+54%	0.242	73	82	+12%	1.000	74	59	-20%	1.000	46	55	+21%	1.000	107	95	-11%	0.491	–
SERINC2	101	160	+58%	0.885	57	72	+25%	1.000	312	327	+5%	1.000	166	160	-3%	1.000	397	630	+58%	0.219	–
ENSG00000290032	65	93	+43%	0.939	132	126	-4%	1.000	1220	1687	+38%	1.000	570	563	-1%	1.000	639	744	+17%	0.303	–
TIMP3	154	232	+51%	0.963	1838	1502	-18%	1.000	1058	1487	+40%	1.000	1110	1051	-5%	1.000	–	–	–	–	–
EDN1	374	270	-28%	0.830	181	203	+13%	1.000	13	20	+49%	1.000	20	17	-14%	1.000	328	236	-28%	0.140	–
OLFML3	61	75	+23%	0.539	–	–	–	–	–	–	–	–	10	9	-14%	1.000	11	17	+60%	0.221	–
NNAT	181	122	-32%	0.380	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
TNNT1	571	400	-30%	0.322	33	32	-2%	1.000	260	271	+4%	1.000	304	325	+7%	1.000	82	122	+49%	0.192	–
FZD1	34	50	+44%	0.980	21	26	+23%	1.000	107	98	-9%	1.000	106	114	+7%	1.000	106	103	-3%	1.000	–
CA9	1891	1184	-37%	0.902	857	910	+6%	1.000	26	34	+30%	1.000	–	–	–	–	144	182	+26%	0.413	–
KCNMA1	309	418	+35%	0.925	207	226	+9%	1.000	44	54	+22%	1.000	12	17	+33%	1.000	22	15	-31%	0.881	–

Table 6. Cross-tissue dose-responsive off-target genes defined by RNA-seq. For every gene nominated by the three-component mixture model at posterior inclusion probability (PIP) > 0.99 in any cell line, expression is tabulated across the five tested cell lines (SVGp12, hCMEC/D3, HEK293, Muscle, THLE-2). Under each cell line, four columns are given: **base**, the normalized mean baseline expression (CPM, library-size–normalized counts per million) at MOI = 0 (no-vector dose), averaged across replicates; **final**, the normalized mean expression (CPM) at the top dose, MOI = 128k; **%**, the percent change from baseline to high dose, 100 × (final − base)/base (negative = knockdown, positive = induction); and **padj**, the Benjamini–Hochberg–adjusted *p*-value from the DESeq2 Wald test for the active-versus-reference construct contrast (PRNP173 or PRNP174 vs PRNP183) at MOI = 128k. Dashes (–) mark genes not expressed above detection in that tissue. **Consistent?** indicates whether every tissue with measurable baseline and padj < 0.05 shows the same direction of change (Y), versus discordant or single-tissue effects (–). *PRNP*, the on-target gene, is shown in the first row as a positive control.

***In silico* CHARM zinc finger binding site analysis:** To complement the RNA-seq based approach for candidate off-target nomination, we used *in silico* approaches to identify genes with cognate or near-cognate binding sites for the CHARM zinc finger DNA-binding domain. Bowtie and BLASTN were used as parallel approaches to identify exact and near-matches to the 19 bp consensus binding site for the CHARM zinc finger, and we subsequently identified the nearest gene to each predicted binding site. The set of nearest genes identified by Bowtie is presented in Table 7, and the nearest genes identified by BLASTN is presented in Table 8. The only gene found in common between the two *in silico* approaches was *PRNP*. *PRNP* was also the only gene which had no mismatches in the predicted CHARM zinc finger binding site. Additionally, *PRNP* was the only gene for which a binding site match was identified and which was nominated by our RNA-seq analyses. Overall, these analyses suggest that *PRNP* is the only gene where predicted gene-proximal CHARM binding has a functional consequence on gene expression in the cell lines evaluated.

Coordinates (hg38)	Strand	True Mismatch	Reported Mismatch	Nearest Gene	Distance (bp)	Location
chr20:4,686,490–4,686,508	-	0	1	*PRNP*	0	in gene
chr13:32,422,782–32,422,800	+	1	2	*N4BP2L1*	0	in gene
chr1:7,652,758–7,652,776	-	2	3	*CAMTA1*	0	in gene
chr1:14,996,679–14,996,697	-	2	3	*KAZN*	0	in gene
chr1:111,938,856–111,938,874	+	2	3	*KCND3*	0	in gene
chr10:102,812,447–102,812,465	-	2	3	*WBP1L*	0	in gene
chr11:75,137,355–75,137,373	+	2	3	*LOC107984358*	0	in gene
chr12:20,396,401–20,396,419	+	2	3	*PDE3A*	0	in gene
chr13:112,072,604–112,072,622	-	2	3	*SOX1-OT*	0	in gene
chr16:87,393,008–87,393,026	+	2	3	*MAP1LC3B*	0	in gene
chr2:2,991,206–2,991,224	-	2	3	*LINC01250*	0	in gene
chr2:112,914,455–112,914,473	-	2	3	*IL37*	0	in gene
chr22:25,452,467–25,452,485	-	2	3	*CRYBB2P1*	0	in gene
chr3:188,027,024–188,027,042	-	2	3	*LOC107986166*	0	in gene
chr4:2,985,020–2,985,038	-	2	3	*GRK4*	0	in gene
chr4:17,637,364–17,637,382	+	2	3	*FAM184B*	0	in gene
chr4:147,481,451–147,481,469	+	2	3	*EDNRA*	0	in gene
chr8:47,574,739–47,574,757	+	2	3	*SPIDR*	0	in gene
chr9:117,405,167–117,405,185	+	2	3	*ASTN2*	0	in gene
chr9:132,411,483–132,411,501	+	2	3	*CFAP77*	0	in gene
chr1:34,791,204–34,791,222	+	2	3	*GJA4*	1,777	intergenic

Table 7. Bowtie-predicted genomic off-target candidates for the CHARM zinc finger binding site. Ungapped alignments of the 19-bp zinc finger target sequence to GRCh38 (hg38) were enumerated using Bowtie 1.3.1 in *-v 3* mode to report all alignments with ≤ 3 mismatches. The 21 hits identified within ±10 kb of a GENCODE gene body are presented, with coordinates indicated as chromosome:start–end. Table columns include: **Coordinates (hg38)**, the genomic span of the alignment; **Strand**, the aligned orientation; **True Mismatch**, the total number of mismatched bases; **Reported Mismatch**, the mismatch count reported by Bowtie (consistent with True Mismatch for one-to-one comparison with BLASTN results); **Nearest Gene**, the closest GENCODE annotation; **Distance (bp)**, the distance from the hit to the gene body (0 = intragenic); and **Location**, the specific genic context of the predicted binding site.

Coordinates (hg38)	Strand	True Mismatch	Reported Mismatch	Nearest Gene	Distance (bp)	Location
chr20:4,686,490–4,686,508	-	1	1	*PRNP*	0	in gene
chr4:70,582,908–70,582,923	-	4	1	*AMBN*	9,333	intergenic
chr1:18,637,568–18,637,582	-	5	1	*PAX7*	0	in gene
chr10:10,665,487–10,665,501	-	5	1	*CELF2*	0	in gene
chr10:128,013,288–128,013,302	+	5	1	*PTPRE*	0	in gene
chr11:2,010,025–2,010,039	+	5	1	*MRPL23*	0	in gene
chr12:30,679,761–30,679,775	-	5	1	*IPO8*	0	in gene
chr14:71,075,317–71,075,331	-	5	1	*PCNX1*	0	in gene
chr4:112,529,739–112,529,753	+	5	1	*NEUROG2-AS1*	0	in gene
chr5:79,457,723–79,457,737	+	5	1	*HOMER1*	0	in gene
chr5:134,759,001–134,759,015	+	5	1	*DDX46*	0	in gene
chr8:23,778,285–23,778,299	+	5	1	*LOC107986930*	0	in gene
chr9:135,585,880–135,585,894	-	5	1	*LINC01502*	0	in gene
chr13:97,475,239–97,475,253	-	5	1	*RAP2A*	6,111	intergenic
chr1:42,747,494–42,747,505	-	7	0	*P3H1*	0	in gene
chr11_KI270832v1_alt:82,506–82,517	-	7	0	*LRRC56*	0	in gene
chr12:96,357,526–96,357,537	+	7	0	*CDK17*	0	in gene
chr15:27,872,524–27,872,535	-	7	0	*OCA2*	0	in gene
chr15_KI270905v1_alt:6,811–6,822	-	7	0	*OCA2*	0	in gene
chr6:45,214,877–45,214,888	-	7	0	*SUPT3H*	0	in gene
chr8:105,415,475–105,415,486	+	7	0	*ZFPM2*	0	in gene
chr1:112,466,335–112,466,346	-	7	0	*WNT2B*	195	intergenic

Table 8. BLASTN-predicted genomic off-target candidates for the CHARM zinc finger binding site. Local alignments of the 19-bp zinc finger target sequence to GRCh38 (hg38) were generated using BLASTN (BLAST+ 2.16.0) with *blastn-short* and a word size of 7. This approach identifies locally aligned regions, which may result in unaligned query ends for lower-stringency matches. The 22 hits identified within ±10 kb of a GENCODE gene body are presented, with coordinates indicated as chromosome:start–end. Table columns include: **Coordinates (hg38)**, the genomic span of the aligned hit; **Strand**, the aligned orientation; **True Mismatch**, the total number of differences across the full 19-bp query (sum of aligned mismatches and unaligned soft-clipped bases) to prevent underestimation of sequence divergence; **Reported Mismatch**, the mismatch count reported by BLASTN within the locally aligned region; **Nearest Gene**, the closest GENCODE annotation; **Distance (bp)**, the distance from the hit to the gene body (0 = intragenic); and **Location**, the specific genic context of the predicted binding site.

7 CONCLUSIONS

We executed *in silico* analysis of potential off-targets and *in vitro* studies to assess potential off-target impacts of PRNP-CHARM-001 activity on gene expression. Off-target analyses consisted of two orthogonal approaches: 1) *in silico* identification of genes proximal to predicted CHARM zinc finger binding sites; 2) RNA-seq performed on a panel of cell lines transduced with increasing amounts of PRNP-CHARM-001 to identify genes showing dose-responsive changes in gene expression similar to *PRNP*.

Using fluorescence imaging, RT-qPCR and cell viability measurements, we confirmed that SVG p12, hCMEC/D3, THLE-2, and skeletal muscle cells were permissive to transduction with hTfR1-binding capsids and responsive to CHARM-based *PRNP* silencing. These cell lines, along with HEK293 cells, were advanced for RNA-seq analyses. We performed RNA-seq on each cell line after transduction with a range of MOIs, identifying dose-responsive candidate off-target genes in each cell line. Importantly, *PRNP* was the only gene showing consistent dose-responsive downregulation across the cell lines tested. *In silico* analyses of predicted PRNP-CHARM-001 binding sites indicated that *PRNP* is the only gene with a gene body-proximal perfect match for the CHARM zinc finger, further supporting the specificity of PRNP-CHARM-001 for *PRNP* silencing. We propose based on this risk assessment that the off-target activity profile of PRNP-CHARM-001 is reasonable to support our proposed clinical plan.

8 REFERENCES

Stephen F. Altschul, Warren Gish, Webb Miller, Eugene W. Myers, David J. Lipman. Basic local alignment search tool. *Journal of molecular biology* vol. 215,3, 1990: 403-10. doi:10.1016/S0022-2836(05)80360-2

Jeff Bezanson, Alan Edelman, Stefan Karpinski, and Viral B. Shah. Julia: A Fresh Approach to Numerical Computing. SIAM Review, 59(1):65-98, 2017. doi: 10.1137/141000671.

Milan Bouchet-Valat, and Bogumił Kamiński. DataFrames.jl: Flexible and Fast Tabular Data in Julia. *Journal of Statistical Software*, *107*(4), 1–32, 2023. doi: 10.18637/jss.v107.i04

Christiam Camacho, George Coulouris, Vahram Avagyan, Ning Ma, Jason Papadopoulos, Kevin Bealer, and Thomas L. Madden. BLAST+: architecture and applications. BMC Bioinformatics, 10:421, 2009. doi: 10.1186/1471-2105-10-421.

Shifu Chen, Yanqing Zhou, Yaru Chen, and Jia Gu. fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics, 34(17):i884–i890, 2018. doi: 10.1093/bioinformatics/bty560.

Petr Danecek, James K. Bonfield, Jennifer Liddle, John Marshall, Valeriu Ohan, Martin O. Pollard, Andrew Whitwham, Thomas Keane, Shane A. McCarthy, Robert M. Davies, and Heng Li. Twelve years of SAMtools and BCFtools. GigaScience, 10(2):giab008, 2021. doi: 10.1093/gigascience/giab008.

Simon Danisch and Julius Krumbiegel. Makie.jl: Flexible high-performance data visualization for Julia. Journal of Open Source Software, 6(65), 3349, 2021. doi: 10.21105/joss.03349

Alexander Dobin, Carrie A. Davis, Felix Schlesinger, Jorg Drenkow, Chris Zaleski, Sonali Jha, Philippe Batut, Mark Chaisson, and Thomas R. Gingeras. STAR: ultrafast universal RNA-seq aligner. Bioinformatics, 29 (1):15–21, 2013. doi: 10.1093/bioinformatics/bts635.

Philip Ewels, Måns Magnusson, Sverker Lundin, and Max Kaller. MultiQC: summarize analysis results for multiple tools and samples in a single report. Bioinformatics, 32(19):3047–3048, 2016. doi: 10.1093/ bioinformatics/btw354.

Ben Langmead and Steven L. Salzberg. Fast gapped-read alignment with Bowtie 2. Nature Methods, 9(4): 357–359, 2012. doi: 10.1038/nmeth.1923.

Ben Langmead, Cole Trapnell, Mihai Pop, and Steven L. Salzberg. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biology, 10(3):R25, 2009. doi: 10.1186/gb-2009- 10-3-r25.

Yang Liao, Gordon K. Smyth, and Wei Shi. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics, 30(7):923–930, 2014. doi: 10.1093/bioinformatics/ btt656.

Daniel Liu. Algorithms for efficiently collapsing reads with unique molecular identifiers. PeerJ, 8:e8275, 2020. doi: 10.7717/peerj.8275.

Michael I. Love, Wolfgang Huber, and Simon Anders. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology, 15(12):550, 2014. doi: 10.1186/s13059-014-0550-8.

Felix Mölder, Kim Philipp Jablonski, Brice Letcher, Michael B. Hall, Christopher H. Tomkins-Tinch, Vanessa Sochat, et al. Sustainable data analysis with Snakemake. F1000Research, 10:33, 2021. doi: 10.12688/f1000research.29032.2.

Edwin N Neumann, Tessa M Bertozzi, Elaine Wu, Fiona Serack, John W Harvey, Pamela P Brauer, Catherine P Pirtle, Alissa Coffey, Michael Howard, Nikita Kamath, Kenney Lenz, Kenia Guzman, Michael H Raymond, Ahmad S Khalil, Benjamin E Deverman, Eric Vallabh Minikel, Sonia M Vallabh, Jonathan S Weissman. Brainwide silencing of prion protein by AAV-mediated delivery of an engineered compact epigenetic editor. Science vol. 384,6703, 2024: ado7082. doi:10.1126/science.ado7082

Aaron R Quinlan and Ira M Hall. BEDTools: a flexible suite of utilities for comparing genomic features. Bioinformatics. 26, 6, pp. 841–842, 2010.

APPENDIX XIII: Draft Study Protocol: PRNP-CHARM-001 Delivery-Device Compatibility and In-Use Stability Study

PRNP-CHARM-001 | DEVICE COMPATIBILITY AND IN-USE STABILITY

Study Protocol Outline — To be submitted to CRO for Protocol Development and Quoting

Sponsor: Broad Institute | CRO: To be determined | Testing Laboratory: Viralgen

1. STUDY OVERVIEW AND PURPOSE

This protocol outline describes the experimental design and procedural requirements for a fit-for-purpose combined Device Compatibility and In-Use Stability (DCIUS) study to support the PRNP-CHARM-001 IND submission. In accordance with FDA feedback, the study is designed to characterize the stability of representative PRNP-CHARM-001 drug product (DP) under post-thaw hold conditions and to assess the impact of the proposed clinical delivery device configuration on DP quality attributes including strength by ITR ddPCR and potency by TCID50. The study is also designed to assess delivery device safety by evaluating the change in endotoxin levels and subvisible particle count after simulating dosing using formulation buffer as a surrogate matrix with the defined delivery device. Ideally, this study is to be conducted under GLP in accordance with 21 CFR Part 58.

The study is composed of three independent modules. This protocol is contemplated as taking place over the course of three separate days with sampling occurring throughout the day at prespecified timepoints to generate performance data across a six (6) hour range:

- Module A — Subvisible Particle and Endotoxin Assessment: Evaluates particle and endotoxin contribution from the delivery device components using formulation buffer as a surrogate matrix. A single six (6) hour time point will also be evaluated.
- Module B — In-Use Stability: Characterizes DP quality attributes over a 6-hour post-thaw window while material remains in the vial under ambient conditions.
- Module C — Device Compatibility: Evaluates DP quality attributes after loading into the clinical delivery device configuration, held for defined time intervals, and expelled through the full device train.

Test materials for this study were selected to be representative of the clinical use scenario while preserving clinical batch material ahead of IND submission. Module A will utilize formulation buffer prepared by Viralgen. Specifically, this formulation buffer will be the same manufactured lot used to prepare and formulate T-POLARIS-123 (PRNP-CHARM-001/TfR1CapX to be utilized in Sponsor's definitive toxicology and biodistribution study). Use of the identical buffer lot ensures compositional equivalence to the DP matrix and provides a well-characterized baseline for device-contributed particle and endotoxin assessment. Modules B and C will utilize residual, unopened DP vials retained from the previously executed toxicology study. This material is appropriate for use in this evaluation for several reasons: (1) it has undergone comprehensive release and characterization testing as part of the toxicology manufacturing program, providing a well-defined quality baseline; (2) it is formulated at the same target concentration as the intended clinical batch (2.5×10^{13} vg/mL), ensuring the stability and compatibility data generated are directly relevant to the clinical dosing scenario; and (3) as intact, unopened vials stored under validated conditions, the material reflects the same container closure system and storage history as vials that would be used in a clinical setting. Use of toxicology batch material at this stage of development is consistent with standard practice for IND-enabling studies and avoids premature consumption of clinical batch inventory.

Module A will assess 2 timepoints (0 and 6 hours) and Modules B and C assess 5 timepoints (0, 1, 2, 4, and 6 hours). All analytical testing (subvisible particle count, endotoxin, ddPCR, and TCID50) is performed by Viralgen. CRO is responsible for all study execution, sample collection, aliquoting, cold storage, and sample shipment to Viralgen.

2. ROLES AND RESPONSIBILITIES

Responsibility	CRO	Viralgen
Protocol Development	Draft full executable protocol from this outline; obtain Sponsor approval prior to execution	Provide method specifications and shipping requirements to CRO as needed
Drug Product Handling	Receive, store, and thaw DP vials per specified conditions	N/A
Formulation Buffer	Receive, store, and thaw buffer material for Module A	N/A
Procurement of study consumables	Order all required consumables for the study execution	N/A
Module Execution	Execute all three modules per approved protocol	N/A
Sample Collection	Collect, aliquot, label, and freeze all samples per plan	N/A
Sample Storage	Store samples at ≤ -60°C until shipment	Confirm receipt and storage upon arrival
Sample Shipment	Ship all samples frozen (dry ice) to Viralgen per agreed logistics	Provide shipping address, chain-of-custody requirements
Analytical Testing	N/A	Perform ddPCR (genome titer), TCID50 (potency), endotoxin, and subvisible particle count per qualified methods
Data Reporting	Provide execution records and deviation documentation	Issue analytical test report with raw data and any deviations
Sponsor Communication	Communicate deviations or material concerns to Sponsor promptly	Report out-of-specification results to Sponsor promptly

It is expected that CRO's execution of the in-use stability and clinical dosing simulations that are a part of Modules A, B, and C will cumulatively require 3 business days of FTE time to execute.

3. TEST ARTICLE AND FORMULATION BUFFER

3.1 Drug Product

Parameter	Specification
Product	PRNP-CHARM-001/TfR1CapX
Source / Batch	T-POLARIS-123
Nominal Concentration	To be determined – projecting 2.5×10^{13} vg/mL
Formulation	20 mM Tris, 1 mM $MgCl_2$, 200 mM NaCl, 0.005% Poloxamer 188, pH 8
Container Closure	Daikyo Crystal Zenith (CZ) COP 2 mL vials with Westar stoppers (West Pharma)
Fill Volume	0.5 mL per vial
Storage Condition	≤ -60°C (frozen)
Modules Used In	Module B (In-Use Stability): 3 vials; Module C (Device Compatibility): 8 vials
Total DP Vials Available	Vials available at Viralgen TBD; 11 Vials required
DP Location / Shipment	Sponsor will coordinate shipment of required DP and formulation buffer from Viralgen.

3.2 Formulation Buffer (Module A Only)

Parameter	Specification
Composition	20 mM Tris, 1 mM $MgCl_2$, 200 mM NaCl, 0.005% Poloxamer 188, pH 8
Containers and fill volumes	To be confirmed
Volume Required	~2.5 mL per run × 3 runs = ~7.5 mL minimum
Storage	Frozen
Source	Buffer will be supplied by Sponsor

NOTE: Acceptance criteria for all analytical outputs are being finalized by Sponsor in parallel with Viralgen input. Criteria will be incorporated into the full executable protocol prior to finalization. CRO should include placeholder acceptance criteria fields in the protocol draft; Sponsor will populate these prior to study execution.

4. REQUIRED MATERIALS

The following materials are required for study execution. Item numbers are provided as the specified source; substitutions are not permitted without Sponsor approval. CRO is responsible for procuring all consumables unless otherwise noted.
NOTE:The current format of this draft study design may require modification to ensure that materials are supported by and carried by the Sponsor's intended clinical site. This will be finalized prior to execution of the formal study.

4.1 Devices and Consumables

Item #	Description	Manufacturer	Catalog #	Used In Module	Total Quantity by Module
1	21G × 1-1/2 in. BD PrecisionGlide™ Needle (for vial withdrawal)	BD	305167	A, B, C	A: 3 B: 5 C: 1
2	General Purpose Syringe BD™ Luer-Lok™ 3 mL Luer Lock Tip Without Safety	BD	309657	A, B	A: 3 B: 5
3	General Purpose Syringe BD™ Luer-Lok™ 5 mL Luer Lock Tip Without Safety	BD	309646	C	C: 1
4	IV Extension Set ICU Small Bore 60 Inch Tubing	ICU Medical	B2048	A, C	A:2 C:5
5	IV Extension Set with Rotating Luer Lock Connector, Small Bore, 6.5in	ICU Medical	B1754	A, C	A:2 C:5
6	BD Insyte Autoguard Winged Shielded IV Catheter, with BD Vialon Catheter Material and BD Instaflash Needle Technology, 22G x 1.00"	BD	381523	A, C	A:2 C:5

4.2 Additional Required Materials and Equipment

Item	Specification / Notes	Responsible Party
Sample collection tubes (Module B and C)	Standard microcentrifuge or cryogenic tubes suitable for ≤ -60°C storage; CRO to recommend specific tube type	CRO (specific tubes to be specified by Viralgen)
Sample collection tubes (Module A)	One tube per run: 300 µL (endotoxin) and 2 mL (subvisible particles); particle-free/low-bind required	CRO (specific tubes to be specified by Viralgen)
Micropipette and tips	Calibrated pipette for 55–60 µL aliquoting; low-bind	CRO
Biosafety cabinet (BSC)	Class II BSC for open manipulation of AAV material	CRO facility
Freezer, ≤ -60°C	For sample storage post-collection	CRO facility
Thermometer / temperature probe	For ambient temperature monitoring and recording during thaw	CRO facility
Timer	For accurate hold time tracking from thaw-complete and syringe-draw timepoints	CRO
Labels / cryo-labels	Legible under cryogenic storage conditions; include sample ID, timepoint, date	CRO
Dry ice and cryo-shipper	For sample shipment to Viralgen at ≤ -60°C	CRO
Chain-of-custody documentation	Temperature monitor (e.g., single-use data logger) included in each shipment	CRO

5. SAMPLE PLAN

5.1 Module A — Subvisible Particle and Endotoxin Sample Plan

Formulation buffer is run through three conditions. Each condition uses a new device set and yields one sample set: 300 µL for endotoxin and 2 mL for subvisible particle analysis.

Condition	Description	Purpose	Samples
1 — Syringe, Immediate	Draw 2.5 mL buffer into syringe; immediately expel into collection tube — no device connected	Syringe baseline; no device contact	1 x 300uL; Endotoxin 1 x 2mL; Subvisible particles
2 — Syringe + Device	Draw 2.5 mL buffer; push through full device train; collect in tube	Device contribution at t=0	1 x 300uL; Endotoxin 1 x 2mL; Subvisible particles
3 — Syringe + 6hr + Device	Draw 2.5 mL buffer; hold in syringe at ambient for 6 hours; then push through fresh device train	Worst-case scenario: 6-hr contact + device	1 x 300uL; Endotoxin 1 x 2mL; Subvisible particles

NOTE: A new delivery line and syringe is used for each individual replicate run. Total buffer volume required: 3 runs × 2.5 mL = 7.5 mL; Samples are frozen post-collection and shipped to the testing laboratory with Module B and C samples.

5.2 Module B — In-Use Stability Sample Plan

Three vials are used across the 5 timepoints as described below. Aliquot volumes are 55 µL per tube.

Timepoint	Time Reference	Vials Used	Extraction Volume	Aliquots (55 µL)	Assays
T0 (0 hr)	At thaw complete	1 dedicated vial	~440 µL total	~8 aliquots	ddPCR TCID50 Retains
T1 (1 hr)	1 hr post-thaw	Shared vial (with T2)	~220 µL	~4 aliquots	ddPCR TCID50 Retains
T2 (2 hr)	2 hr post-thaw	Shared vial (with T1)	~220 µL	~4 aliquots	ddPCR TCID50 Retains
T4 (4 hr)	4 hr post-thaw	Shared vial (with T6)	~220 µL	~4 aliquots	ddPCR TCID50 Retains
T6 (6 hr)	6 hr post-thaw	Shared vial (with T4)	~220 µL	~4 aliquots	ddPCR TCID50 Retains

NOTE: Vial pairing rationale: fill volume is 0.5 mL per vial. T0 requires 8 aliquots × 55 µL = 440 µL, consuming a full vial. T1+T2 paired in one vial (2 × 220 µL = 440 µL); T4+T6 paired in a second vial. Total: 3 vials for Module B. Withdrawal is performed with syringe and needle (21G) piercing the stopper at each timepoint.

5.3 Module C — Device Compatibility Sample Plan

Eight vials are pooled into a single syringe. Sampling occurs at each timepoint by ejecting ~0.5 mL through the full device train (or directly for T0 loaded sample). Aliquot volume is 55 µL per tube.

Timepoint	Sample Type	Time Reference	Volume Ejected	Aliquots (55 µL)	Assays
T0 Loaded	Pre-device (syringe only)	Immediately after pooling complete	0.5 mL direct eject	~8 aliquots (≤480 µL)	ddPCR TCID50 Retains
T0 Expelled	Post-device	Immediately after T0 loaded	Target of 500uL; ≥400 µL through device	~6–7 aliquots	ddPCR TCID50 Retains
T1 Expelled	Post-device	1 hr post syringe draw	Target of 500uL; ≥400 µL through device	~6–7 aliquots	ddPCR TCID50 Retains
T2 Expelled	Post-device	2 hr post syringe draw	Target of 500uL; ≥400 µL through device	~6–7 aliquots	ddPCR TCID50 Retains
T4 Expelled	Post-device	4 hr post syringe draw	Target of 500uL; ≥400 µL through device	~6–7 aliquots	ddPCR TCID50 Retains
T6 Expelled	Post-device	6 hr post syringe draw	Target of 500uL; ≥400 µL through device	~6–7 aliquots	ddPCR TCID50 Retains

NOTE: Device configuration for expelled samples: Syringe → 60-inch IV extension set (ICU Medical B2048) → 6.5-inch small bore extension (ICU Medical B1754) → 22G IV catheter (BD 381523). A fresh delivery line is assembled for each timepoint. Minimum ejection pulse volume is 400 µL. After ejecting DP into the line, a separate air-filled syringe is used to chase the remaining DP in the line through the catheter and into the collection tube.

6. MODULE PROCEDURES

The following procedures describe the intended workflow for each module. These serve as the basis for CRO to develop the full executable protocol. Steps are written at the level of detail needed to communicate intent; CRO should expand each step with SOPs, instrument references, and site-specific operational details.

6.1 Pre-Study Setup (All Modules)

6.1.1. Prior to study start, coordinate with Sponsor and Viralgen for cryo-shipment of DP vials from Viralgen to CRO. Sponsor will specify the number to transfer.

6.1.2. Upon receipt of DP vials at CRO: confirm shipment temperature monitor readings are within acceptable range, perform visual inspection of all vials, and record lot number, vial count, and condition. Notify Sponsor immediately of any concerns.

6.1.3. Confirm all consumables in Section 4 are on-hand and within acceptable expiry dates. Document catalog and lot numbers.

6.1.4. Calibrate or verify calibration of pipettes and thermometers prior to study execution.

6.1.5. Prepare sample labels in advance. Labels must include: sample ID, module, timepoint, condition (for Module A), date, and initials.

6.1.6. Confirm BSC operation and record BSC certification status in the study record.

6.2 Module A — Subvisible Particle and Endotoxin Assessment

Purpose: Assess particle burden and endotoxin contribution from delivery device components using formulation buffer as a surrogate. Three conditions are tested.

Module Preparation:

6.2.1. Gather all consumables, documentation, and labels required for the execution of Module A

6.2.2. Prepare BSC per standard procedure. All Module A operations are to take place inside of a BSC.

Thaw Procedure:

6.2.3. Thaw at least 8mL of formulation buffer per CRO's standard thaw procedure.

6.2.4. Allow buffer to equilibrate to ambient temperature. Record ambient temperature at time of use.

Condition A — Syringe Baseline:

6.2.5. In a BSC, draw 2.5 mL of formulation buffer into a new 3 mL syringe using a new 21G needle.

6.2.6. Remove needle. Immediately expel the full 2.5 mL into a clean collection tube.

6.2.7. Aliquot: transfer 300 µL into endotoxin tube; transfer 2 mL into sub-visible particle tube and record the time.

6.2.8. Label, immediately transfer to ≤ -60°C freezer, and record time placed in freezer.

Condition B — Syringe + Device:

6.2.9. Draw 2.5 mL of formulation buffer into a new 3 mL syringe using a new 21G needle.

6.2.10. In BSC, assemble a fresh delivery line: connect the 60-inch IV extension set (ICU Medical B2048), then the 6.5-inch small bore extension (ICU Medical B1754), then the 22G IV catheter (BD 381523).

6.2.11. Remove the 21G needle then connect the syringe (with buffer) to the assembled device via Luer connection.

6.2.12. Place and secure the catheter tip into a collection tube. Push the full 2.5 mL through the device slowly in one continuous manual press.

6.2.13. Disconnect the buffer syringe. Then using a new 3mL syringe, fill with approximately 2mL of air then connect to the delivery line and slowly depress the plunger to expel any remaining buffer in the line

6.2.14. Aliquot: transfer 300 µL into endotoxin tube; transfer 2 mL into sub-visible particle tube and record the time.

6.2.15. Label, immediately transfer to ≤ -60°C freezer, and record time placed in freezer.

Condition C — Syringe Hold 6hr + Device:

6.2.16. Draw 2.5 mL of formulation buffer into a new 3 mL syringe using a new 21G needle. Remove needle and cap syringe.

6.2.17. Record the time as the start of hold. Hold the loaded syringe at ambient temperature for 6 hours.

6.2.18. In BSC, assemble a fresh delivery line: connect the 60-inch IV extension set (ICU Medical B2048), then the 6.5-inch small bore extension (ICU Medical B1754), then the 22G IV catheter (BD 381523).

6.2.19. Remove the 21G needle then connect the syringe (with buffer) to the assembled device via Luer connection.

6.2.20. Place and secure the catheter tip into a collection tube. Push the full 2.5 mL through the device slowly in one continuous manual press.

6.2.21. Disconnect the buffer syringe. Then using a new 3mL syringe, fill with approximately 2mL of air then connect to the delivery line and slowly depress the plunger to expel any remaining buffer in the line

6.2.22. Aliquot: transfer 300 µL into endotoxin tube; transfer 2 mL into sub-visible particle tube and record the time.

6.2.23. Label, immediately transfer to ≤ -60°C freezer, and record time placed in freezer.

6.3 Module B — In-Use Stability

Purpose: Assess PRNP-CHARM-001/TfR1CapX quality attributes over a 6-hour post-thaw hold in the vial at ambient temperature using clinical container closure.

Module Preparation:

6.3.1. Gather all consumables, documentation, and labels required for the execution of Module B

6.3.2. Prepare BSC per standard procedure. All Module B operations are to take place inside of a BSC.

Thaw Procedure:

6.3.3. Remove 3 PRNP-CHARM-001/TfR1CapX vials from ≤ -60°C storage.

6.3.4. Thaw vials at ambient room temperature per CRO's standard thaw procedure.

6.3.5. Monitor thaw visually. Record the time at which each vial is fully thawed (thaw-complete time). This is T=0 for Module B.

6.3.6. Record ambient temperature at time of thaw.

6.3.7. Allow thawed vials to remain at ambient temperature for the duration of the study.

Sampling Procedure (per timepoint):
T0 (Vial 1 — dedicated):

6.3.8. At T=0 (thaw complete), gently invert vial 1 no less than 5 times before using a new 3 mL syringe fitted with a 21G needle to pierce the vial stopper.

6.3.9. Withdraw the total extractable volume (~440 µL target, based on 0.5 mL fill).

6.3.10. In BSC, expel material into a microcentrifuge/cryotube.

6.3.11. Using a calibrated micropipette, aliquot into a maximum number of 55 µL aliquots (~8 aliquots) into individual labeled tubes and record the aliquot time.

6.3.12. Immediately transfer to ≤ -60°C freezer, and record time placed in freezer.

6.3.13. Dispose of Vial 1 and any residual contents.

T1hr (Vial 2 — shared with T2):

6.3.14. At T=1hr post-thaw: using a new 3mL syringe and needle, pierce Vial 2 stopper and withdraw ~250 µL.

6.3.15. Expel into collection tube. Aliquot into a maximum number of 55 µL aliquots (~4) into labeled tubes and record the aliquot time. Any residual material less than 55uL should be aliquoted in a tube and labeled appropriately.

6.3.16. Immediately transfer to ≤ -60°C freezer, and record time placed in freezer.

T2hr (Vial 2 — shared):

6.3.17. At T=2hr: Using a new 3mL syringe and needle withdraw the remaining extractable volume from vial 2.

6.3.18. Expel into collection tube. Aliquot into a maximum number of 55 µL aliquots (~4) into labeled tubes and record the aliquot time. Any residual material less than 55uL should be aliquoted in a tube and labeled appropriately.

6.3.19. Immediately transfer to ≤ -60°C freezer, and record time placed in freezer.

6.3.20. Dispose of Vial 2 and any remaining contents.

T4hr (Vial 3 — shared with T6):

6.4.1. At T=4hr post-thaw: using a new 3mL syringe and needle, pierce Vial 3 stopper and withdraw ~250 µL.

6.4.2. Expel into collection tube. Aliquot into a maximum number of 55 µL aliquots (~4) into labeled tubes and record the aliquot time. Any residual material less than 55uL should be aliquoted in a tube and labeled appropriately.

6.4.3. Immediately transfer to ≤ -60°C freezer, and record time placed in freezer.

T6hr (Vial 3 — shared):

6.4.4. At T=6hr: Using a new 3mL syringe and needle withdrawal the remaining extractable volume from vial 3.

6.4.5. Expel into collection tube. Aliquot into a maximum number of 55 µL aliquots (~4) into labeled tubes and record the aliquot time. Any residual material less than 55uL should be aliquoted in a tube and labeled appropriately.

6.4.6. Immediately transfer to ≤ -60°C freezer, and record time placed in freezer.

6.4.7. Dispose of Vial 3 and any remaining contents.

NOTE: For each withdrawal, a new needle and syringe is used. Any needle coring, particulates, or unusual observations should be noted in the study record. Vials should not be agitated.

6.4 Module C — Device Compatibility

Purpose: Assess PRNP-CHARM-001/TfR1CapX quality attributes after residence in the clinical delivery syringe and expulsion through the full device train at defined time intervals.

Module Preparation:

6.4.1. Gather all consumables, documentation, and labels required for the execution of Module C

6.4.2. Prepare BSC per standard procedure. All Module C operations are to take place inside of a BSC.

Thaw and Pool Procedure:

6.4.3. Remove 8 DP vials from ≤ -60°C storage and place in a BSC for thawing.

6.4.4. Thaw all 8 vials at ambient temperature per CRO's standard thaw procedure. Record thaw-complete time for each vial.

6.4.5. Once all vials are thawed, begin pooling immediately using a single 5 mL Luer-Lok syringe with a 21G needle by sequentially withdrawing the extractable volume from each vial by piercing the stopper and drawing into the syringe. Continue until all 8 vials are emptied into the single syringe.

6.4.6. Record the time at which the final vial volume is drawn into the syringe. This is T=0 for Module C.

T0 — Loaded Sample (pre-device):

6.4.7. Immediately after pooling is complete (T=0), in the BSC, slowly eject 0.5 mL of DP directly from the syringe (no device connected, needle still attached) into a collection tube.

6.4.8. Aliquot into maximum number of 55 µL aliquots from the 0.5 mL volume (~8 aliquots) and record aliquot time.

6.4.9. Label and freeze at ≤ -60°C and record time placed in freezer

T0 — Expelled Sample (through device):

6.4.10. Assemble a fresh delivery line in the BSC: connect the 60-inch IV extension set (ICU Medical B2048), then the 6.5-inch small bore extension (ICU Medical B1754), then the 22G IV catheter (BD 381523).

6.4.11. Remove the needle on the syringe and connect to the delivery line

6.4.12. Place the catheter tip into a collection tube. Slowly, manually push ≥ 400 µL (target 0.5 mL) of DP through the line.

6.4.13. Disconnect the DP syringe and store on its side inside the BSC.

6.4.14. With the catheter still secured in the collection tube, connect a new syringe pre-filled with ~2mL of air to the charged catheter line.

6.4.15. Slowly push the air to chase the remaining DP in the line through the catheter and into the collection tube. Avoid introducing any bubbles.

6.4.16. Using a calibrated pipette and low-bind tips, aliquot the collected material into the maximum number of 55 µL aliquots.

6.4.17. Label and freeze at ≤ -60°C and record time placed in freezer.

6.4.18. Discard the used delivery line.

T1hr, T2hr, T4hr, T6hr — Expelled Samples:

6.4.19. Assemble a fresh delivery line in the BSC for the subsequent time point: connect the 60-inch IV extension set (ICU Medical B2048), then the 6.5-inch small bore extension (ICU Medical B1754), then the 22G IV catheter (BD 381523).

6.4.20. Connect the filled syringe to the delivery line and wait until the target expulsion time (T1hr, T2hr, T4hr, T6hr).

6.4.21. Place the catheter tip into a collection tube. Slowly, manually push ≥ 400 µL (target 0.5 mL) of DP through the line.

6.4.22. Disconnect the DP syringe and store on its side inside the BSC.

6.4.23. With the catheter still secured in the collection tube, connect a new syringe pre-filled with ~2mL of air to the charged catheter line.

6.4.24. Slowly push the air to chase the remaining DP in the line through the catheter and into the collection tube. Avoid introducing any bubbles.

6.4.25. Using a calibrated pipette and low-bind tips, aliquot the collected material into the maximum number of 55 µL aliquots.

6.4.26. Label and freeze at ≤ -60°C and record time placed in freezer.

6.4.27. Discard the used delivery line.

6.4.28. Repeat the expelled sample procedure as described in steps 6.4.19 – 6.4.27 at each timepoint (T2hr, T4hr, T6hr)

6.4.29. Aliquot any remaining DP in the syringe into sample tubes and record the time.

NOTE: The DP syringe remains loaded throughout the study. Only expelled material — not direct syringe ejects — is collected for T1 through T6. A new delivery line is assembled at each timepoint. DP syringe should be labeled and handled under BSC conditions where open manipulation occurs.

7. SAMPLE HANDLING, STORAGE, AND SHIPMENT

7.1 Post-Collection Handling

- All samples are aliquoted in a BSC and transferred to labeled cryogenic tubes immediately after collection.
- Samples are placed at ≤ -60°C immediately after aliquoting. Do not hold samples at ambient temperature after collection.
- Do not subject samples to freeze-thaw cycles prior to shipment. Samples should be shipped once all modules are complete unless otherwise directed by Sponsor.

7.2 Labeling Requirements

All sample tubes must be labeled with cryo-compatible labels. Labels must include at minimum:

Module A

- Study ID / Protocol ID
- Module A
- Condition
- Aliquot Volume
- Aliquot number (e.g., 1, 2)
- Collection date and initials of operator

Module B

- Study ID / Protocol ID
- Module B
- Timepoint (T0hr, T1hr, T2hr…)
- Aliquot Volume
- Aliquot number (e.g., 1, 2)
- Collection date and initials of operator

Module C

- Study ID / Protocol ID
- Module C
- Condition (Loaded sample or Expelled sample)
- Timepoint (T0hr, T1hr, T2hr…)
- Aliquot Volume
- Aliquot number (e.g., 1, 2)
- Collection date and initials of operator

7.3 Shipment to Viralgen

- All samples from all three modules will be shipped together to Viralgen.
- Samples must be shipped on dry ice in a validated cryo-shipper. Include a calibrated temperature monitor in the shipment.
- CRO to prepare a chain-of-custody manifest listing all sample IDs, aliquot counts, timepoints, and modules.
- Viralgen to confirm receipt of samples, temperature monitor readings, and sample integrity within 24 hours of receipt.

> **TBD:** Viralgen shipping address, courier account, and chain-of-custody form format to be confirmed between CRO and Viralgen prior to study execution. Sponsor to facilitate initial introduction between CRO and Viralgen logistics contacts.

8. DOCUMENTATION AND DEVIATIONS

CRO is responsible for maintaining complete records of all study activities. At minimum, the following must be documented:

- DP and buffer receipt records (lot, vial count, visual inspection)
- Thaw logs: vial IDs, thaw-start time, thaw-complete time, ambient temperature
- Module execution logs: actual timepoints, operator initials, ambient temperature at each action
- Sample collection log: sample IDs, tube types, aliquot volumes, freeze time
- Consumable lot numbers for all devices used
- Equipment calibration/verification records (pipettes, thermometers)
- Deviation log: any departure from the protocol with description, assessment, and disposition

Any deviation from the approved protocol must be documented and communicated to Sponsor prior to or at the time of occurrence where possible. Deviations affecting sample integrity or analytical validity must be evaluated for their impact on data interpretation.

APPENDIX XIV: PRNP-CHARM-001 In Vitro Off-target Analysis RNA-seq Sample Information

sample_id	batch	cell_line	construct	moi	replicate	input_reads	mapped_reads	unique_mapped_	multi_mapped_re	unmapped_reads	mapping_rate_pc	dedup_mapped_	dedup_unique_ri	dedup_multi_rea	dedup_rate_pct	assigned_reads	assigned_pct	outlier_flag	outlier_reason	failure_flag	failure_reasons	included_in_analysis
068R29_1_samp	068R29	CMEC	PRNP173	128000	R1	20361737	20059017	18379072	1679945	278517	98.51	12583335	11493941	1089394	61.8	9210495	61.95	FALSE		FALSE		TRUE
068R29_2_samp	068R29	CMEC	PRNP183	8000	R1	20516638	20227779	18471376	1756403	268410	98.59	12828298	11671572	1156726	62.53	9842516	64.22	FALSE		FALSE		TRUE
068R29_3_samp	068R29	CMEC	PRNP173	500	R1	20731721	20416304	18380092	2036212	292979	98.48	13345718	11969986	1375732	64.37	9888938	56.95	FALSE		FALSE		TRUE
068R29_4_samp	068R29	CMEC	PRNP183	128000	R2	20769061	20472366	18734709	1737657	274871	98.57	13061866	11947484	1114382	62.89	9929586	65	FALSE		FALSE		TRUE
068R29_5_samp	068R29	CMEC	PRNP174	0	R4	20107563	19789852	18025627	1764225	292235	98.42	13104880	11894105	1210775	65.17	9527365	58.88	FALSE		FALSE		TRUE
068R29_6_samp	068R29	CMEC	PRNP173	8000	R3	19502350	19230004	17462220	1767784	252647	98.6	12180094	11007981	1172113	62.45	9393633	63.03	FALSE		FALSE		TRUE
068R29_7_samp	068R29	CMEC	PRNP183	2000	R2	19363043	19050703	17510740	1539963	287676	98.39	12689939	11636924	1053015	65.54	9283081	63.34	FALSE		FALSE		TRUE
068R29_8_samp	068R29	CMEC	PRNP173	500	R3	20865197	20497395	18758642	1738753	338264	98.24	13146322	11977445	1168877	63.01	9171299	57.45	FALSE		FALSE		TRUE
068R29_9_samp	068R29	CMEC	PRNP183	500	R4	20443471	20137794	18481868	1655926	280773	98.5	12596845	11527990	1068855	61.62	9262811	63.23	FALSE		FALSE		TRUE
068R29_10_sam	068R29	CMEC	PRNP173	2000	R4	19085232	18822726	17181483	1641243	243727	98.62	12847122	11698147	1148975	67.31	9996283	65.3	FALSE		FALSE		TRUE
068R29_11_sam	068R29	CMEC	PRNP183	0	R4	19521218	19219961	17563323	1656638	276729	98.46	12348731	11241583	1107148	63.26	8926706	59.93	FALSE		FALSE		TRUE
068R29_12_sam	068R29	CMEC	PRNP174	128000	R4	19007301	18724799	17232269	1492530	259381	98.51	12603647	11572071	1031576	66.31	9238043	63.33	FALSE		FALSE		TRUE
068R29_13_sam	068R29	CMEC	PRNP183	0	R4	19204107	18952519	17402179	1550340	230965	98.69	12504866	11454840	1050026	65.12	9357603	65.02	FALSE		FALSE		TRUE
068R29_14_sam	068R29	CMEC	PRNP183	2000	R4	20561584	20281293	18495148	1786145	258032	98.64	13464290	12245201	1219089	65.48	10201463	63.32	FALSE		FALSE		TRUE
068R29_15_sam	068R29	CMEC	PRNP173	2000	R3	20355588	20039170	18363632	1675538	291009	98.45	12017217	10977262	1039955	59.04	8801103	62.96	FALSE		FALSE		TRUE
068R29_16_sam	068R29	CMEC	PRNP174	2000	R3	20805397	20518034	18807035	1710999	262293	98.62	14093462	12883714	1209748	67.74	10308036	62.3	FALSE		FALSE		TRUE
068R29_17_sam	068R29	CMEC	PRNP183	0	R2	19887884	19597621	17964213	1633408	266476	98.54	12013397	10980590	1032807	60.41	8968002	64.23	FALSE		FALSE		TRUE
068R29_18_sam	068R29	CMEC	PRNP173	32000	R4	20807332	20519524	18863264	1656660	264838	98.62	13126602	12035380	1091222	63.09	10033798	66.76	FALSE		FALSE		TRUE
068R29_19_sam	068R29	CMEC	PRNP173	2000	R4	19219151	18928749	17339429	1589320	267545	98.49	11134825	10160376	974449	57.94	8247314	62.78	FALSE		FALSE		TRUE
068R29_20_sam	068R29	CMEC	PRNP174	2000	R4	20148350	19884102	18153898	1730204	243171	98.69	11801462	10744364	1057098	58.57	8891344	64.47	FALSE		FALSE		TRUE
068R29_21_sam	068R29	CMEC	PRNP174	128000	R3	19420412	19169880	17546209	1623671	229385	98.71	12022709	10974888	1047821	61.91	8955923	64.4	FALSE		FALSE		TRUE
068R29_22_sam	068R29	CMEC	PRNP183	8000	R2	19027451	18713369	17047029	1666340	287643	98.35	11542868	10479548	1063320	60.66	7899793	56.09	FALSE		FALSE		TRUE
068R29_23_sam	068R29	CMEC	PRNP173	32000	R3	20399592	20087567	18201557	1886010	284564	98.47	13035376	11763061	1272315	63.9	9268770	57.23	FALSE		FALSE		TRUE
068R29_24_sam	068R29	CMEC	PRNP174	0	R3	20413109	20143330	18417522	1725808	248022	98.68	13121448	11967840	1153608	64.28	9842492	64.05	FALSE		FALSE		TRUE
068R29_25_sam	068R29	CMEC	PRNP183	2000	R3	18987066	18724999	17108613	1616386	238789	98.62	11922244	10866627	1055617	62.79	8822959	63.89	FALSE		FALSE		TRUE
068R29_26_sam	068R29	CMEC	PRNP173	500	R4	20830147	20532487	18788178	1744309	274248	98.57	12641720	11536580	1105140	60.69	9461542	63.53	FALSE		FALSE		TRUE
068R29_27_sam	068R29	CMEC	PRNP183	500	R2	18797164	18424446	16998404	1426042	349272	98.02	10290419	9465671	824748	54.74	7488810	65.31	FALSE		FALSE		TRUE
068R29_28_sam	068R29	CMEC	PRNP183	2000	R1	19879431	19613965	17962411	1651554	243572	98.66	11879942	10843724	1036218	59.76	8838854	64.12	FALSE		FALSE		TRUE
068R29_29_sam	068R29	CMEC	PRNP183	8000	R3	20320061	20052379	18361552	1690827	246045	98.68	12701143	11601837	1099306	62.51	9664217	66	FALSE		FALSE		TRUE
068R29_30_sam	068R29	CMEC	PRNP174	500	R1	19483500	19237867	17689543	1548324	224480	98.74	13477252	12372098	1105154	69.17	10200017	66.38	FALSE		FALSE		TRUE
068R29_31_sam	068R29	CMEC	PRNP173	0	R3	20335461	20016214	18266902	1749312	291882	98.43	12646405	11503006	1143399	62.19	8838062	58.22	FALSE		FALSE		TRUE
068R29_32_sam	068R29	CMEC	PRNP183	8000	R4	19371509	19086010	17114292	1971718	263975	98.53	12561571	11172601	1388970	64.85	9213580	56.52	FALSE		FALSE		TRUE
068R29_33_sam	068R29	CMEC	PRNP183	32000	R1	19557685	19287449	17698362	1589087	249760	98.62	11587357	10601989	985368	59.25	8845541	66.77	FALSE		FALSE		TRUE
068R29_34_sam	068R29	CMEC	PRNP183	2000	R1	19490688	19212419	17502579	1709840	255196	98.57	11370981	10340575	1030406	58.34	8442049	63.66	FALSE		FALSE		TRUE
068R29_35_sam	068R29	CMEC	PRNP173	500	R1	19896708	19583605	17887090	1696515	287560	98.43	11686305	10655291	1031014	58.73	8444700	61.35	FALSE		FALSE		TRUE
068R29_36_sam	068R29	CMEC	PRNP174	32000	R3	19612265	19314974	17669551	1645423	270059	98.48	11444925	10439462	1005463	58.36	8145181	61.14	FALSE		FALSE		TRUE
068R29_37_sam	068R29	CMEC	PRNP174	32000	R4	19125433	18881662	17343315	1538347	225644	98.73	11992726	10989066	1003660	62.71	9514075	69.55	FALSE		FALSE		TRUE
068R29_38_sam	068R29	CMEC	PRNP183	0	R1	20227001	19965010	18291139	1673871	241105	98.7	14092580	12882366	1210214	69.67	10844469	65.68	FALSE		FALSE		TRUE
068R29_39_sam	068R29	CMEC	PRNP174	128000	R1	19472875	19226813	17620318	1606495	225937	98.74	12618628	11537574	1081054	64.8	9662007	66.35	FALSE		FALSE		TRUE
068R29_40_sam	068R29	CMEC	PRNP173	32000	R1	19687683	19410624	17661845	1748779	254192	98.59	11867838	10762934	1104904	60.28	8776492	62.75	FALSE		FALSE		TRUE
068R29_41_sam	068R29	CMEC	PRNP174	500	R4	19556048	19319390	17773102	1546288	216433	98.79	12465754	11441255	1024499	63.74	9551680	67.51	FALSE		FALSE		TRUE
068R29_42_sam	068R29	CMEC	PRNP174	32000	R4	20470377	20159060	18502548	1656512	285242	98.48	11836662	10829748	1006914	57.82	8528652	61.61	FALSE		FALSE		TRUE
068R29_43_sam	068R29	CMEC	PRNP173	0	R4	19683059	19409490	17707566	1701924	248822	98.61	11441496	10438350	1003146	58.13	8302960	62.46	FALSE		FALSE		TRUE
068R29_44_sam	068R29	CMEC	PRNP174	500	R2	18953186	18690430	17148892	1541538	242712	98.61	11648103	10662938	985165	61.46	8915801	66.55	FALSE		FALSE		TRUE
068R29_45_sam	068R29	CMEC	PRNP174	500	R3	19094459	18817855	17263453	1554402	251640	98.55	12498834	11446172	1052662	65.46	9113017	63.13	FALSE		FALSE		TRUE
068R29_46_sam	068R29	CMEC	PRNP174	0	R1	19897920	19634814	17895959	1738855	241825	98.68	13036302	11847168	1189134	65.52	9906824	63.36	FALSE		FALSE		TRUE
068R29_47_sam	068R29	CMEC	PRNP183	128000	R3	20847351	20571709	18842236	1729473	253466	98.68	13102721	11972285	1130436	62.85	9851212	65.07	FALSE		FALSE		TRUE
068R29_48_sam	068R29	CMEC	PRNP174	8000	R1	19046575	18808567	17215219	1593348	218019	98.75	12263929	11204747	1059182	64.39	9474119	67.76	FALSE		FALSE		TRUE
068R29_49_sam	068R29	CMEC	PRNP174	32000	R1	19986847	19713430	18171768	1541662	249949	98.63	12586736	11572925	1013811	62.98	9299027	65.01	FALSE		FALSE		TRUE
068R29_50_sam	068R29	CMEC	PRNP183	0	R3	19986391	19740873	18094829	1646044	224694	98.77	13617753	12456128	1161625	68.14	10357104	65	FALSE		FALSE		TRUE
068R29_51_sam	068R29	CMEC	PRNP173	128000	R4	18999433	18738117	16973928	1764189	240872	98.62	12117969	10910776	1207193	63.78	9027309	59.03	FALSE		FALSE		TRUE
068R29_52_sam	068R29	CMEC	PRNP173	128000	R1	19863419	19606549	17973975	1632574	237616	98.71	12743959	11645911	1098048	64.16	9905111	66.82	FALSE		FALSE		TRUE
068R29_53_sam	068R29	CMEC	PRNP173	0	R4	20798332	20545512	18888263	1657249	233698	98.78	13543061	12423683	1119378	65.12	10710640	69.43	FALSE		FALSE		TRUE
068R29_54_sam	068R29	CMEC	PRNP183	500	R1	20382004	20111817	18486313	1625504	250206	98.67	13023078	11943312	1079766	63.89	10145732	68.28	FALSE		FALSE		TRUE
068R29_55_sam	068R29	CMEC	PRNP183	32000	R4	19609068	19348148	17702623	1645525	239282	98.67	12072196	11015431	1056765	61.56	9012869	64.13	FALSE		FALSE		TRUE
068R29_56_sam	068R29	CMEC	PRNP183	8000	R4	20186425	19890800	18140876	1749924	271109	98.54	12344734	11129558	1115176	60.66	8982856	62.01	FALSE		FALSE		TRUE
068R29_57_sam	068R29	CMEC	PRNP174	32000	R3	20824258	20566119	18876624	1689495	235216	98.76	13810116	12650656	1159460	66.32	10470592	66.28	FALSE		FALSE		TRUE
068R29_58_sam	068R29	CMEC	PRNP173	2000	R1	20837539	20577621	18869637	1707984	239467	98.75	12742029	11655157	1086872	61.15	9983802	68.69	FALSE		FALSE		TRUE
068R29_59_sam	068R29	CMEC	PRNP174	8000	R4	19489975	19246896	17611134	1635762	224655	98.75	12712503	11605639	1106864	65.23	10088829	68.38	FALSE		FALSE		TRUE
068R29_60_sam	068R29	CMEC	PRNP173	32000	R3	20457300	20203674	18525217	1678457	233853	98.76	12529777	11461421	1068356	61.25	9940748	69.52	FALSE		FALSE		TRUE
068R29_61_sam	068R29	CMEC	PRNP174	2000	R1	20520006	20240942	18533087	1707855	258196	98.64	13845939	12657770	1188169	67.48	10838846	67.28	FALSE		FALSE		TRUE
068R29_62_sam	068R29	CMEC	PRNP183	32000	R3	19006724	18742276	17191817	1550459	245515	98.61	12550574	11487409	1063165	66.03	9780807	68.1	FALSE		FALSE		TRUE
068R29_63_sam	068R29	CMEC	PRNP183	500	R3	19897738	19601115	17734145	1866970	269808	98.51	13316332	12032624	1283708	66.92	9532797	57.43	FALSE		FALSE		TRUE
068R29_64_sam	068R29	CMEC	PRNP174	8000	R3	20280224	20025883	18324980	1700903	235362	98.75	12268746	11194678	1074068	60.5	9769267	69.24	FALSE		FALSE		TRUE
068R29_65_sam	068R29	CMEC	PRNP173	0	R3	20393772	20144214	18483912	1660302	230232	98.78	13163970	12055427	1108543	64.55	10437803	69.79	FALSE		FALSE		TRUE
068R29_66_sam	068R29	CMEC	PRNP174	0	R4	20447379	20168032	18578240	1589792	259961	98.63	12040007	11055865	984142	58.88	9394725	68.73	FALSE		FALSE		TRUE
068R29_67_sam	068R29	CMEC	PRNP183	128000	R4	19156394	18871234	17330189	1541045	262902	98.51	11193207	10247520	945687	58.43	8243869	63.86	FALSE		FALSE		TRUE
068R29_68_sam	068R29	CMEC	PRNP174	2000	R3	20539597	20247399	18512477	1734922	266544	98.58	12482880	11381499	1101381	60.77	9203984	62.84	FALSE		FALSE		TRUE
068R29_69_sam	068R29	CMEC	PRNP183	0	R3	19082030	18792942	17201098	1591844	266583	98.49	10730624	9789997	940627	56.23	7983142	64.25	FALSE		FALSE		TRUE
068R29_70_sam	068R29	CMEC	PRNP183	32000	R4	19277390	19004127	17404398	1599729	250482	98.58	11918505	10883839	1034666	61.83	8869502	63.66	FALSE		FALSE		TRUE
068R29_71_sam	068R29	CMEC	PRNP183	500	R3	20594104	20331859	18674325	1657534	241903	98.73	13334037	12219613	1114424	64.75	10445818	68.32	FALSE		FALSE		TRUE
068R29_72_sam	068R29	CMEC	PRNP183	32000	R3	17853836	17469040	15960838	1508202	359973	97.84	10056859	9148669	908190	56.33	7004778	59.7	FALSE		FALSE		TRUE
068R29_73_sam	068R29	CMEC	PRNP174	500	R3	20212561	19942543	18355295	1587248	248625	98.66	12599172	11569841	1029331	62.33	9712054	68.07	FALSE		FALSE		TRUE
068R29_74_sam	068R29	CMEC	PRNP174	2000	R2	19371178	19110377	17526031	1584346	239731	98.65	12142542	11116209	1026333	62.68	9484862	68.77	FALSE		FALSE		TRUE
068R29_75_sam	068R29	CMEC	PRNP183	2000	R3	20743048	20491879	18830222	1661657	230319	98.79	13457341	12344223	1113118	64.88	10475990	68.89	FALSE		FALSE		TRUE
068R29_76_sam	068R29	CMEC	PRNP173	0	R1	20086453	19816482	18153920	1662562	250386	98.66	11688374	10686270	1002104	58.19	9139602	68.84	FALSE		FALSE		TRUE
068R29_77_sam	068R29	CMEC	PRNP174	500	R4	19934779	19644056	17994962	1649094	266266	98.54	11194348	10228009	966339	56.15	8296160	64.76	FALSE		FALSE		TRUE
068R29_78_sam	068R29	CMEC	PRNP174	8000	R2	20143940	19851531	18162523	1689008	268098	98.55	11582858	10567726	1015132	57.5	8643517	64.21	FALSE		FALSE		TRUE
068R29_79_sam	068R29	CMEC	PRNP173	8000	R1	20353649	20062801	18358151	1704650	269724	98.57	12275251	11203180	1072071	60.31	9541459	67.65	FALSE		FALSE		TRUE
068R29_80_sam	068R29	CMEC	PRNP174	128000	R3	19736196	19462164	17894533	1567631	250497	98.61	11784527	10799552	984975	59.71	8799165	65.07	FALSE		FALSE		TRUE
068R29_81_sam	068R29	CMEC	PRNP174	128000	R4	20498409	20248618	18585389	1663229	228669	98.78	13001119	11904911	1096208	63.43	10009844	67.18	FALSE		FALSE		TRUE
068R29_82_sam	068R29	CMEC	PRNP174	128000	R2	20000791	19746130	18047784	1698346	235455	98.73	13187824	12039031	1148793	65.94	10520855	68.86	FALSE		FALSE		TRUE
068R29_83_sam	068R29	CMEC	PRNP173	500	R4	20472927	20208922	18553733	1655189	245742	98.71	12066711	11053141	1013570	58.94	9751636	71.42	FALSE		FALSE		TRUE

sample_id	batch	cell_line	construct	moi	replicate	input_reads	mapped_reads	unique_mapped_	multi_mapped_r	unmapped_reads	mapping_rate_pc	dedup_mapped_	dedup_unique_r	dedup_multi_rea	dedup_rate_pct	assigned_reads	assigned_pct	outlier_flag	outlier_reason	failure_flag	failure_reasons	included_in_analysis
S68R29_84_sam	S68R29	CMEC	PRNP173	128000	R3	20285153	20010492	18378382	1632110	251884	98.65	12331107	11303591	1027516	60.79	9480892	67.71	FALSE		FALSE		TRUE
S68R29_85_sam	S68R29	CMEC	PRNP174	0	R2	20718263	20391450	18657877	1733573	303289	98.42	10853150	9894126	959024	52.38	8166758	65.24	FALSE		FALSE		TRUE
S68R29_86_sam	S68R29	CMEC	PRNP183	128000	R3	19600794	19341432	17719826	1621606	238981	98.68	12272912	11234369	1038543	62.61	9386996	66.74	FALSE		FALSE		TRUE
S68R29_87_sam	S68R29	CMEC	PRNP183	32000	R2	19223856	18962378	17414798	1547580	241140	98.64	12208572	11194180	1014392	63.51	9522957	68.83	FALSE		FALSE		TRUE
S68R29_88_sam	S68R29	CMEC	PRNP173	128000	R4	19220500	18974712	17456225	1518487	226746	98.72	12499204	11476120	1023084	65.03	9795106	69.51	FALSE		FALSE		TRUE
S68R29_89_sam	S68R29	CMEC	PRNP173	8000	R4	19354409	19120309	17545498	1574811	214790	98.79	12533552	11493071	1040481	64.76	9868254	69.83	FALSE		FALSE		TRUE
S68R29_90_sam	S68R29	CMEC	PRNP174	0	R3	20429304	20167829	18527312	1640517	241164	98.72	12884887	11819074	1065813	63.07	10198026	69.99	FALSE		FALSE		TRUE
S68R29_91_sam	S68R29	CMEC	PRNP174	8000	R4	19651422	19382901	17796831	1586070	248923	98.63	12105764	11108012	997752	61.6	9511837	69.11	FALSE		FALSE		TRUE
S68R29_92_sam	S68R29	CMEC	PRNP173	32000	R1	19085894	18842128	17257274	1584854	226571	98.72	9931229	9068719	862510	52.03	8025135	71.86	FALSE		FALSE		TRUE
S68R29_93_sam	S68R29	CMEC	PRNP173	0	R1	20716847	20436592	18677451	1759141	258000	98.65	11493426	10481248	1012178	55.48	8805064	67.12	FALSE		FALSE		TRUE
S68R29_94_sam	S68R29	CMEC	PRNP183	8000	R3	19953040	19686389	17992787	1693602	245053	98.66	11964084	10911793	1052291	59.96	9214315	67.25	FALSE		FALSE		TRUE
S68R29_95_sam	S68R29	CMEC	PRNP183	128000	R1	20205831	19942917	18268724	1674193	244228	98.7	14039377	12837618	1201759	69.48	11099933	67.74	FALSE		FALSE		TRUE
S68R29_96_sam	S68R29	CMEC	PRNP173	8000	R3	20139685	19854645	17931867	1922778	261528	98.58	12257253	11149731	1107522	60.86	9179382	64.27	FALSE		FALSE		TRUE
S68R29_97_sam	S68R29	CMEC	PRNP174	8000	R3	471658	463618	399191	64427	7339	98.3	305825	261497	44328	64.84	237672	51.48	FALSE		TRUE	fc_post_dedup_u	FALSE
S68R29_98_sam	S68R29	CMEC	PRNP183	2000	R4	61775	60129	45332	14797	1569	97.34	40976	30243	10733	66.33	26509	28.87	TRUE		TRUE	fc_assigned_pct;	FALSE
S68R29_99_sam	S68R29	CMEC	PRNP173	500	R3	165541	159403	115758	43645	5657	96.29	109673	77767	31906	66.25	64789	24.31	TRUE		TRUE	fc_assigned_pct;	FALSE
S68R29_100_sa	S68R29	CMEC	PRNP174	2000	R4	142281	140221	113980	26241	1942	98.55	92454	74303	18151	64.98	67080	40.92	TRUE		TRUE	fc_assigned_pct;	FALSE
S68R29_101_sa	S68R29	CMEC	PRNP183	128000	R3	197152	193216	163319	29897	3700	98	130319	108956	21363	66.1	98543	46.42	TRUE		TRUE	fc_post_dedup_u	FALSE
S68R29_102_sa	S68R29	CMEC	PRNP173	32000	R4	222041	218550	185982	32568	3291	98.43	144577	122170	22407	65.11	113707	51.03	TRUE		TRUE	fc_post_dedup_u	FALSE
S68R29_103_sa	S68R29	CMEC	PRNP183	500	R4	92639	90597	76161	14436	1943	97.8	61031	50779	10252	65.88	45314	45.77	TRUE		TRUE	fc_post_dedup_u	FALSE
S68R29_104_sa	S68R29	CMEC	PRNP183	128000	R4	131733	129889	108934	20955	1676	98.6	86136	71331	14805	65.39	67292	46.78	TRUE		TRUE	fc_post_dedup_u	FALSE
S68R29_105_sa	S68R29	CMEC	PRNP174	32000	R2	491363	426559	355984	70575	64211	86.81	233584	200903	32681	47.54	102051	31.65	TRUE		TRUE	fc_assigned_pct;	FALSE
S68R29_106_sa	S68R29	CMEC	PRNP173	8000	R1	699410	569715	484946	84769	128765	81.46	340528	292658	47870	48.69	158017	32.91	TRUE		TRUE	fc_assigned_pct;	FALSE
S68R29_107_sa	S68R29	CMEC	PRNP173	2000	R3	717238	599543	513699	85844	116726	83.59	361878	311462	50416	50.45	162999	32.33	TRUE		TRUE	fc_assigned_pct;	FALSE
S68R29_108_sa	S68R29	CMEC	PRNP173	8000	R4	499205	427110	360862	66248	71450	85.56	268319	225854	42465	53.75	97298	27.39	TRUE		TRUE	fc_assigned_pct;	FALSE
GG83WH_1_san	GG83WH	HEK293	PRNP173	128000	R1	20092912	19829284	17798236	2031048	236247	98.69	11347583	10161298	1186285	56.48	8682760	64.12	FALSE		FALSE		TRUE
GG83WH_2_san	GG83WH	HEK293	PRNP183	8000	R1	19006424	18758539	17134064	1624475	218056	98.7	12560321	11455227	1105094	66.08	9058772	61.96	FALSE		FALSE		TRUE
GG83WH_3_san	GG83WH	HEK293	PRNP173	500	R1	19806524	19532486	17801353	1731133	242448	98.62	13965031	12713923	1251108	70.51	9912173	59.22	FALSE		FALSE		TRUE
GG83WH_4_san	GG83WH	HEK293	PRNP183	128000	R2	20719491	20448758	18445732	2003026	244085	98.69	10503948	9458501	1045447	50.7	7971699	64.13	FALSE		FALSE		TRUE
GG83WH_5_san	GG83WH	HEK293	PRNP174	0	R4	18836590	18553915	16586320	1967595	246820	98.5	9654048	8592034	1062014	51.25	7200786	59.76	FALSE		FALSE		TRUE
GG83WH_6_san	GG83WH	HEK293	PRNP173	8000	R3	20785282	20494020	18697299	1796721	261477	98.6	13930309	12698128	1232181	67.02	10131092	61.97	FALSE		FALSE		TRUE
GG83WH_7_san	GG83WH	HEK293	PRNP183	2000	R2	19986510	19689649	17844712	1844937	265372	98.51	12935425	11707608	1227817	64.72	9294622	61.86	FALSE		FALSE		TRUE
GG83WH_8_san	GG83WH	HEK293	PRNP173	500	R3	19969484	19700845	17729244	1971601	243402	98.65	10985602	9860777	1124825	55.01	8409178	64.34	FALSE		FALSE		TRUE
GG83WH_9_san	GG83WH	HEK293	PRNP183	500	R4	19338969	19063370	17227612	1835758	235205	98.57	11554084	10423519	1130565	59.75	8465763	62.42	FALSE		FALSE		TRUE
GG83WH_10_sa	GG83WH	HEK293	PRNP173	2000	R4	20717929	20444294	18635312	1808982	244796	98.68	12411085	11295860	1115225	59.91	9274553	64.08	FALSE		FALSE		TRUE
GG83WH_11_sa	GG83WH	HEK293	PRNP183	0	R4	20760154	20437413	18458926	1978487	269329	98.45	12569573	11334015	1235558	60.55	8495442	56.95	FALSE		FALSE		TRUE
GG83WH_12_sa	GG83WH	HEK293	PRNP174	128000	R4	19384960	19119329	17095219	2024110	229273	98.63	9137605	8145431	992174	47.14	7031767	64.02	FALSE		FALSE		TRUE
GG83WH_13_sa	GG83WH	HEK293	PRNP183	0	R4	18915758	18676135	17021675	1654460	213138	98.73	12712128	11572913	1139215	67.2	9353734	63.56	FALSE		FALSE		TRUE
GG83WH_14_sa	GG83WH	HEK293	PRNP183	2000	R4	20635358	20345401	18470167	1875234	252633	98.59	11832985	10711066	1121919	57.34	8882504	63.36	FALSE		FALSE		TRUE
GG83WH_15_sa	GG83WH	HEK293	PRNP173	2000	R3	20620977	20328489	18425710	1902779	250847	98.58	12738629	11527718	1210911	61.78	9402061	63.26	FALSE		FALSE		TRUE
GG83WH_16_sa	GG83WH	HEK293	PRNP174	2000	R3	19975048	19719585	17953626	1765959	227303	98.72	11408407	10357660	1050747	57.11	8615042	64.38	FALSE		FALSE		TRUE
GG83WH_17_sa	GG83WH	HEK293	PRNP183	0	R2	20536023	20280474	18426658	1853816	220914	98.76	10931694	9905301	1026393	53.23	8527574	66.84	FALSE		FALSE		TRUE
GG83WH_18_sa	GG83WH	HEK293	PRNP173	32000	R4	19546470	19288892	17313074	1975818	233759	98.68	10747170	9626150	1121020	54.98	8440878	66.98	FALSE		FALSE		TRUE
GG83WH_19_sa	GG83WH	HEK293	PRNP173	2000	R4	18929762	18658645	16752734	1905911	236249	98.57	9718764	8699388	1019376	51.34	7422990	63.63	FALSE		FALSE		TRUE
GG83WH_20_sa	GG83WH	HEK293	PRNP174	2000	R4	20266233	19997660	18250008	1747652	233616	98.67	11135323	10135485	999838	54.95	8490007	65.67	FALSE		FALSE		TRUE
GG83WH_21_sa	GG83WH	HEK293	PRNP174	128000	R3	19161232	18907313	17232961	1674352	218296	98.67	9802287	8906415	895872	51.16	7248740	63.62	FALSE		FALSE		TRUE
GG83WH_22_sa	GG83WH	HEK293	PRNP183	8000	R2	19411715	19116083	17113070	2003013	260214	98.48	9496418	8469420	1026998	48.92	7177047	61.67	FALSE		FALSE		TRUE
GG83WH_23_sa	GG83WH	HEK293	PRNP173	32000	R3	19461508	19200516	17190188	2010328	236047	98.66	10212167	9105688	1106479	52.47	7828967	62.77	FALSE		FALSE		TRUE
GG83WH_24_sa	GG83WH	HEK293	PRNP174	0	R3	19410578	19172636	17518513	1654123	213063	98.77	11592829	10567889	1024940	59.72	8761237	64.94	FALSE		FALSE		TRUE
GG83WH_25_sa	GG83WH	HEK293	PRNP183	2000	R3	18930628	18689579	16842501	1847078	208489	98.73	9640969	8665297	975672	50.93	7550116	66.6	FALSE		FALSE		TRUE
GG83WH_26_sa	GG83WH	HEK293	PRNP173	500	R4	19467779	19210937	17248994	1961943	221092	98.68	11858964	10629182	1229782	60.92	9255414	65.48	FALSE		FALSE		TRUE
GG83WH_27_sa	GG83WH	HEK293	PRNP183	500	R2	20194781	19923970	17979623	1944347	234507	98.66	10122838	9111661	1011177	50.13	7820475	65.81	FALSE		FALSE		TRUE
GG83WH_28_sa	GG83WH	HEK293	PRNP183	2000	R1	19926728	19671344	17929980	1741364	220816	98.72	10627919	9653498	974421	53.33	8077130	64.97	FALSE		FALSE		TRUE
GG83WH_29_sa	GG83WH	HEK293	PRNP183	8000	R3	20041069	19778074	18023809	1754265	228358	98.69	11057550	10051919	1005631	55.17	8341345	65.13	FALSE		FALSE		TRUE
GG83WH_30_sa	GG83WH	HEK293	PRNP174	500	R1	19920388	19666247	17979320	1686927	219721	98.72	11397068	10393837	1003231	57.21	8607750	65.46	FALSE		FALSE		TRUE
GG83WH_31_sa	GG83WH	HEK293	PRNP173	0	R3	18841154	18579163	16683515	1895648	227720	98.61	11316721	10133431	1183290	60.06	8741143	63.81	FALSE		FALSE		TRUE
GG83WH_32_sa	GG83WH	HEK293	PRNP183	8000	R4	19657888	19374415	17428500	1945915	246173	98.56	11929723	10679963	1249760	60.69	8668612	57.8	FALSE		FALSE		TRUE
GG83WH_33_sa	GG83WH	HEK293	PRNP183	32000	R1	19412123	19163385	17500812	1662573	222361	98.72	10277292	9356991	920301	52.94	7861278	66.37	FALSE		FALSE		TRUE
GG83WH_34_sa	GG83WH	HEK293	PRNP173	2000	R1	20714089	20434604	18412970	2021634	240847	98.65	11829541	10640067	1189474	57.11	9211673	66.26	FALSE		FALSE		TRUE
GG83WH_35_sa	GG83WH	HEK293	PRNP173	500	R1	19960007	19687640	17737227	1950413	235301	98.64	11079889	9960901	1118988	55.51	8572030	65.23	FALSE		FALSE		TRUE
GG83WH_36_sa	GG83WH	HEK293	PRNP174	32000	R3	19157195	18910749	17036628	1874121	222689	98.71	10052840	9031690	1021150	52.48	7790342	65.88	FALSE		FALSE		TRUE
GG83WH_37_sa	GG83WH	HEK293	PRNP174	32000	R4	20633440	20370403	18605660	1764743	233500	98.73	12671829	11556952	1114877	61.41	9525738	65.76	FALSE		FALSE		TRUE
GG83WH_38_sa	GG83WH	HEK293	PRNP183	0	R1	20639625	20386369	18602125	1784244	225287	98.77	12420939	11308000	1112939	60.18	9245939	63.71	FALSE		FALSE		TRUE
GG83WH_39_sa	GG83WH	HEK293	PRNP174	128000	R1	19857016	19623034	17912941	1710093	206718	98.82	11859194	10802083	1057111	59.72	9223606	67.85	FALSE		FALSE		TRUE
GG83WH_40_sa	GG83WH	HEK293	PRNP173	32000	R1	19143460	18882294	16999171	1883123	224426	98.64	10377200	9317039	1060161	54.21	7882022	64.01	FALSE		FALSE		TRUE
GG83WH_41_sa	GG83WH	HEK293	PRNP174	500	R4	19517879	19281814	17604365	1677449	209263	98.79	10792181	9826701	965480	55.29	8135067	65.67	FALSE		FALSE		TRUE
GG83WH_42_sa	GG83WH	HEK293	PRNP174	32000	R4	20600143	20307285	18256569	2050716	254442	98.58	10535968	9439004	1096964	51.15	8005727	63.33	FALSE		FALSE		TRUE
GG83WH_43_sa	GG83WH	HEK293	PRNP173	0	R4	20326584	20060848	18027227	2033621	229864	98.69	9971886	8945399	1026487	49.06	7721171	65.33	FALSE		FALSE		TRUE
GG83WH_44_sa	GG83WH	HEK293	PRNP174	500	R2	18702251	18456410	16632513	1823897	213141	98.69	8354102	7502016	852086	44.67	6482709	65.77	FALSE		FALSE		TRUE
GG83WH_45_sa	GG83WH	HEK293	PRNP174	500	R3	20642618	20369550	18374967	1994583	235217	98.68	11109479	9995847	1113632	53.82	8467123	64.52	FALSE		FALSE		TRUE
GG83WH_46_sa	GG83WH	HEK293	PRNP174	0	R1	19330780	19081241	17375024	1706217	214601	98.71	11117467	10092365	1025102	57.51	8345742	63.28	FALSE		FALSE		TRUE
GG83WH_47_sa	GG83WH	HEK293	PRNP183	128000	R3	19034055	18792750	17094137	1698613	214826	98.73	11880931	10787349	1093582	62.42	9037634	65.74	FALSE		FALSE		TRUE
GG83WH_48_sa	GG83WH	HEK293	PRNP174	8000	R1	20258375	20004986	18285285	1719701	218480	98.75	12465065	11371613	1093452	61.53	9726151	67.94	FALSE		FALSE		TRUE
GG83WH_49_sa	GG83WH	HEK293	PRNP174	32000	R1	20271018	20021799	18355134	1666665	221835	98.77	11082300	10130282	952018	54.67	8446835	66.49	FALSE		FALSE		TRUE
GG83WH_50_sa	GG83WH	HEK293	PRNP183	0	R3	3053938	2999512	2741735	257777	47686	98.22	1412669	1286329	126340	46.26	1031456	64.74	FALSE		FALSE		TRUE
GG83WH_51_sa	GG83WH	HEK293	PRNP173	128000	R4	19538962	19265310	17104564	2160746	236562	98.6	10490889	9260564	1230325	53.69	7844933	58.17	FALSE		FALSE		TRUE
GG83WH_52_sa	GG83WH	HEK293	PRNP173	128000	R1	20432117	20178658	18407987	1770671	217622	98.76	9377282	8524379	852903	45.89	7238749	66.95	FALSE		FALSE		TRUE
GG83WH_53_sa	GG83WH	HEK293	PRNP173	0	R4	20714849	20465143	18757995	1707148	222733	98.79	11492354	10507625	984729	55.48	8702887	66.07	FALSE		FALSE		TRUE
GG83WH_54_sa	GG83WH	HEK293	PRNP183	500	R1	20423052	20162376	18395749	1766627	230923	98.72	12489521	11375780	1113741	61.15	9323115	64.63	FALSE		FALSE		TRUE
GG83WH_55_sa	GG83WH	HEK293	PRNP183	32000	R4	19022368	18765439	17066729	1698710	222255	98.65	11969791	10864406	1105385	62.92	9107577	64.93	FALSE		FALSE		TRUE
GG83WH_56_sa	GG83WH	HEK293	PRNP183	8000	R4	19103761	18841899	16913260	1928639	227747	98.63	10942492	9796850	1145642	57.28	8513775	65.1	FALSE		FALSE		TRUE
GG83WH_57_sa	GG83WH	HEK293	PRNP174	32000	R3	20719465	20468905	18715738	1753167	221986	98.79	11882881	10836932	1045949	57.35	8941248	65.07	FALSE		FALSE		TRUE
GG83WH_58_sa	GG83WH	HEK293	PRNP173	2000	R1	20060267	19825140	18135798	1689342	208310	98.83	13435730	12275232	1160498	66.98	10262117	66.5	FALSE		FALSE		TRUE

sample_id	batch	cell_line	construct	moi	replicate	input_reads	mapped_reads	unique_mapped_	multi_mapped_r	unmapped_reads	mapping_rate_pc	dedup_mapped_	dedup_unique_r	dedup_multi_rea	dedup_rate_pct	assigned_reads	assigned_pct	outlier_flag	outlier_reason	failure_flag	failure_reasons	included_in_analysis
GG83WH_59_sa	GG83WH	HEK293	PRNP174	8000	R4	20169078	19911674	18196422	1715252	221288	98.72	11465716	10449084	1016632	56.85	8723026	65.46	FALSE		FALSE		TRUE
GG83WH_60_sa	GG83WH	HEK293	PRNP173	32000	R3	19971309	19725254	18035497	1689757	215879	98.77	12165571	11102303	1063268	60.92	9188904	66	FALSE		FALSE		TRUE
GG83WH_61_sa	GG83WH	HEK293	PRNP174	2000	R1	7333876	7233468	6611127	622341	91345	98.63	3447032	3141250	305782	47	2624568	68.2	FALSE		FALSE		TRUE
GG83WH_62_sa	GG83WH	HEK293	PRNP183	32000	R3	19590029	19337260	17624661	1712599	217771	98.71	11595639	10543157	1052482	59.19	8841289	65.78	FALSE		FALSE		TRUE
GG83WH_63_sa	GG83WH	HEK293	PRNP183	500	R3	20294075	20021232	17916908	2104324	234386	98.66	12226785	10919901	1306884	60.25	9248159	60.92	FALSE		FALSE		TRUE
GG83WH_64_sa	GG83WH	HEK293	PRNP174	8000	R3	19679905	19447587	17787703	1659884	206427	98.82	13042071	11911373	1130698	66.27	10010695	66.67	FALSE		FALSE		TRUE
GG83WH_65_sa	GG83WH	HEK293	PRNP173	0	R3	20067216	19823937	18154991	1668946	216929	98.79	11345709	10367659	978050	56.54	8567828	65.7	FALSE		FALSE		TRUE
GG83WH_66_sa	GG83WH	HEK293	PRNP174	0	R4	19237580	18979030	17388787	1590243	225713	98.66	11795972	10788344	1007628	61.32	8534802	63.04	FALSE		FALSE		TRUE
GG83WH_67_sa	GG83WH	HEK293	PRNP183	128000	R4	20259987	19988591	17936352	2052239	246014	98.66	10247582	9171507	1076075	50.58	8040800	66.36	FALSE		FALSE		TRUE
GG83WH_68_sa	GG83WH	HEK293	PRNP174	2000	R3	19166531	18915891	16998957	1916934	216095	98.69	10172722	9117374	1055348	53.08	8010801	66.32	FALSE		FALSE		TRUE
GG83WH_69_sa	GG83WH	HEK293	PRNP183	0	R3	19141738	18875145	17057511	1817634	231866	98.61	9352439	8426807	925632	48.86	7282868	66.33	FALSE		FALSE		TRUE
GG83WH_70_sa	GG83WH	HEK293	PRNP183	32000	R4	18984917	18720794	16907197	1813597	219931	98.61	11506993	10374742	1132251	60.61	8340353	61.29	FALSE		FALSE		TRUE
GG83WH_71_sa	GG83WH	HEK293	PRNP183	500	R3	19175476	18933454	17217487	1715967	209011	98.74	11964626	10856909	1107717	62.4	9362322	67.01	FALSE		FALSE		TRUE
GG83WH_72_sa	GG83WH	HEK293	PRNP183	32000	R3	19323335	19052279	17066013	1986266	235534	98.6	11077551	9890044	1187507	57.33	8431271	61.84	FALSE		FALSE		TRUE
GG83WH_73_sa	GG83WH	HEK293	PRNP174	500	R3	19267283	19025800	17378768	1647032	215570	98.75	12342588	11253632	1088956	64.06	9619029	68.12	FALSE		FALSE		TRUE
GG83WH_74_sa	GG83WH	HEK293	PRNP174	2000	R2	18902314	18658076	16848651	1809425	209257	98.71	11760990	10606961	1154029	62.22	9289980	67.88	FALSE		FALSE		TRUE
GG83WH_75_sa	GG83WH	HEK293	PRNP183	2000	R3	19291735	19053272	17377829	1675443	205210	98.76	11080997	10083634	997363	57.44	8597614	67.49	FALSE		FALSE		TRUE
GG83WH_76_sa	GG83WH	HEK293	PRNP173	0	R1	19844844	19594833	17996610	1598223	219964	98.74	12179172	11170381	1008791	61.37	9060160	65.29	FALSE		FALSE		TRUE
GG83WH_77_sa	GG83WH	HEK293	PRNP174	500	R4	19160890	18887709	16978575	1909134	236639	98.57	9316183	8349898	966285	48.62	7226247	65.78	FALSE		FALSE		TRUE
GG83WH_78_sa	GG83WH	HEK293	PRNP174	8000	R2	19766355	19510134	17542110	1968024	231739	98.7	10724816	9622257	1102559	54.26	8449047	67.04	FALSE		FALSE		TRUE
GG83WH_79_sa	GG83WH	HEK293	PRNP173	8000	R1	19953400	19678822	17707651	1971171	236195	98.62	11800821	10601114	1199707	59.14	9144304	65.82	FALSE		FALSE		TRUE
GG83WH_80_sa	GG83WH	HEK293	PRNP174	128000	R3	20444412	20177901	18065359	2112542	228682	98.7	11141548	9951359	1190189	54.5	8826059	66.39	FALSE		FALSE		TRUE
GG83WH_81_sa	GG83WH	HEK293	PRNP174	128000	R4	19297121	19066819	17406968	1659851	200531	98.81	12461932	11366453	1095479	64.58	9171430	64.03	FALSE		FALSE		TRUE
GG83WH_82_sa	GG83WH	HEK293	PRNP174	128000	R2	19831352	19579466	17516770	2062696	216621	98.73	9873150	8814039	1059111	49.79	7879280	66.89	FALSE		FALSE		TRUE
GG83WH_83_sa	GG83WH	HEK293	PRNP173	500	R4	20474216	20198920	18404965	1793955	238915	98.66	11430612	10392105	1038507	55.83	8930429	67.38	FALSE		FALSE		TRUE
GG83WH_84_sa	GG83WH	HEK293	PRNP173	128000	R3	19493075	19233472	17250954	1982518	224210	98.67	10404548	9313677	1090871	53.38	8286661	67.5	FALSE		FALSE		TRUE
GG83WH_85_sa	GG83WH	HEK293	PRNP174	0	R2	19440783	19157270	17233940	1923330	248194	98.54	9860659	8847824	1012835	50.72	7753767	66.65	FALSE		FALSE		TRUE
GG83WH_86_sa	GG83WH	HEK293	PRNP183	128000	R3	19361172	19115909	17133193	1982716	210050	98.73	10381314	9292031	1089283	53.62	8318286	67.76	FALSE		FALSE		TRUE
GG83WH_87_sa	GG83WH	HEK293	PRNP183	32000	R2	19794598	19534100	17581292	1952808	225276	98.68	10677766	9592358	1085408	53.94	8532868	68.22	FALSE		FALSE		TRUE
GG83WH_88_sa	GG83WH	HEK293	PRNP173	128000	R4	19123965	18871217	17227132	1644085	214000	98.68	13395726	12214250	1181476	70.05	10213239	66.44	FALSE		FALSE		TRUE
GG83WH_89_sa	GG83WH	HEK293	PRNP173	8000	R4	19630798	19387851	17642408	1745443	210086	98.76	12153425	11046926	1106499	61.91	9798380	69.9	FALSE		FALSE		TRUE
GG83WH_90_sa	GG83WH	HEK293	PRNP174	0	R3	19683513	19442037	17479088	1962949	207373	98.77	10925716	9806213	1119503	55.51	8911956	69.74	FALSE		FALSE		TRUE
GG83WH_91_sa	GG83WH	HEK293	PRNP174	8000	R4	20201945	19945608	17843187	2102421	232781	98.73	9972626	8909458	1063168	49.36	7936599	67.5	FALSE		FALSE		TRUE
GG83WH_92_sa	GG83WH	HEK293	PRNP173	32000	R1	14270332	13988675	12770041	1218634	259814	98.03	7487712	6819726	667986	52.47	5634587	67.41	FALSE		FALSE		TRUE
GG83WH_93_sa	GG83WH	HEK293	PRNP173	0	R1	19783112	19560425	17591241	1969184	189582	98.87	9134159	8199355	934804	46.17	7652913	72.18	FALSE		FALSE		TRUE
GG83WH_94_sa	GG83WH	HEK293	PRNP183	8000	R3	20523537	20252421	18172388	2080033	232990	98.68	10341945	9261346	1080599	50.39	8220699	67.52	FALSE		FALSE		TRUE
GG83WH_95_sa	GG83WH	HEK293	PRNP183	128000	R1	20001519	19738676	17977548	1761128	224948	98.69	13289727	12086907	1202820	66.44	9973498	64.06	FALSE		FALSE		TRUE
GG83WH_96_sa	GG83WH	HEK293	PRNP173	8000	R3	19701891	19422635	17436323	1986312	242789	98.58	10600072	9527571	1072501	53.8	8205867	65.67	FALSE		FALSE		TRUE
GG83WH_97_sa	GG83WH	HEK293	PRNP174	8000	R3	7330	7014	5902	1112	307	95.69	4841	4043	798	66.04	3525	46.11	TRUE		TRUE	fc_post_dedup_u	FALSE
GG83WH_98_sa	GG83WH	HEK293	PRNP183	2000	R4	17165	16632	13790	2842	497	96.89	11645	9559	2086	67.84	7686	39.87	TRUE		TRUE	fc_assigned_pct;	FALSE
GG83WH_99_sa	GG83WH	HEK293	PRNP173	500	R3	25671	24784	19727	5057	826	96.54	17572	13824	3748	68.45	10971	32.93	TRUE		TRUE	fc_assigned_pct;	FALSE
GG83WH_100_s	GG83WH	HEK293	PRNP174	2000	R4	9481	9165	8047	1118	308	96.67	6263	5452	811	66.06	4942	59.99	TRUE		TRUE	fc_post_dedup_u	FALSE
GG83WH_101_s	GG83WH	HEK293	PRNP173	128000	R3	12619	12235	10515	1720	367	96.96	8482	7250	1232	67.22	6814	61.7	TRUE		TRUE	fc_post_dedup_u	FALSE
GG83WH_102_s	GG83WH	HEK293	PRNP173	32000	R4	21076	20478	17521	2957	580	97.16	14066	11932	2134	66.74	10817	54.03	TRUE		TRUE	fc_post_dedup_u	FALSE
GG83WH_103_s	GG83WH	HEK293	PRNP183	500	R4	307976	302986	259205	43781	4557	98.38	206233	175020	31213	66.96	146533	46.3	TRUE		TRUE	fc_post_dedup_u	FALSE
GG83WH_104_s	GG83WH	HEK293	PRNP183	128000	R4	662651	653718	571011	82707	7920	98.65	437660	379223	58437	66.05	327808	53.84	TRUE		TRUE	fc_post_dedup_u	FALSE
GG83WH_105_s	GG83WH	HEK293	PRNP174	32000	R2	514556	372682	271022	101660	141279	72.43	188623	153651	34972	36.66	64063	19.48	TRUE		TRUE	star_unique_pct;	FALSE
GG83WH_106_s	GG83WH	HEK293	PRNP173	8000	R1	958206	670936	530378	140558	286145	70.02	379879	310297	69582	39.64	130351	19.34	TRUE		TRUE	star_unique_pct;	FALSE
GG83WH_107_s	GG83WH	HEK293	PRNP173	2000	R3	1162336	836234	663985	172249	324833	71.94	453744	369760	83984	39.04	153414	18.95	TRUE		TRUE	star_unique_pct;	FALSE
GG83WH_108_s	GG83WH	HEK293	PRNP173	8000	R4	473390	333516	269184	64332	139248	70.45	202856	166186	36670	42.85	69946	20.23	TRUE		TRUE	star_unique_pct;	FALSE
59DNQR_1_sam	59DNQR	Muscle	PRNP174	128000	R2	19445012	19190981	17456817	1734164	217187	98.69	12905616	11718817	1186799	66.37	8838882	58.08	FALSE		FALSE		TRUE
59DNQR_2_sam	59DNQR	Muscle	PRNP173	0	R3	19986479	19706796	17917104	1789692	242217	98.6	13259511	12034370	1225141	66.34	8968654	57.22	FALSE		FALSE		TRUE
59DNQR_3_sam	59DNQR	Muscle	PRNP173	0	R3	20666918	20362307	18329201	2033106	265968	98.53	13631435	12250456	1380979	65.96	9192900	54.15	FALSE		FALSE		TRUE
59DNQR_4_sam	59DNQR	Muscle	PRNP183	2000	R4	20432562	20162747	18379398	1783349	231231	98.68	13832341	12598553	1233788	67.7	9409537	58.62	FALSE		FALSE		TRUE
59DNQR_5_sam	59DNQR	Muscle	PRNP173	2000	R1	19640924	19349205	17567748	1781457	250730	98.51	12718041	11520137	1197904	64.75	8241278	53.76	FALSE		FALSE		TRUE
59DNQR_6_sam	59DNQR	Muscle	PRNP173	2000	R4	19446630	19202007	17323763	1878244	213236	98.74	12417578	11175439	1242139	63.85	9144471	61.28	FALSE		FALSE		TRUE
59DNQR_7_sam	59DNQR	Muscle	PRNP174	2000	R1	20077610	19807946	18011210	1796736	235550	98.66	12598369	11434605	1163764	62.75	9108949	62.19	FALSE		FALSE		TRUE
59DNQR_8_sam	59DNQR	Muscle	PRNP174	8000	R4	19893743	19628819	17841082	1787737	228414	98.67	12694773	11516938	1177835	63.81	8791921	58.88	FALSE		FALSE		TRUE
59DNQR_9_sam	59DNQR	Muscle	PRNP183	128000	R3	19371556	19127224	17353815	1773409	210776	98.74	12890652	11701224	1189428	66.54	9116742	60.62	FALSE		FALSE		TRUE
59DNQR_10_sa	59DNQR	Muscle	PRNP183	128000	R3	20211406	19939561	18148233	1791328	234433	98.65	13836555	12580673	1255882	68.46	9549049	59.04	FALSE		FALSE		TRUE
59DNQR_11_sa	59DNQR	Muscle	PRNP173	32000	R2	20317244	20049716	18234470	1815246	229271	98.68	13587419	12335281	1252138	66.88	9246570	57.69	FALSE		FALSE		TRUE
59DNQR_12_sa	59DNQR	Muscle	PRNP183	0	R2	18921169	18674742	17008255	1666487	211112	98.7	13007335	11835332	1172003	68.74	8949929	59.23	FALSE		FALSE		TRUE
59DNQR_13_sa	59DNQR	Muscle	PRNP174	2000	R2	19932809	19678690	17910242	1768448	217125	98.73	12481117	11340314	1140803	62.62	8868229	61.41	FALSE		FALSE		TRUE
59DNQR_14_sa	59DNQR	Muscle	PRNP173	2000	R3	19710106	19446077	17634255	1811822	225973	98.66	13149414	11910959	1238455	66.71	8903186	56.69	FALSE		FALSE		TRUE
59DNQR_15_sa	59DNQR	Muscle	PRNP173	2000	R3	19061788	18800085	17107234	1692851	226428	98.63	12127628	11018724	1108904	63.62	8320545	59.02	FALSE		FALSE		TRUE
59DNQR_16_sa	59DNQR	Muscle	PRNP173	2000	R2	19535193	19271914	17558096	1713818	224813	98.65	14401474	13107418	1294056	73.72	9743750	57.95	FALSE		FALSE		TRUE
59DNQR_17_sa	59DNQR	Muscle	PRNP173	500	R3	18947953	18704634	17043631	1661003	199477	98.72	13175925	11989875	1186050	69.54	9362599	61.51	FALSE		FALSE		TRUE
59DNQR_18_sa	59DNQR	Muscle	PRNP174	8000	R3	19480739	19240886	17486084	1754802	208022	98.77	12653677	11482577	1171100	64.95	9344830	64.38	FALSE		FALSE		TRUE
59DNQR_19_sa	59DNQR	Muscle	PRNP183	32000	R4	20687204	20409981	18595148	1814833	238017	98.66	13144600	11951002	1193598	63.54	9038838	58.76	FALSE		FALSE		TRUE
59DNQR_20_sa	59DNQR	Muscle	PRNP174	32000	R2	19613858	19361404	17631591	1729813	214127	98.71	12741783	11584266	1157517	64.96	8845684	60.03	FALSE		FALSE		TRUE
59DNQR_21_sa	59DNQR	Muscle	PRNP174	0	R1	19281909	19057773	17293325	1764448	190906	98.84	11628242	10530463	1097779	60.31	8509865	63.51	FALSE		FALSE		TRUE
59DNQR_22_sa	59DNQR	Muscle	PRNP174	500	R4	19802954	19512985	17694179	1818806	249420	98.54	12871848	11656552	1215296	65	8408206	54.54	FALSE		FALSE		TRUE
59DNQR_23_sa	59DNQR	Muscle	PRNP183	32000	R3	19018386	18764380	17005353	1759027	217226	98.66	12809138	11582621	1226517	67.35	8696897	56.69	FALSE		FALSE		TRUE
59DNQR_24_sa	59DNQR	Muscle	PRNP183	500	R3	20828461	20553300	18659660	1893640	235768	98.68	13684552	12407711	1276841	65.7	9367568	58.23	FALSE		FALSE		TRUE
59DNQR_25_sa	59DNQR	Muscle	PRNP174	8000	R4	20450354	20195211	18405062	1790149	207553	98.75	14158551	12886689	1271862	69.23	10001960	61.38	FALSE		FALSE		TRUE
59DNQR_26_sa	59DNQR	Muscle	PRNP174	500	R1	19658233	19422756	17576486	1846270	192640	98.8	13212382	11952322	1260060	67.21	9834272	63.5	FALSE		FALSE		TRUE
59DNQR_27_sa	59DNQR	Muscle	PRNP173	32000	R3	20686742	20438099	18554810	1883289	203150	98.8	12452252	11285709	1166543	60.19	9171793	63.75	FALSE		FALSE		TRUE
59DNQR_28_sa	59DNQR	Muscle	PRNP174	32000	R1	19567914	19324777	17544939	1779838	208703	98.76	12073992	10943268	1130724	61.7	8551207	60.78	FALSE		FALSE		TRUE
59DNQR_29_sa	59DNQR	Muscle	PRNP174	128000	R4	19127733	18878819	17154242	1724577	214217	98.7	11769999	10677856	1092143	61.53	8119637	59.33	FALSE		FALSE		TRUE
59DNQR_30_sa	59DNQR	Muscle	PRNP183	2000	R2	20480614	20225807	18433370	1792437	217760	98.76	14528548	13231337	1297211	70.94	10183805	60.71	FALSE		FALSE		TRUE
59DNQR_31_sa	59DNQR	Muscle	PRNP183	128000	R1	20492506	20225725	18327999	1897726	230523	98.7	13339678	12069769	1269909	65.1	9294873	58.62	FALSE		FALSE		TRUE
59DNQR_32_sa	59DNQR	Muscle	PRNP183	32000	R1	20168519	19871933	17822278	2049655	256319	98.53	13886395	12423322	1463073	68.85	9262526	52.64	FALSE		FALSE		TRUE
59DNQR_33_sa	59DNQR	Muscle	PRNP183	0	R3	19479253	19217959	17492345	1725614	227016	98.66	12246053	11131619	1114434	62.87	8613023	60.9	FALSE		FALSE		TRUE

sample_id	batch	cell_line	construct	moi	replicate	input_reads	mapped_reads	unique_mapped_	multi_mapped_r	unmapped_reads	mapping_rate_pc	dedup_mapped_	dedup_unique_r	dedup_multi_rea	dedup_rate_pct	assigned_reads	assigned_pct	outlier_flag	outlier_reason	failure_flag	failure_reasons	included_in_analysis
59DNQR_34_sai	59DNQR	Muscle	PRNP183	8000	R3	20456896	20220301	18318665	1901636	202788	98.84	12612461	11410975	1201486	61.65	9461843	65.1	FALSE		FALSE		TRUE
59DNQR_35_sai	59DNQR	Muscle	PRNP183	128000	R2	19613071	19365564	17570636	1794928	212489	98.74	12877195	11668231	1208964	65.66	9268951	61.94	FALSE		FALSE		TRUE
59DNQR_36_sai	59DNQR	Muscle	PRNP173	128000	R4	20169295	19918148	18084475	1833673	215323	98.75	13132902	11910137	1222765	65.11	9287254	60.82	FALSE		FALSE		TRUE
59DNQR_37_sai	59DNQR	Muscle	PRNP173	128000	R2	20524594	20269999	18417147	1852852	220280	98.76	13112121	11898507	1213614	63.88	9500837	62.76	FALSE		FALSE		TRUE
59DNQR_38_sai	59DNQR	Muscle	PRNP173	500	R2	19123287	18877328	17150096	1727232	209494	98.71	13391479	12150351	1241128	70.03	9188408	58.31	FALSE		FALSE		TRUE
59DNQR_39_sai	59DNQR	Muscle	PRNP174	2000	R4	20629600	20368976	18537060	1831916	222864	98.74	14111384	12829986	1281398	68.4	9814811	60.04	FALSE		FALSE		TRUE
59DNQR_40_sai	59DNQR	Muscle	PRNP174	128000	R4	20075914	19818797	18029405	1789392	220711	98.72	13744647	12484908	1259739	68.46	9612316	60.16	FALSE		FALSE		TRUE
59DNQR_41_sai	59DNQR	Muscle	PRNP173	500	R3	18857111	18640365	16952996	1687369	184555	98.85	12768325	11596950	1171375	67.71	9357361	63.49	FALSE		FALSE		TRUE
59DNQR_42_sai	59DNQR	Muscle	PRNP183	8000	R3	20474676	20221852	18305157	1916695	217298	98.77	12710657	11492125	1218532	62.08	9192947	61.68	FALSE		FALSE		TRUE
59DNQR_43_sai	59DNQR	Muscle	PRNP183	0	R3	19264496	19045854	17251760	1794094	186314	98.87	11938444	10805989	1132455	61.97	8808402	63.98	FALSE		FALSE		TRUE
59DNQR_44_sai	59DNQR	Muscle	PRNP173	8000	R1	19410199	19171953	17442617	1729336	204445	98.77	12667027	11514249	1152778	65.26	8923137	61.05	FALSE		FALSE		TRUE
59DNQR_45_sai	59DNQR	Muscle	PRNP173	128000	R1	20733657	20460930	18653990	1806940	230476	98.68	14753605	13443041	1310564	71.16	9858621	57.9	FALSE		FALSE		TRUE
59DNQR_46_sai	59DNQR	Muscle	PRNP183	8000	R4	20610773	20353278	18407657	1945621	223633	98.75	13287525	11994512	1293013	64.47	9599709	60.55	FALSE		FALSE		TRUE
59DNQR_47_sai	59DNQR	Muscle	PRNP183	128000	R4	19823551	19570653	17832631	1738022	217466	98.72	13556747	12340266	1216481	68.39	9521568	60.76	FALSE		FALSE		TRUE
59DNQR_48_sai	59DNQR	Muscle	PRNP183	0	R1	20575339	20315770	18529180	1786590	222439	98.74	14207447	12948717	1258730	69.05	10062735	61.76	FALSE		FALSE		TRUE
59DNQR_49_sai	59DNQR	Muscle	PRNP173	0	R1	18876690	18637895	16961537	1676358	207269	98.73	12560726	11419895	1140831	66.54	9015718	62.19	FALSE		FALSE		TRUE
59DNQR_50_sai	59DNQR	Muscle	PRNP183	32000	R2	20509854	20265529	18438547	1826982	208817	98.81	14244849	12947350	1297499	69.45	10170378	61.42	FALSE		FALSE		TRUE
59DNQR_51_sai	59DNQR	Muscle	PRNP183	500	R4	19126485	18876758	17031268	1845490	215069	98.69	12756461	11487199	1269262	66.7	8761132	56.57	FALSE		FALSE		TRUE
59DNQR_52_sai	59DNQR	Muscle	PRNP174	8000	R2	19745417	19497207	17703901	1793306	204712	98.74	12496861	11323144	1173717	63.29	9064218	62.13	FALSE		FALSE		TRUE
59DNQR_53_sai	59DNQR	Muscle	PRNP173	32000	R3	20361857	20105917	18292452	1813465	209253	98.74	13677304	12430778	1246526	67.17	9841860	62.46	FALSE		FALSE		TRUE
59DNQR_54_sai	59DNQR	Muscle	PRNP173	8000	R4	19754247	19520022	17678744	1841278	202805	98.81	11910266	10769083	1141183	60.29	8914881	64.6	FALSE		FALSE		TRUE
59DNQR_55_sai	59DNQR	Muscle	PRNP183	0	R4	20625659	20347746	18492427	1855319	241259	98.65	13865279	12589763	1275516	67.22	9733205	60.18	FALSE		FALSE		TRUE
59DNQR_56_sai	59DNQR	Muscle	PRNP183	2000	R1	19000396	18763596	17023643	1739953	205148	98.75	12804216	11600594	1203622	67.39	9275415	62.06	FALSE		FALSE		TRUE
59DNQR_57_sai	59DNQR	Muscle	PRNP173	0	R1	19722809	19491586	17712519	1779067	198683	98.83	13589904	12339623	1250281	68.9	9866213	62.71	FALSE		FALSE		TRUE
59DNQR_58_sai	59DNQR	Muscle	PRNP174	32000	R4	19935321	19698346	17946528	1751818	200907	98.81	13450081	12242151	1207930	67.47	9660586	62.9	FALSE		FALSE		TRUE
59DNQR_59_sai	59DNQR	Muscle	PRNP183	8000	R1	20043145	19791526	17956759	1834767	207075	98.74	13108318	11878568	1229750	65.4	9586524	62.85	FALSE		FALSE		TRUE
59DNQR_60_sai	59DNQR	Muscle	PRNP173	8000	R3	19447057	19207153	17460615	1746538	205994	98.77	12696131	11527068	1169063	65.29	9216637	63.33	FALSE		FALSE		TRUE
59DNQR_61_sai	59DNQR	Muscle	PRNP173	32000	R1	20283475	20024743	18192180	1832563	222616	98.72	14191211	12888612	1302599	69.96	10122620	61.3	FALSE		FALSE		TRUE
59DNQR_62_sai	59DNQR	Muscle	PRNP183	2000	R4	19275412	19041618	17314278	1727340	201447	98.79	12790322	11614397	1175925	66.36	9419062	63.81	FALSE		FALSE		TRUE
59DNQR_63_sai	59DNQR	Muscle	PRNP183	8000	R2	19651235	19390230	17575368	1814862	223041	98.67	13993684	12670269	1323415	71.21	9527639	56.86	FALSE		FALSE		TRUE
59DNQR_64_sai	59DNQR	Muscle	PRNP174	500	R4	18960355	18720475	17049996	1670479	207843	98.73	13353499	12150408	1203091	70.43	9580068	62.2	FALSE		FALSE		TRUE
59DNQR_65_sai	59DNQR	Muscle	PRNP174	0	R4	19758394	19521795	17676976	1844819	206250	98.8	11937473	10792167	1145306	60.42	8834085	63.9	FALSE		FALSE		TRUE
59DNQR_66_sai	59DNQR	Muscle	PRNP173	8000	R2	20529660	20261052	18406678	1854374	223349	98.69	12285259	11137257	1148002	59.84	9025569	63.75	FALSE		FALSE		TRUE
59DNQR_67_sai	59DNQR	Muscle	PRNP173	8000	R1	20370752	20107874	18232810	1875064	227892	98.71	12504651	11316784	1187867	61.39	9190418	63.37	FALSE		FALSE		TRUE
59DNQR_68_sai	59DNQR	Muscle	PRNP173	32000	R1	20386608	20155965	18268949	1887016	196644	98.87	12884796	11661933	1222863	63.2	9647383	64.92	FALSE		FALSE		TRUE
59DNQR_69_sai	59DNQR	Muscle	PRNP174	128000	R3	19497997	19237941	17484574	1753367	226218	98.67	12508083	11357317	1150766	64.15	8832783	61.09	FALSE		FALSE		TRUE
59DNQR_70_sai	59DNQR	Muscle	PRNP183	0	R4	20448874	20202727	18347139	1855588	211070	98.8	12866433	11661942	1204491	62.92	9437261	63.22	FALSE		FALSE		TRUE
59DNQR_71_sai	59DNQR	Muscle	PRNP174	32000	R3	20365403	20103445	18337580	1765865	225511	98.71	14276415	13013198	1263217	70.1	10040508	61.12	FALSE		FALSE		TRUE
59DNQR_72_sai	59DNQR	Muscle	PRNP174	500	R2	19959563	19730802	17900382	1830420	198105	98.85	12690337	11498312	1192025	63.58	9476918	65.6	FALSE		FALSE		TRUE
59DNQR_73_sai	59DNQR	Muscle	PRNP174	8000	R2	20193986	19949432	18125815	1823617	211355	98.79	13109587	11895860	1213727	64.92	9720058	64.8	FALSE		FALSE		TRUE
59DNQR_74_sai	59DNQR	Muscle	PRNP173	500	R4	19031927	18823205	17109739	1713466	178566	98.9	13088114	11885665	1202449	68.77	9899124	66.01	FALSE		FALSE		TRUE
59DNQR_75_sai	59DNQR	Muscle	PRNP174	0	R3	19821889	19575304	17808043	1767261	201973	98.76	13320454	12104074	1216380	67.2	9570561	62.55	FALSE		FALSE		TRUE
59DNQR_76_sai	59DNQR	Muscle	PRNP183	500	R4	18893636	18668707	16843463	1825244	196240	98.81	11243078	10174474	1068604	59.51	8413573	64.93	FALSE		FALSE		TRUE
59DNQR_77_sai	59DNQR	Muscle	PRNP183	500	R3	19860748	19614400	17789413	1824987	214060	98.76	12032578	10901676	1130902	60.58	8820395	63.86	FALSE		FALSE		TRUE
59DNQR_78_sai	59DNQR	Muscle	PRNP183	500	R1	19697492	19455647	17680592	1775055	198531	98.77	12888888	11697192	1191696	65.43	9505357	63.99	FALSE		FALSE		TRUE
59DNQR_79_sai	59DNQR	Muscle	PRNP174	500	R3	19206397	18963973	17290414	1673559	208041	98.74	13248959	12066230	1182729	68.98	9446586	62.19	FALSE		FALSE		TRUE
59DNQR_80_sai	59DNQR	Muscle	PRNP174	128000	R2	19142939	18910884	17192298	1718586	198182	98.79	12533854	11376386	1157468	65.48	9091909	62.91	FALSE		FALSE		TRUE
59DNQR_81_sai	59DNQR	Muscle	PRNP174	2000	R2	20235052	19985167	18176211	1808956	211806	98.77	13561878	12322268	1239610	67.02	9647879	61.59	FALSE		FALSE		TRUE
59DNQR_82_sai	59DNQR	Muscle	PRNP183	2000	R3	19828808	19625250	17712652	1912598	175248	98.97	12925443	11663379	1262064	65.19	9944482	66.33	FALSE		FALSE		TRUE
59DNQR_83_sai	59DNQR	Muscle	PRNP174	8000	R1	19168059	18932734	17181323	1751411	202582	98.77	11969407	10846032	1123375	62.44	8837913	64.08	FALSE		FALSE		TRUE
59DNQR_84_sai	59DNQR	Muscle	PRNP174	0	R2	19875122	19642256	17890244	1752012	198082	98.83	13675580	12445588	1229992	68.81	10011169	63.98	FALSE		FALSE		TRUE
59DNQR_85_sai	59DNQR	Muscle	PRNP173	500	R1	18904885	18642941	16956524	1686417	228602	98.61	11855888	10771073	1084815	62.71	8343110	61.24	FALSE		FALSE		TRUE
59DNQR_86_sai	59DNQR	Muscle	PRNP174	128000	R1	19983547	19750375	17932933	1817442	200034	98.83	13870032	12592157	1277875	69.41	10099018	63.18	FALSE		FALSE		TRUE
59DNQR_87_sai	59DNQR	Muscle	PRNP174	32000	R4	19235830	19011509	17235857	1775652	196071	98.83	12651370	11459677	1191693	65.77	9567687	65.67	FALSE		FALSE		TRUE
59DNQR_88_sai	59DNQR	Muscle	PRNP173	2000	R1	20840458	20575115	18739061	1836054	228720	98.73	14575635	13267427	1308208	69.94	10377514	62.14	FALSE		FALSE		TRUE
59DNQR_89_sai	59DNQR	Muscle	PRNP183	32000	R3	20431583	20239318	18194278	2045040	167900	99.06	12643771	11370251	1273520	61.88	10211114	70.1	FALSE		FALSE		TRUE
59DNQR_90_sai	59DNQR	Muscle	PRNP173	128000	R3	19497264	19302257	17370781	1931476	159149	99	12113341	10893030	1220311	62.13	9965554	71.54	FALSE		FALSE		TRUE
59DNQR_91_sai	59DNQR	Muscle	PRNP174	32000	R2	20097056	19847585	18011311	1836274	207462	98.76	12434875	11271426	1163449	61.87	9288917	64.93	FALSE		FALSE		TRUE
59DNQR_92_sai	59DNQR	Muscle	PRNP183	32000	R4	18904219	18687970	16981282	1706688	184876	98.86	13025633	11826552	1199081	68.9	9758360	65.63	FALSE		FALSE		TRUE
59DNQR_93_sai	59DNQR	Muscle	PRNP173	8000	R3	20565476	20339279	18430684	1908595	196426	98.9	12886009	11667533	1218476	62.66	9857409	66.9	FALSE		FALSE		TRUE
59DNQR_94_sai	59DNQR	Muscle	PRNP173	32000	R4	18966101	18749746	16940884	1808862	189085	98.86	12254198	11070996	1183202	64.61	9294173	65.47	FALSE		FALSE		TRUE
59DNQR_95_sai	59DNQR	Muscle	PRNP173	128000	R3	20828167	20562058	18615258	1946800	230187	98.72	14975479	13558571	1416908	71.9	10753060	61.05	FALSE		FALSE		TRUE
59DNQR_96_sai	59DNQR	Muscle	PRNP173	128000	R1	19180615	18949914	17208879	1741035	197883	98.8	13901821	12644429	1257392	72.48	9976961	62.21	FALSE		FALSE		TRUE
59DNQR_97_sai	59DNQR	Muscle	PRNP183	8000	R4	19147282	18896646	17208483	1688163	212269	98.69	11489522	10440261	1049261	60.01	8414855	61.82	FALSE		FALSE		TRUE
59DNQR_98_sai	59DNQR	Muscle	PRNP174	500	R2	19136230	18865733	17218054	1647679	228748	98.59	11673700	10624055	1049645	61	8218033	59.38	FALSE		FALSE		TRUE
59DNQR_99_sai	59DNQR	Muscle	PRNP174	0	R4	19728231	19430669	17552765	1877904	253638	98.49	12490596	11246053	1244543	63.31	8483494	53.63	FALSE		FALSE		TRUE
59DNQR_100_si	59DNQR	Muscle	PRNP183	128000	R4	19397396	19140119	17426729	1713390	216778	98.67	12012077	10922682	1089395	61.93	8737111	61.84	FALSE		FALSE		TRUE
59DNQR_101_si	59DNQR	Muscle	PRNP174	2000	R3	20064423	19780647	18014050	1766597	239388	98.59	13058676	11864450	1194226	65.08	9036268	57.25	FALSE		FALSE		TRUE
59DNQR_102_si	59DNQR	Muscle	PRNP183	500	R2	19201360	18931010	17258264	1672746	229080	98.59	13128206	11942594	1185612	68.37	9308078	59.41	FALSE		FALSE		TRUE
59DNQR_103_si	59DNQR	Muscle	PRNP173	500	R1	20294163	20015446	18396002	1619444	238360	98.63	14328581	13155052	1173529	70.6	9422985	57.23	FALSE		FALSE		TRUE
59DNQR_104_si	59DNQR	Muscle	PRNP183	2000	R3	19399340	19143243	17541136	1602107	222314	98.68	12908992	11810244	1098748	66.54	8859537	59.03	FALSE		FALSE		TRUE
59DNQR_105_si	59DNQR	Muscle	PRNP183	128000	R3	20616632	20350860	18593012	1757848	222982	98.71	12690953	11574377	1116576	61.56	9241832	62.59	FALSE		FALSE		TRUE
59DNQR_106_si	59DNQR	Muscle	PRNP174	0	R2	19943630	19674397	17991836	1682561	227565	98.65	12396708	11312175	1084533	62.16	8918941	61.5	FALSE		FALSE		TRUE
59DNQR_107_si	59DNQR	Muscle	PRNP173	0	R4	19460646	19194047	17478875	1715172	225139	98.63	12132238	11021032	1111206	62.34	8785006	60.9	FALSE		FALSE		TRUE
59DNQR_108_si	59DNQR	Muscle	PRNP174	2000	R4	20713600	20495050	18612514	1882536	177282	98.94	12452486	11290630	1161856	60.12	9715535	67.25	FALSE		FALSE		TRUE
PMJTDJ_1_sam	PMJTDJ	SVGp12	PRNP173	128000	R1	19966187	19687213	18028619	1658594	249847	98.6	10140854	9252392	888462	50.79	7784772	64.57	FALSE		FALSE		TRUE
PMJTDJ_2_sam	PMJTDJ	SVGp12	PRNP183	8000	R1	18393822	18178431	16353825	1824606	191183	98.83	9022964	8083119	939845	49.05	7446595	66.88	FALSE		FALSE		TRUE
PMJTDJ_3_sam	PMJTDJ	SVGp12	PRNP173	500	R1	20018609	19754290	17744202	2010088	244679	98.68	10698583	9564518	1134065	53.44	8107183	57.73	FALSE		FALSE		TRUE
PMJTDJ_4_sam	PMJTDJ	SVGp12	PRNP183	128000	R2	20285914	20008516	18473139	1535377	252766	98.63	13806441	12740555	1065886	68.06	10357068	65.46	FALSE		FALSE		TRUE
PMJTDJ_5_sam	PMJTDJ	SVGp12	PRNP174	0	R4	19136287	18856229	17260044	1596185	260277	98.54	11796072	10768549	1027523	61.64	8991549	63.49	FALSE		FALSE		TRUE
PMJTDJ_6_sam	PMJTDJ	SVGp12	PRNP173	8000	R3	20357623	20097552	18358212	1739340	240019	98.72	11842306	10782744	1059562	58.17	9097651	64.15	FALSE		FALSE		TRUE
PMJTDJ_7_sam	PMJTDJ	SVGp12	PRNP183	2000	R2	4832285	4755637	4269869	485768	68486	98.41	2359203	2108844	250359	48.82	1806330	61.48	FALSE		FALSE		TRUE
PMJTDJ_8_sam	PMJTDJ	SVGp12	PRNP173	500	R3	19172057	18867309	17436715	1430594	276260	98.41	13455991	12420719	1035272	70.19	9351938	60.64	FALSE		FALSE		TRUE

sample_id	batch	cell_line	construct	moi	replicate	input_reads	mapped_reads	unique_mapped_	multi_mapped_r	unmapped_reads	mapping_rate_pc	dedup_mapped_	dedup_unique_r	dedup_multi_rea	dedup_rate_pct	assigned_reads	assigned_pct	outlier_flag	outlier_reason	failure_flag	failure_reasons	included_in_analysis
PMJTDJ_9_sam	PMJTDJ	SVGp12	PRNP183	500	R4	19357088	19070508	17402917	1667591	257847	98.52	9676274	8804714	871560	49.99	7538530	65.68	FALSE		FALSE		TRUE
PMJTDJ_10_san	PMJTDJ	SVGp12	PRNP173	2000	R4	20073902	19824206	18194825	1629381	231444	98.76	12862431	11785987	1076444	64.08	10152045	67.69	FALSE		FALSE		TRUE
PMJTDJ_11_san	PMJTDJ	SVGp12	PRNP183	0	R4	20593558	20304440	18581040	1723400	267393	98.6	11793593	10761294	1032299	57.27	9070096	64.58	FALSE		FALSE		TRUE
PMJTDJ_12_san	PMJTDJ	SVGp12	PRNP174	128000	R4	20251119	19979411	18330410	1649001	253501	98.66	11789700	10791216	998484	58.22	9257849	67.2	FALSE		FALSE		TRUE
PMJTDJ_13_san	PMJTDJ	SVGp12	PRNP183	0	R4	18856112	18631337	17049880	1581457	208617	98.81	10726501	9796173	930328	56.89	8539243	68.14	FALSE		FALSE		TRUE
PMJTDJ_14_san	PMJTDJ	SVGp12	PRNP183	2000	R4	19478545	19234809	17544306	1690503	224977	98.75	11687527	10631469	1056058	60	9009637	63.7	FALSE		FALSE		TRUE
PMJTDJ_15_san	PMJTDJ	SVGp12	PRNP173	2000	R3	19319559	19033253	17397612	1635641	255662	98.52	9217599	8392718	824881	47.71	6995932	64	FALSE		FALSE		TRUE
PMJTDJ_16_san	PMJTDJ	SVGp12	PRNP174	2000	R3	20480861	20217988	18500554	1717434	239808	98.72	11206873	10223255	983618	54.72	8318224	62.35	FALSE		FALSE		TRUE
PMJTDJ_17_san	PMJTDJ	SVGp12	PRNP183	0	R2	20260805	19972725	18330590	1642135	260355	98.58	11111709	10168151	943558	54.84	8877515	68.54	FALSE		FALSE		TRUE
PMJTDJ_18_san	PMJTDJ	SVGp12	PRNP173	32000	R4	19607492	19337041	17808793	1528248	253073	98.62	13450173	12373688	1076485	68.6	10807544	70.5	FALSE		FALSE		TRUE
PMJTDJ_19_san	PMJTDJ	SVGp12	PRNP173	2000	R4	18923329	18643396	17169866	1473530	256157	98.52	12409500	11407780	1001720	65.58	9159633	63.38	FALSE		FALSE		TRUE
PMJTDJ_20_san	PMJTDJ	SVGp12	PRNP174	2000	R4	19501990	19272766	17644684	1628082	214045	98.82	12270967	11215914	1055053	62.92	9985624	69.89	FALSE		FALSE		TRUE
PMJTDJ_21_san	PMJTDJ	SVGp12	PRNP174	128000	R3	20733696	20502942	18846466	1656476	212603	98.89	13245176	12156805	1088371	63.88	10581302	69.23	FALSE		FALSE		TRUE
PMJTDJ_22_san	PMJTDJ	SVGp12	PRNP183	8000	R2	19375632	19087239	17435872	1651367	260268	98.51	12118339	11042441	1075898	62.54	9518478	65.18	FALSE		FALSE		TRUE
PMJTDJ_23_san	PMJTDJ	SVGp12	PRNP173	32000	R3	19536742	19236679	17585741	1650938	277762	98.46	10568009	9623442	944567	54.09	7758637	60.82	FALSE		FALSE		TRUE
PMJTDJ_24_san	PMJTDJ	SVGp12	PRNP174	0	R3	19776866	19517103	17692399	1824704	231110	98.69	9900229	8947480	952749	50.06	7880133	65.67	FALSE		FALSE		TRUE
PMJTDJ_25_san	PMJTDJ	SVGp12	PRNP183	2000	R3	19578899	19321258	17698409	1622849	230623	98.68	11247740	10275791	971949	57.45	9125029	69.47	FALSE		FALSE		TRUE
PMJTDJ_26_san	PMJTDJ	SVGp12	PRNP173	500	R4	20418093	20136884	18494382	1642502	252817	98.62	12666613	11621527	1045086	62.04	9995518	68.08	FALSE		FALSE		TRUE
PMJTDJ_27_san	PMJTDJ	SVGp12	PRNP183	500	R2	19335968	19052221	17587328	1464893	258320	98.53	13862158	12785323	1076835	71.69	10166174	64.35	FALSE		FALSE		TRUE
PMJTDJ_28_san	PMJTDJ	SVGp12	PRNP183	2000	R1	20139042	19904983	18192064	1712919	205697	98.84	10319933	9401276	918657	51.24	8326681	68.59	FALSE		FALSE		TRUE
PMJTDJ_29_san	PMJTDJ	SVGp12	PRNP183	8000	R3	19401237	19138354	17667628	1470726	235159	98.65	12132880	11185141	947739	62.54	8494177	61.29	FALSE		FALSE		TRUE
PMJTDJ_30_san	PMJTDJ	SVGp12	PRNP174	500	R1	19833003	19644300	17862969	1781331	176251	99.05	11294588	10253555	1041033	56.95	9651843	72.81	FALSE		FALSE		TRUE
PMJTDJ_31_san	PMJTDJ	SVGp12	PRNP173	0	R3	19449351	19162484	17523235	1639249	258423	98.53	11707660	10674823	1032837	60.2	9156384	65.36	FALSE		FALSE		TRUE
PMJTDJ_32_san	PMJTDJ	SVGp12	PRNP183	8000	R4	16709972	16504269	14836924	1667345	180721	98.77	8177176	7293050	884126	48.94	6520314	61.52	FALSE		FALSE		TRUE
PMJTDJ_33_san	PMJTDJ	SVGp12	PRNP183	32000	R1	345318	333530	300071	33459	10859	96.59	153782	138010	15772	44.53	92594	46.18	TRUE		TRUE	fc_post_dedup_u	FALSE
PMJTDJ_34_san	PMJTDJ	SVGp12	PRNP173	2000	R1	5979483	5902477	5310468	592009	72095	98.71	2997518	2692802	304716	50.13	2512748	70.56	FALSE		FALSE		TRUE
PMJTDJ_35_san	PMJTDJ	SVGp12	PRNP173	500	R1	19498660	19195165	17699582	1495583	273273	98.44	12860061	11842613	1017448	65.95	8898661	60.1	FALSE		FALSE		TRUE
PMJTDJ_36_san	PMJTDJ	SVGp12	PRNP174	32000	R3	19819926	19539706	17900744	1638962	252340	98.59	11613470	10615504	997966	58.59	9294009	68.25	FALSE		FALSE		TRUE
PMJTDJ_37_san	PMJTDJ	SVGp12	PRNP174	32000	R4	19208952	18991041	17412411	1578630	203265	98.87	12080226	11062807	1017419	62.89	10003810	72.05	FALSE		FALSE		TRUE
PMJTDJ_38_san	PMJTDJ	SVGp12	PRNP183	0	R1	19804805	19579592	17839160	1740432	209761	98.86	11117254	10099881	1017373	56.13	9023193	67.81	FALSE		FALSE		TRUE
PMJTDJ_39_san	PMJTDJ	SVGp12	PRNP174	128000	R1	20148741	19943849	18096089	1847760	181547	98.98	10543146	9540863	1002283	52.33	9089408	73.24	FALSE		FALSE		TRUE
PMJTDJ_40_san	PMJTDJ	SVGp12	PRNP173	32000	R1	20605985	20314641	18619754	1694887	261167	98.59	11573711	10577409	996302	56.17	9102586	66.9	FALSE		FALSE		TRUE
PMJTDJ_41_san	PMJTDJ	SVGp12	PRNP174	500	R4	20587294	20343630	18592843	1750787	227402	98.82	11210115	10221349	988766	54.45	9083518	69.33	FALSE		FALSE		TRUE
PMJTDJ_42_san	PMJTDJ	SVGp12	PRNP174	32000	R4	19054163	18784779	17243322	1541457	243494	98.59	12123090	11106400	1016690	63.62	9847639	69.68	FALSE		FALSE		TRUE
PMJTDJ_43_san	PMJTDJ	SVGp12	PRNP173	0	R4	19810645	19537601	18000622	1536979	250353	98.62	13255195	12204669	1050526	66.91	10125051	66.85	FALSE		FALSE		TRUE
PMJTDJ_44_san	PMJTDJ	SVGp12	PRNP174	500	R2	5117933	5046019	4524512	521507	64005	98.59	2485229	2222714	262515	48.56	2057159	68.15	FALSE		FALSE		TRUE
PMJTDJ_45_san	PMJTDJ	SVGp12	PRNP174	500	R3	19377644	19104491	17511949	1592542	246754	98.59	11722588	10722445	1000143	60.5	9529388	69.69	FALSE		FALSE		TRUE
PMJTDJ_46_san	PMJTDJ	SVGp12	PRNP174	0	R1	19237750	18999705	17297704	1702001	213282	98.76	10527559	9547880	979679	54.72	8695598	68.45	FALSE		FALSE		TRUE
PMJTDJ_47_san	PMJTDJ	SVGp12	PRNP183	128000	R3	19959166	19740662	18116613	1624049	200632	98.91	11736305	10748656	987649	58.8	9137227	66.89	FALSE		FALSE		TRUE
PMJTDJ_48_san	PMJTDJ	SVGp12	PRNP174	8000	R1	20638763	20395959	18639047	1756912	224007	98.82	10794605	9843538	951067	52.3	8686712	69.76	FALSE		FALSE		TRUE
PMJTDJ_49_san	PMJTDJ	SVGp12	PRNP174	32000	R1	19462965	19227937	17643751	1584186	217603	98.79	11095086	10160300	934786	57.01	8670466	67.46	FALSE		FALSE		TRUE
PMJTDJ_50_san	PMJTDJ	SVGp12	PRNP183	0	R3	19997315	19757243	18095003	1662240	222988	98.8	12239567	11185288	1054279	61.21	9764401	68.27	FALSE		FALSE		TRUE
PMJTDJ_51_san	PMJTDJ	SVGp12	PRNP173	128000	R4	20016675	19756076	17954375	1801701	231891	98.7	10425778	9442783	982995	52.09	8659731	69.62	FALSE		FALSE		TRUE
PMJTDJ_52_san	PMJTDJ	SVGp12	PRNP173	128000	R1	19772114	19557655	17758134	1799521	201764	98.92	10645458	9642188	1003270	53.84	9079473	72.02	FALSE		FALSE		TRUE
PMJTDJ_53_san	PMJTDJ	SVGp12	PRNP173	0	R4	19139153	18901916	17186216	1715700	221979	98.76	10348131	9396564	951567	54.07	8458940	69.29	FALSE		FALSE		TRUE
PMJTDJ_54_san	PMJTDJ	SVGp12	PRNP183	500	R1	20266585	20025916	18396353	1629563	213507	98.81	13008805	11932223	1076582	64.19	10675488	71.42	FALSE		FALSE		TRUE
PMJTDJ_55_san	PMJTDJ	SVGp12	PRNP183	32000	R4	19190717	18981058	17402661	1578397	193423	98.91	12559013	11499405	1059608	65.44	10073577	68.92	FALSE		FALSE		TRUE
PMJTDJ_56_san	PMJTDJ	SVGp12	PRNP183	8000	R4	19384805	19098468	17521826	1576642	269328	98.52	12110351	11090097	1020254	62.47	9604682	68.33	FALSE		FALSE		TRUE
PMJTDJ_57_san	PMJTDJ	SVGp12	PRNP174	32000	R3	20639885	20413284	18647077	1766207	209974	98.9	11115644	10132878	982766	53.86	8965022	68.93	FALSE		FALSE		TRUE
PMJTDJ_58_san	PMJTDJ	SVGp12	PRNP173	2000	R1	2770476	2728374	2457095	271279	37533	98.48	1317297	1186434	130863	47.55	1060059	66.82	FALSE		FALSE		TRUE
PMJTDJ_59_san	PMJTDJ	SVGp12	PRNP174	8000	R4	19729068	19504218	17845378	1658840	210207	98.86	12562274	11476795	1085479	63.67	10313162	70.43	FALSE		FALSE		TRUE
PMJTDJ_60_san	PMJTDJ	SVGp12	PRNP173	32000	R3	20500569	20269992	18447829	1822163	204909	98.88	10782024	9788698	993326	52.59	9116478	72.15	FALSE		FALSE		TRUE
PMJTDJ_61_san	PMJTDJ	SVGp12	PRNP174	2000	R1	18813517	18595123	16995350	1599773	194732	98.84	12242604	11176926	1065678	65.07	10181329	70.54	FALSE		FALSE		TRUE
PMJTDJ_62_san	PMJTDJ	SVGp12	PRNP183	32000	R3	19442381	19246978	17624885	1622093	181176	98.99	11945042	10920193	1024849	61.44	9832503	71.38	FALSE		FALSE		TRUE
PMJTDJ_63_san	PMJTDJ	SVGp12	PRNP183	500	R3	20589350	20311553	18498540	1813013	248750	98.65	13011270	11822789	1188481	63.19	10540411	67	FALSE		FALSE		TRUE
PMJTDJ_64_san	PMJTDJ	SVGp12	PRNP174	8000	R3	19352412	19106257	17462608	1643649	217936	98.73	10947017	9978545	968472	56.57	8696214	67.83	FALSE		FALSE		TRUE
PMJTDJ_65_san	PMJTDJ	SVGp12	PRNP173	0	R3	19369973	19160433	17545736	1614697	195601	98.92	11904978	10886142	1018836	61.46	9876232	71.81	FALSE		FALSE		TRUE
PMJTDJ_66_san	PMJTDJ	SVGp12	PRNP174	0	R4	19329152	19106499	17432267	1674232	209345	98.85	9756186	8877807	878379	50.47	8173390	71.75	FALSE		FALSE		TRUE
PMJTDJ_67_san	PMJTDJ	SVGp12	PRNP183	128000	R4	19248247	19011788	17491001	1520787	220328	98.77	12049663	11067319	982344	62.6	9702877	70.17	FALSE		FALSE		TRUE
PMJTDJ_68_san	PMJTDJ	SVGp12	PRNP174	2000	R3	19257547	19016431	17301325	1715106	213905	98.75	10151563	9213883	937680	52.71	8540044	71.77	FALSE		FALSE		TRUE
PMJTDJ_69_san	PMJTDJ	SVGp12	PRNP183	0	R3	18961097	18704894	17100176	1604718	240821	98.65	10758855	9814893	943962	56.74	8943775	71.7	FALSE		FALSE		TRUE
PMJTDJ_70_san	PMJTDJ	SVGp12	PRNP183	32000	R4	20684220	20421535	18685046	1736489	245610	98.73	12007318	10959390	1047928	58.05	9719567	69.07	FALSE		FALSE		TRUE
PMJTDJ_71_san	PMJTDJ	SVGp12	PRNP183	500	R3	19076873	18876354	17298789	1577565	186715	98.95	12163641	11128197	1035444	63.76	10089224	72.01	FALSE		FALSE		TRUE
PMJTDJ_72_san	PMJTDJ	SVGp12	PRNP183	32000	R3	19259674	19017054	17387515	1629539	216699	98.74	12515684	11419457	1096227	64.98	10199649	68.52	FALSE		FALSE		TRUE
PMJTDJ_73_san	PMJTDJ	SVGp12	PRNP174	500	R3	19538425	19309653	17658549	1651104	214787	98.83	10938205	9984959	953246	55.98	9010695	70.98	FALSE		FALSE		TRUE
PMJTDJ_74_san	PMJTDJ	SVGp12	PRNP174	2000	R2	19530540	19292240	17686939	1605301	213115	98.78	12051293	11029673	1021620	61.7	10161966	73.35	FALSE		FALSE		TRUE
PMJTDJ_75_san	PMJTDJ	SVGp12	PRNP183	2000	R3	19955214	19751192	18042184	1709008	189571	98.98	11261289	10269780	991509	56.43	9317908	71.4	FALSE		FALSE		TRUE
PMJTDJ_76_san	PMJTDJ	SVGp12	PRNP173	0	R1	19639508	19414197	17752099	1662098	212354	98.85	11746138	10740944	1005194	59.81	9839056	72.87	FALSE		FALSE		TRUE
PMJTDJ_77_san	PMJTDJ	SVGp12	PRNP174	500	R4	18957857	18695803	17130785	1565018	248478	98.62	11195386	10241412	953974	59.05	9281242	71.73	FALSE		FALSE		TRUE
PMJTDJ_78_san	PMJTDJ	SVGp12	PRNP174	8000	R2	20180582	19910824	18213880	1696944	252075	98.66	11239919	10257084	982835	55.7	9157102	70.17	FALSE		FALSE		TRUE
PMJTDJ_79_san	PMJTDJ	SVGp12	PRNP173	8000	R1	19086726	18828138	17261003	1567135	232109	98.65	11083960	10137935	946025	58.07	9028003	70.37	FALSE		FALSE		TRUE
PMJTDJ_80_san	PMJTDJ	SVGp12	PRNP174	128000	R3	20712212	20468144	18798758	1669386	228273	98.82	12498962	11456810	1042152	60.35	10274435	71.33	FALSE		FALSE		TRUE
PMJTDJ_81_san	PMJTDJ	SVGp12	PRNP174	128000	R4	19416353	19219777	17456250	1763527	172822	98.99	10541913	9559439	982474	54.29	9093050	74.09	FALSE		FALSE		TRUE
PMJTDJ_82_san	PMJTDJ	SVGp12	PRNP174	128000	R2	20145039	19911423	18176053	1735370	219770	98.84	11327352	10326913	1000439	56.23	9476519	71.76	FALSE		FALSE		TRUE
PMJTDJ_83_san	PMJTDJ	SVGp12	PRNP173	500	R4	19132369	18949799	17141033	1808766	172728	99.05	9519501	8594483	925018	49.76	8346531	74.7	FALSE		FALSE		TRUE
PMJTDJ_84_san	PMJTDJ	SVGp12	PRNP173	128000	R3	19692608	19458029	17870127	1587902	218572	98.81	12104981	11101223	1003758	61.47	10121594	73.12	FALSE		FALSE		TRUE
PMJTDJ_85_san	PMJTDJ	SVGp12	PRNP174	0	R2	19334901	19053581	17465587	1587994	255221	98.55	10551249	9647897	903352	54.57	8733444	71.71	FALSE		FALSE		TRUE
PMJTDJ_86_san	PMJTDJ	SVGp12	PRNP183	128000	R3	19101719	18886795	17301952	1584843	201056	98.87	11708520	10715362	993158	61.3	9748867	72.22	FALSE		FALSE		TRUE
PMJTDJ_87_san	PMJTDJ	SVGp12	PRNP183	32000	R2	20325044	20061674	18427459	1634215	236402	98.7	11958061	10963521	994540	58.83	9952400	72.7	FALSE		FALSE		TRUE
PMJTDJ_88_san	PMJTDJ	SVGp12	PRNP173	128000	R4	18868704	18668579	17014342	1654237	187777	98.94	11005302	10014148	991154	58.33	9385625	73.75	FALSE		FALSE		TRUE
PMJTDJ_89_san	PMJTDJ	SVGp12	PRNP173	8000	R4	20130931	19902133	18138007	1764126	214695	98.86	10801973	9833221	968752	53.66	8976854	71.43	FALSE		FALSE		TRUE
PMJTDJ_90_san	PMJTDJ	SVGp12	PRNP174	0	R3	20413938	20155286	18463196	1692090	234294	98.73	15351284	14053829	1297455	75.2	13328940	75.39	FALSE		FALSE		TRUE
PMJTDJ_91_san	PMJTDJ	SVGp12	PRNP174	8000	R4	19730497	19475306	17764487	1710819	230041	98.71	10258711	9336510	922201	51.99	8623007	72.05	FALSE		FALSE		TRUE

sample_id	batch	cell_line	construct	moi	replicate	input_reads	mapped_reads	unique_mapped_	multi_mapped_r	unmapped_reads	mapping_rate_pc	dedup_mapped_	dedup_unique_r	dedup_multi_rea	dedup_rate_pct	assigned_reads	assigned_pct	outlier_flag	outlier_reason	failure_flag	failure_reasons	included_in_analysis
PMJTDJ_92_sam	PMJTDJ	SVGp12	PRNP173	32000	R1	19888088	19662488	17968816	1693672	200718	98.87	11726131	10702647	1023484	58.96	9893465	72.68	FALSE		FALSE		TRUE
PMJTDJ_93_sam	PMJTDJ	SVGp12	PRNP173	0	R1	20321847	20052635	18338880	1713755	244569	98.68	11334992	10344980	990012	55.78	9678579	73.76	FALSE		FALSE		TRUE
PMJTDJ_94_sam	PMJTDJ	SVGp12	PRNP183	8000	R3	18456974	18228964	16489855	1739109	199976	98.76	8915748	8047090	868658	48.31	7460062	70.92	FALSE		FALSE		TRUE
PMJTDJ_95_sam	PMJTDJ	SVGp12	PRNP183	128000	R1	20518292	20287600	18550481	1737119	214654	98.88	12170616	11116221	1054395	59.32	9934917	69.67	FALSE		FALSE		TRUE
PMJTDJ_96_sam	PMJTDJ	SVGp12	PRNP173	8000	R3	19171422	18903917	17174388	1729529	239393	98.6	9874306	8980581	893725	51.51	7876368	67.87	FALSE		FALSE		TRUE
PMJTDJ_97_sam	PMJTDJ	SVGp12	PRNP174	8000	R3	19581204	19255064	17752309	1502755	305311	98.33	12106257	11144229	962028	61.83	9104688	64.35	FALSE		FALSE		TRUE
PMJTDJ_98_sam	PMJTDJ	SVGp12	PRNP183	2000	R4	20316169	19955664	18447585	1508079	336752	98.23	14014386	12940463	1073923	68.98	10444363	64.2	FALSE		FALSE		TRUE
PMJTDJ_99_sam	PMJTDJ	SVGp12	PRNP173	500	R3	19481074	19112846	17247921	1864925	342482	98.11	10161038	9127009	1034029	52.16	8149058	62.05	FALSE		FALSE		TRUE
PMJTDJ_100_sa	PMJTDJ	SVGp12	PRNP174	2000	R4	19995287	19662400	17985558	1676842	308388	98.34	9835004	8979399	855605	49.19	8049546	68.69	FALSE		FALSE		TRUE
PMJTDJ_101_sa	PMJTDJ	SVGp12	PRNP173	128000	R3	18994218	18682848	17006790	1676058	288103	98.36	9209696	8349097	860599	48.49	7647987	67.49	FALSE		FALSE		TRUE
PMJTDJ_102_sa	PMJTDJ	SVGp12	PRNP173	32000	R4	20507416	20193830	18446651	1747179	299342	98.47	10815084	9848043	967041	52.74	8815019	67.64	FALSE		FALSE		TRUE
PMJTDJ_103_sa	PMJTDJ	SVGp12	PRNP183	500	R4	20645867	20332065	18762439	1569626	294143	98.48	13014715	11995220	1019495	63.04	10060179	66.61	FALSE		FALSE		TRUE
PMJTDJ_104_sa	PMJTDJ	SVGp12	PRNP183	128000	R4	20349846	20054999	18379991	1675008	279409	98.55	10173078	9292171	880907	49.99	8110075	66.92	FALSE		FALSE		TRUE
PMJTDJ_105_sa	PMJTDJ	SVGp12	PRNP174	32000	R2	20742150	20384047	18814033	1570014	327397	98.27	12875852	11867870	1007982	62.08	10067540	67.5	FALSE		FALSE		TRUE
PMJTDJ_106_sa	PMJTDJ	SVGp12	PRNP173	8000	R1	19212914	18904035	17341974	1562061	283573	98.39	10534157	9642126	892031	54.83	8452389	67.61	FALSE		FALSE		TRUE
PMJTDJ_107_sa	PMJTDJ	SVGp12	PRNP173	2000	R3	19516671	19202011	17636485	1565526	287204	98.39	13127813	12038811	1089002	67.26	10478726	67.45	FALSE		FALSE		TRUE
PMJTDJ_108_sa	PMJTDJ	SVGp12	PRNP173	8000	R4	19358193	19036684	17543668	1493016	293776	98.34	12460787	11468521	992266	64.37	9950753	68.61	FALSE		FALSE		TRUE
B48LG7_1_samp	B48LG7	CMEC	PRNP174	32000	R1	19850419	19574630	17874015	1700615	253563	98.61	10140081	9229784	910297	51.08	7699855	64.04	FALSE		FALSE		TRUE
B48LG7_2_samp	B48LG7	CMEC	PRNP174	8000	R3	20068641	19762425	17968166	1794259	274239	98.47	9538726	8632791	905935	47.53	7272213	63.04	FALSE		FALSE		TRUE
B48LG7_3_samp	B48LG7	CMEC	PRNP183	8000	R4	19361145	19057850	17267757	1790093	278667	98.43	9895742	8926759	968983	51.11	7026457	56.37	FALSE		FALSE		TRUE
B48LG7_4_samp	B48LG7	CMEC	PRNP173	0	R2	19578780	19284770	17596292	1688478	263515	98.5	10031924	9144080	887844	51.24	7712144	65.32	FALSE		FALSE		TRUE
B48LG7_5_samp	B48LG7	CMEC	PRNP183	0	R2	20401855	20106880	18258012	1848868	264413	98.55	10348889	9348083	1000806	50.73	8009672	62.44	FALSE		FALSE		TRUE
B48LG7_6_samp	B48LG7	CMEC	PRNP183	128000	R4	19576130	19288627	17591932	1696695	257077	98.53	10613049	9635754	977295	54.21	8042902	62.51	FALSE		FALSE		TRUE
B48LG7_7_samp	B48LG7	CMEC	PRNP174	8000	R4	20735047	20425120	18722485	1702635	284968	98.51	9956411	9102277	854134	48.02	7348199	63.72	FALSE		FALSE		TRUE
B48LG7_8_samp	B48LG7	CMEC	PRNP174	32000	R3	20156299	19850247	18068048	1782199	272261	98.48	9610628	8714658	895970	47.68	7276048	63.21	FALSE		FALSE		TRUE
B48LG7_9_samp	B48LG7	CMEC	PRNP174	2000	R1	19736624	19458854	17883074	1575780	247950	98.59	10239947	9385722	854225	51.88	7830340	66.33	FALSE		FALSE		TRUE
B48LG7_10_sam	B48LG7	CMEC	PRNP173	8000	R4	20707037	20411838	18613221	1798617	265010	98.57	10410895	9465171	945724	50.28	8173034	66.2	FALSE		FALSE		TRUE
B48LG7_11_sam	B48LG7	CMEC	PRNP174	500	R2	18946810	18669376	17005445	1663931	247769	98.54	10098191	9160119	938072	53.3	7741121	63.72	FALSE		FALSE		TRUE
B48LG7_12_sam	B48LG7	CMEC	PRNP173	128000	R3	20842707	20535067	18802117	1732950	276565	98.52	11050588	10092116	958472	53.02	8514157	66.04	FALSE		FALSE		TRUE
B48LG7_13_sam	B48LG7	CMEC	PRNP183	128000	R3	19511821	19238591	17618520	1620071	243837	98.6	9895149	9040403	854746	50.71	7755407	67.84	FALSE		FALSE		TRUE
B48LG7_14_sam	B48LG7	CMEC	PRNP183	2000	R1	20633982	20348159	18534408	1813751	263219	98.61	10509187	9534570	974617	50.93	8051838	63.5	FALSE		FALSE		TRUE
B48LG7_15_sam	B48LG7	CMEC	PRNP173	128000	R4	19093525	18819702	17273370	1546332	245616	98.57	9626603	8811718	814885	50.42	7480746	67.26	FALSE		FALSE		TRUE
B48LG7_16_sam	B48LG7	CMEC	PRNP174	128000	R3	20599878	20296939	18473531	1823408	275869	98.53	10328829	9373804	955025	50.14	7352890	59.48	FALSE		FALSE		TRUE
B48LG7_17_sam	B48LG7	CMEC	PRNP183	2000	R4	20005928	19734362	18074387	1659975	239241	98.64	10987252	10042766	944486	54.92	8388982	65.99	FALSE		FALSE		TRUE
B48LG7_18_sam	B48LG7	CMEC	PRNP183	0	R1	20492269	20228310	18564104	1664206	245268	98.71	10919474	10008284	911190	53.29	8655989	69.42	FALSE		FALSE		TRUE
B48LG7_19_sam	B48LG7	CMEC	PRNP173	500	R4	19396984	19115623	17492885	1622738	251510	98.55	9593125	8751107	842018	49.46	7519020	66.93	FALSE		FALSE		TRUE
B48LG7_20_sam	B48LG7	CMEC	PRNP173	2000	R4	20400307	20120444	18388783	1731661	245858	98.63	10255645	9348958	906687	50.27	7661601	64.39	FALSE		FALSE		TRUE
B48LG7_21_sam	B48LG7	CMEC	PRNP183	32000	R3	20182899	19906490	18166028	1740462	242625	98.63	9691746	8818269	873477	48.02	7219197	64.14	FALSE		FALSE		TRUE
B48LG7_22_sam	B48LG7	CMEC	PRNP174	500	R4	19958356	19651153	17866547	1784606	275197	98.46	9707095	8789163	917932	48.64	7247066	61.6	FALSE		FALSE		TRUE
B48LG7_23_sam	B48LG7	CMEC	PRNP173	32000	R4	19886571	19611995	17863554	1748441	244277	98.62	10515874	9539716	976158	52.88	8251803	65.12	FALSE		FALSE		TRUE
B48LG7_24_sam	B48LG7	CMEC	PRNP183	2000	R2	20208481	19916698	18225625	1691073	257310	98.56	10089828	9205359	884469	49.93	7539103	63.91	FALSE		FALSE		TRUE
B48LG7_25_sam	B48LG7	CMEC	PRNP174	8000	R1	19990558	19750208	18128657	1621551	218779	98.8	12462544	11427861	1034683	62.34	9783648	69.17	FALSE		FALSE		TRUE
B48LG7_26_sam	B48LG7	CMEC	PRNP174	500	R1	19564097	19299002	17685408	1613594	237199	98.64	10738479	9818887	919592	54.89	8482823	67.76	FALSE		FALSE		TRUE
B48LG7_27_sam	B48LG7	CMEC	PRNP183	500	R2	20512646	20251707	18526265	1725442	239902	98.73	10470279	9558022	912257	51.04	8335012	69.81	FALSE		FALSE		TRUE
B48LG7_28_sam	B48LG7	CMEC	PRNP173	0	R1	20133675	19847377	18132572	1714805	253942	98.58	9391348	8549651	841697	46.64	6956735	63.41	FALSE		FALSE		TRUE
B48LG7_29_sam	B48LG7	CMEC	PRNP183	32000	R1	20347347	20071274	18386632	1684642	243810	98.64	10828805	9899814	928991	53.22	8286999	66.5	FALSE		FALSE		TRUE
B48LG7_30_sam	B48LG7	CMEC	PRNP174	2000	R4	19337680	19081721	17474975	1606746	223116	98.68	10423302	9527129	896173	53.9	7814297	65.45	FALSE		FALSE		TRUE
B48LG7_31_sam	B48LG7	CMEC	PRNP183	32000	R4	20468881	20173165	18430167	1742998	270750	98.56	10224644	9309580	915064	49.95	7591009	63.05	FALSE		FALSE		TRUE
B48LG7_32_sam	B48LG7	CMEC	PRNP173	128000	R1	20679960	20339819	18398436	1941383	314596	98.36	10640962	9538278	1102684	51.46	7494239	53.98	FALSE		FALSE		TRUE
B48LG7_33_sam	B48LG7	CMEC	PRNP173	0	R4	19979500	19713423	18105593	1607830	237710	98.67	9906523	9077434	829089	49.58	7969896	70.83	FALSE		FALSE		TRUE
B48LG7_34_sam	B48LG7	CMEC	PRNP173	8000	R1	19587201	19315993	17710748	1605245	242349	98.62	9450809	8642652	808157	48.25	7394216	68.21	FALSE		FALSE		TRUE
B48LG7_35_sam	B48LG7	CMEC	PRNP183	128000	R1	20870969	20582298	18815870	1766428	256693	98.62	10309352	9404610	904742	49.4	7943982	66.23	FALSE		FALSE		TRUE
B48LG7_36_sam	B48LG7	CMEC	PRNP173	2000	R1	20540455	20272827	18569577	1703250	236667	98.7	9958094	9096197	861897	48.48	7783868	68.25	FALSE		FALSE		TRUE
B48LG7_37_sam	B48LG7	CMEC	PRNP173	32000	R3	20823701	20526636	18801022	1725614	262586	98.57	10337363	9446312	891051	49.64	8017705	67.78	FALSE		FALSE		TRUE
B48LG7_38_sam	B48LG7	CMEC	PRNP173	500	R1	19508174	19254892	17596050	1658842	232364	98.7	10876402	9918082	958320	55.75	8463300	66.82	FALSE		FALSE		TRUE
B48LG7_39_sam	B48LG7	CMEC	PRNP183	0	R4	20472366	20205478	18528237	1677241	236185	98.7	10118274	9254334	863940	49.42	7806507	67.04	FALSE		FALSE		TRUE
B48LG7_40_sam	B48LG7	CMEC	PRNP173	8000	R3	20655300	20370286	18651053	1719233	259850	98.62	11362389	10386629	975760	55.01	8247277	62.7	FALSE		FALSE		TRUE
B48LG7_41_sam	B48LG7	CMEC	PRNP183	8000	R3	20504964	20266283	18543593	1722690	218895	98.84	11583301	10584278	999023	56.49	9462827	71.95	FALSE		FALSE		TRUE
B48LG7_42_sam	B48LG7	CMEC	PRNP174	0	R4	19346423	19082525	17456962	1625563	235210	98.64	10016948	9140124	876824	51.78	7838985	67.23	FALSE		FALSE		TRUE
B48LG7_43_sam	B48LG7	CMEC	PRNP173	500	R2	19710928	19444083	17713375	1730708	234699	98.65	10113625	9203257	910368	51.31	7834160	67.02	FALSE		FALSE		TRUE
B48LG7_44_sam	B48LG7	CMEC	PRNP174	32000	R4	20784592	20510367	18785447	1724920	251281	98.68	9398564	8580238	818326	45.22	7238664	67.12	FALSE		FALSE		TRUE
B48LG7_45_sam	B48LG7	CMEC	PRNP174	128000	R1	19450321	19211151	17602964	1608187	218515	98.77	11439700	10467911	971789	58.81	8998591	69.22	FALSE		FALSE		TRUE
B48LG7_46_sam	B48LG7	CMEC	PRNP183	8000	R1	20564438	20273157	18467851	1805306	264694	98.58	9426515	8546038	880477	45.84	6538669	57.81	FALSE		FALSE		TRUE
B48LG7_47_sam	B48LG7	CMEC	PRNP173	32000	R1	19073271	18810844	17254384	1556460	233833	98.62	9563799	8750408	813391	50.14	7440981	67.66	FALSE		FALSE		TRUE
B48LG7_48_sam	B48LG7	CMEC	PRNP173	2000	R2	20143026	19888023	18194242	1693781	233479	98.73	11117939	10158897	959042	55.19	8850607	69.88	FALSE		FALSE		TRUE
B48LG7_49_sam	B48LG7	CMEC	PRNP174	0	R1	19938048	19681504	18098921	1582583	228469	98.71	10849763	9961470	888293	54.42	8677749	70.94	FALSE		FALSE		TRUE
B48LG7_50_sam	B48LG7	CMEC	PRNP174	128000	R4	18965959	18726717	17105034	1621683	212038	98.74	10194569	9285099	909470	53.75	8079881	68.06	FALSE		FALSE		TRUE
B48LG7_51_sam	B48LG7	CMEC	PRNP174	0	R2	19987820	19736599	17980661	1755938	223453	98.74	11490701	10430593	1060108	57.49	9221184	66.98	FALSE		FALSE		TRUE
B48LG7_52_sam	B48LG7	CMEC	PRNP183	500	R4	19915857	19649044	18016163	1632881	233908	98.66	9992426	9132711	859715	50.17	7365882	63.9	FALSE		FALSE		TRUE
B48LG7_53_sam	B48LG7	CMEC	PRNP174	2000	R2	19860007	19598944	17898882	1700062	229860	98.69	10745321	9794994	950327	54.11	8424329	68.2	FALSE		FALSE		TRUE
B48LG7_54_sam	B48LG7	CMEC	PRNP183	500	R1	20556476	20289447	18557006	1732441	244720	98.7	9385710	8560762	824948	45.66	6983602	64.8	FALSE		FALSE		TRUE
DBJDCT_1_sam	DBJDCT	Muscle	PRNP174	32000	R1	7417663	7332867	6637310	695557	70688	98.86	3333938	3005533	328405	44.95	2571935	64.1	FALSE		FALSE		TRUE
DBJDCT_2_sam	DBJDCT	Muscle	PRNP174	8000	R3	3525637	3467673	3120936	346737	50107	98.36	1572153	1407277	164876	44.59	1159492	59.57	FALSE		FALSE		TRUE
DBJDCT_3_sam	DBJDCT	Muscle	PRNP183	8000	R4	798996	758007	692631	65376	37767	94.87	330690	301846	28844	41.39	81972	19.92	TRUE		TRUE	fc_assigned_pct;	FALSE
DBJDCT_4_sam	DBJDCT	Muscle	PRNP173	0	R2	19293761	19024125	17367434	1656691	232634	98.6	13908179	12695065	1213114	72.09	9207354	56.32	FALSE		FALSE		TRUE
DBJDCT_5_sam	DBJDCT	Muscle	PRNP183	0	R2	19026823	18758869	17122811	1636058	231687	98.59	13294059	12122987	1171072	69.87	8891532	56.18	FALSE		FALSE		TRUE
DBJDCT_6_sam	DBJDCT	Muscle	PRNP183	128000	R4	19613380	19353811	17673597	1680214	226974	98.68	14068488	12831100	1237388	71.73	9835147	59.06	FALSE		FALSE		TRUE
DBJDCT_7_sam	DBJDCT	Muscle	PRNP174	8000	R4	896896	869161	791809	77352	25570	96.91	386057	349287	36770	43.04	197078	42.13	FALSE		TRUE	fc_assigned_pct;	FALSE
DBJDCT_8_sam	DBJDCT	Muscle	PRNP174	32000	R3	19091983	18829467	17246395	1583072	226382	98.62	14398758	13180599	1218159	75.42	9599430	57.14	FALSE		FALSE		TRUE
DBJDCT_9_sam	DBJDCT	Muscle	PRNP174	2000	R1	19978766	19728837	18057380	1671457	217298	98.75	14428650	13197179	1231471	72.22	10188172	60.75	FALSE		FALSE		TRUE
DBJDCT_10_sar	DBJDCT	Muscle	PRNP173	8000	R4	20261800	19989594	18271423	1718171	235391	98.66	13745471	12555008	1190463	67.84	9430372	58.7	FALSE		FALSE		TRUE
DBJDCT_11_sar	DBJDCT	Muscle	PRNP174	500	R2	20052450	19802331	17970050	1832281	207005	98.75	10943905	9900552	1043353	54.58	8255227	62.74	FALSE		FALSE		TRUE
DBJDCT_12_sar	DBJDCT	Muscle	PRNP173	128000	R3	19423793	19172968	17468792	1704176	220286	98.71	13307408	12113397	1194011	68.51	9452985	60.23	FALSE		FALSE		TRUE

sample_id	batch	cell_line	construct	moi	replicate	input_reads	mapped_reads	unique_mapped_	multi_mapped_re	unmapped_reads	mapping_rate_pc	dedup_mapped_	dedup_unique_re	dedup_multi_rea	dedup_rate_pct	assigned_reads	assigned_pct	outlier_flag	outlier_reason	failure_flag	failure_reasons	included_in_analysis
DBJDCT_13_sar	DBJDCT	Muscle	PRNP183	128000	R3	20546327	20278704	18544542	1734162	233466	98.7	14058230	12843193	1215037	68.42	9807032	59.76	FALSE		FALSE		TRUE
DBJDCT_14_sar	DBJDCT	Muscle	PRNP183	2000	R1	18869257	18605429	16964843	1640586	230024	98.6	12930262	11778143	1152119	68.53	8888261	57.82	FALSE		FALSE		TRUE
DBJDCT_15_sar	DBJDCT	Muscle	PRNP173	128000	R4	19395584	19142065	17508962	1633103	220936	98.69	13604802	12431084	1173718	70.14	9456649	59.5	FALSE		FALSE		TRUE
DBJDCT_16_sar	DBJDCT	Muscle	PRNP174	128000	R3	20363361	20092074	18398289	1693785	231940	98.67	14728734	13478127	1250607	72.33	9838137	57.26	FALSE		FALSE		TRUE
DBJDCT_17_sar	DBJDCT	Muscle	PRNP183	2000	R4	19942418	19696775	18042607	1654168	211056	98.77	14808492	13556327	1252165	74.26	10245155	59.65	FALSE		FALSE		TRUE
DBJDCT_18_sar	DBJDCT	Muscle	PRNP183	0	R1	474074	453979	413961	40018	18610	95.76	204565	185802	18763	43.15	89956	36.43	TRUE		TRUE	fc_assigned_pct;	FALSE
DBJDCT_19_sar	DBJDCT	Muscle	PRNP173	500	R4	19642146	19383269	17634228	1749041	228137	98.68	11974270	10873443	1100827	60.96	8609829	60.3	FALSE		FALSE		TRUE
DBJDCT_20_sar	DBJDCT	Muscle	PRNP173	2000	R4	729426	706348	647837	58511	20320	96.84	323678	295473	28205	44.37	106795	27.52	TRUE		TRUE	fc_assigned_pct;	FALSE
DBJDCT_21_sar	DBJDCT	Muscle	PRNP183	32000	R3	20326345	20069195	18323788	1745407	222727	98.73	13579998	12389470	1190528	66.81	9540860	60.23	FALSE		FALSE		TRUE
DBJDCT_22_sar	DBJDCT	Muscle	PRNP174	500	R4	19313618	19044236	17355440	1688796	235745	98.61	13525910	12316698	1209212	70.03	9231416	57.3	FALSE		FALSE		TRUE
DBJDCT_23_sar	DBJDCT	Muscle	PRNP173	32000	R4	716586	693999	638617	55382	20304	96.85	306533	280050	26483	42.78	111139	29.99	TRUE		TRUE	fc_assigned_pct;	FALSE
DBJDCT_24_sar	DBJDCT	Muscle	PRNP183	2000	R2	3485131	3433347	3097177	336170	46017	98.51	1543142	1392895	150247	44.28	1076288	58.32	FALSE		FALSE		TRUE
DBJDCT_25_sar	DBJDCT	Muscle	PRNP174	8000	R1	20550167	20315376	18566760	1748616	204656	98.86	14172716	12939297	1233419	68.97	10376533	62.77	FALSE		FALSE		TRUE
DBJDCT_26_sar	DBJDCT	Muscle	PRNP174	500	R1	1363850	1335711	1194483	141228	25261	97.94	592314	532866	59448	43.43	384404	53.03	FALSE		TRUE	fc_post_dedup_u	FALSE
DBJDCT_27_sar	DBJDCT	Muscle	PRNP183	500	R2	19637708	19399173	17733790	1665383	209147	98.79	13340675	12184587	1156088	67.93	9733535	62.82	FALSE		FALSE		TRUE
DBJDCT_28_sar	DBJDCT	Muscle	PRNP173	0	R1	20228858	19983256	18254065	1729191	213560	98.79	14282320	13036724	1245596	70.6	10230621	61.38	FALSE		FALSE		TRUE
DBJDCT_29_sar	DBJDCT	Muscle	PRNP183	32000	R1	4101548	4048791	3659530	389261	43923	98.71	1823567	1643021	180546	44.46	1396711	64.87	FALSE		FALSE		TRUE
DBJDCT_30_sar	DBJDCT	Muscle	PRNP174	2000	R4	19029603	18799270	17230292	1568978	196560	98.79	14725158	13491176	1233982	77.38	10302944	60.57	FALSE		FALSE		TRUE
DBJDCT_31_sar	DBJDCT	Muscle	PRNP183	32000	R4	19845247	19593203	17889560	1703643	219061	98.73	14637914	13353140	1284774	73.76	10262865	59.34	FALSE		FALSE		TRUE
DBJDCT_32_sar	DBJDCT	Muscle	PRNP173	128000	R1	19269566	19009442	17161384	1848058	227529	98.65	12570920	11311539	1259381	65.24	8679595	54.8	FALSE		FALSE		TRUE
DBJDCT_33_sar	DBJDCT	Muscle	PRNP173	0	R4	19689111	19449689	17773707	1675982	209866	98.78	13972752	12756305	1216447	70.97	10110627	62.28	FALSE		FALSE		TRUE
DBJDCT_34_sar	DBJDCT	Muscle	PRNP173	8000	R1	20667730	20419424	18651823	1767601	214878	98.8	14377463	13124827	1252636	69.56	10394627	62.41	FALSE		FALSE		TRUE
DBJDCT_35_sar	DBJDCT	Muscle	PRNP183	128000	R1	20806882	20538373	18655268	1883105	227554	98.71	12463617	11299245	1164372	59.9	9437145	63.64	FALSE		FALSE		TRUE
DBJDCT_36_sar	DBJDCT	Muscle	PRNP173	2000	R1	18918462	18691611	17065820	1625791	194987	98.8	13815360	12607033	1208327	73.03	9938275	61.95	FALSE		FALSE		TRUE
DBJDCT_37_sar	DBJDCT	Muscle	PRNP173	32000	R3	242419	233532	210995	22537	7844	96.33	99569	89422	10147	41.07	47930	37.46	TRUE		TRUE	fc_assigned_pct;	FALSE
DBJDCT_38_sar	DBJDCT	Muscle	PRNP173	500	R1	18462016	18281438	16630833	1650605	145534	99.02	8516314	7726986	789328	46.13	6963831	71.14	FALSE		FALSE		TRUE
DBJDCT_39_sar	DBJDCT	Muscle	PRNP183	0	R4	7765844	7691656	6955845	735811	65575	99.04	3515656	3170548	345108	45.27	2892794	70.86	FALSE		FALSE		TRUE
DBJDCT_40_sar	DBJDCT	Muscle	PRNP173	8000	R3	20753260	20537106	18677859	1859247	173763	98.96	9132408	8281936	850472	44	7089934	66.61	FALSE		FALSE		TRUE
DBJDCT_41_sar	DBJDCT	Muscle	PRNP183	8000	R3	20342871	20103754	18351065	1752689	196874	98.82	13724916	12509727	1215189	67.47	10252125	64.12	FALSE		FALSE		TRUE
DBJDCT_42_sar	DBJDCT	Muscle	PRNP174	0	R4	385826	372257	335950	36307	11713	96.48	160312	143649	16663	41.55	90552	44.32	TRUE		TRUE	fc_assigned_pct;	FALSE
DBJDCT_43_sar	DBJDCT	Muscle	PRNP173	500	R2	354480	343943	308516	35427	9610	97.03	146667	130559	16108	41.38	79504	41.67	TRUE		TRUE	fc_assigned_pct;	FALSE
DBJDCT_44_sar	DBJDCT	Muscle	PRNP174	32000	R4	19955041	19715202	17998030	1717172	198455	98.8	12748048	11620719	1127329	63.88	9514733	64.34	FALSE		FALSE		TRUE
DBJDCT_45_sar	DBJDCT	Muscle	PRNP174	128000	R1	20246432	20011976	18259522	1752454	205225	98.84	15181399	13843161	1338238	74.98	11185860	62.98	FALSE		FALSE		TRUE
DBJDCT_46_sar	DBJDCT	Muscle	PRNP183	8000	R1	488916	472281	424085	48196	15295	96.6	198951	177029	21922	40.69	107969	41.58	TRUE		TRUE	fc_assigned_pct;	FALSE
DBJDCT_47_sar	DBJDCT	Muscle	PRNP173	32000	R1	421065	407346	365951	41395	12465	96.74	169444	150836	18608	40.24	93859	42.07	TRUE		TRUE	fc_assigned_pct;	FALSE
DBJDCT_48_sar	DBJDCT	Muscle	PRNP173	2000	R2	20435787	20196054	18441863	1754191	209462	98.83	15051649	13738222	1313427	73.65	11032711	63.08	FALSE		FALSE		TRUE
DBJDCT_49_sar	DBJDCT	Muscle	PRNP174	0	R1	12718316	12587434	11422698	1164736	106958	98.97	5674542	5134705	539837	44.62	4629421	70.15	FALSE		FALSE		TRUE
DBJDCT_50_sar	DBJDCT	Muscle	PRNP174	128000	R4	2750840	2717339	2446209	271130	28040	98.78	1237956	1108739	129217	45	1012874	68	FALSE		FALSE		TRUE
DBJDCT_51_sar	DBJDCT	Muscle	PRNP174	0	R2	19408950	19183066	17354256	1828810	197528	98.84	13864296	12523959	1340337	71.43	10055602	59.26	FALSE		FALSE		TRUE
DBJDCT_52_sar	DBJDCT	Muscle	PRNP183	500	R4	705666	683637	612962	70675	19722	96.88	294207	261429	32778	41.69	163595	43.28	TRUE		TRUE	fc_assigned_pct;	FALSE
DBJDCT_53_sar	DBJDCT	Muscle	PRNP174	2000	R2	260371	249318	221971	27347	10042	95.75	102286	90832	11454	39.28	42753	31.05	TRUE		TRUE	fc_assigned_pct;	FALSE
DBJDCT_54_sar	DBJDCT	Muscle	PRNP183	500	R1	1064556	1041301	931996	109305	20346	97.82	471759	419958	51801	44.32	344453	59.81	FALSE		TRUE	fc_post_dedup_u	FALSE
X26MQB_1_sam	X26MQB	THLE2	PRNP174	32000	R6	19908440	19716155	18205314	1510841	167131	99.03	11932490	10995136	937354	59.94	9837043	72.5	FALSE		FALSE		TRUE
X26MQB_2_sam	X26MQB	THLE2	PRNP173	8000	R3	20388479	20153830	18496822	1657008	221155	98.85	11521286	10543279	978007	56.51	9397147	69.38	FALSE		FALSE		TRUE
X26MQB_3_sam	X26MQB	THLE2	PRNP183	2000	R7	20162596	19955730	18354447	1601283	183381	98.97	11478826	10524321	954505	56.93	9300363	69	FALSE		FALSE		TRUE
X26MQB_4_sam	X26MQB	THLE2	PRNP183	8000	R1	20600699	20398143	18773941	1624202	179809	99.02	11551040	10606068	944972	56.07	9879056	74.83	FALSE		FALSE		TRUE
X26MQB_5_sam	X26MQB	THLE2	PRNP183	0	R1	20632294	20428660	18800985	1627675	179682	99.01	11847946	10871255	976691	57.42	9839560	71.49	FALSE		FALSE		TRUE
X26MQB_6_sam	X26MQB	THLE2	PRNP183	0	R2	20380079	20196940	18591784	1605156	160079	99.1	12858246	11810140	1048106	63.09	10913026	73.97	FALSE		FALSE		TRUE
X26MQB_7_sam	X26MQB	THLE2	PRNP174	2000	R3	20614950	20429953	18833737	1596216	159383	99.1	12517125	11515761	1001364	60.72	10497980	73.73	FALSE		FALSE		TRUE
X26MQB_8_sam	X26MQB	THLE2	PRNP173	128000	R6	19999531	19819025	18266961	1552064	167117	99.1	10976325	10086695	889630	54.88	9301437	74.37	FALSE		FALSE		TRUE
X26MQB_9_sam	X26MQB	THLE2	PRNP183	128000	R3	20253745	20056597	18554016	1502581	182248	99.03	11354603	10472355	882248	56.06	9262232	73.4	FALSE		FALSE		TRUE
X26MQB_10_sa	X26MQB	THLE2	PRNP174	0	R1	1159587	1121336	1045199	76137	37156	96.7	464547	431391	33156	40.06	197549	37.72	FALSE		TRUE	fc_assigned_pct;	FALSE
X26MQB_11_sa	X26MQB	THLE2	PRNP173	32000	R7	19936315	19748417	18155590	1592827	164806	99.06	10754710	9856443	898267	53.95	8966656	72.97	FALSE		FALSE		TRUE
X26MQB_12_sa	X26MQB	THLE2	PRNP173	8000	R6	19111607	18931196	17427447	1503749	165838	99.06	12144259	11160511	983748	63.54	10088444	72.86	FALSE		FALSE		TRUE
X26MQB_13_sa	X26MQB	THLE2	PRNP174	8000	R1	18836519	18648691	17144205	1504486	175494	99	11768223	10800834	967389	62.48	10036366	74.48	FALSE		FALSE		TRUE
X26MQB_14_sa	X26MQB	THLE2	PRNP174	128000	R1	20119910	19918483	18274644	1643839	178032	99	10390543	9503510	887033	51.64	8855667	74.57	FALSE		FALSE		TRUE
X26MQB_15_sa	X26MQB	THLE2	PRNP183	0	R7	19198548	18988515	17520664	1467851	186408	98.91	11786724	10851220	935504	61.39	9575136	70.91	FALSE		FALSE		TRUE
X26MQB_16_sa	X26MQB	THLE2	PRNP174	0	R6	19688679	19489400	17980224	1509176	172623	98.99	12752908	11742977	1009931	64.77	10005380	67.82	FALSE		FALSE		TRUE
X26MQB_17_sa	X26MQB	THLE2	PRNP183	128000	R1	19966006	19776498	18215435	1561063	165727	99.05	11376520	10453462	923058	56.98	9561793	74.31	FALSE		FALSE		TRUE
X26MQB_18_sa	X26MQB	THLE2	PRNP173	8000	R7	20528425	20328921	18682196	1646725	186592	99.03	10485382	9603374	882008	51.08	8927100	75.54	FALSE		FALSE		TRUE
X26MQB_19_sa	X26MQB	THLE2	PRNP173	2000	R3	19739369	19509625	17926181	1583444	215951	98.84	11502595	10545043	957552	58.27	9375753	69.99	FALSE		FALSE		TRUE
X26MQB_20_sa	X26MQB	THLE2	PRNP174	32000	R3	12064439	11934697	11040927	893770	116678	98.92	5742199	5286640	455559	47.6	4558400	71.22	FALSE		FALSE		TRUE
X26MQB_21_sa	X26MQB	THLE2	PRNP183	2000	R1	7715042	7620750	7088220	532530	87596	98.78	3667349	3394393	272956	47.54	2802227	69.31	FALSE		FALSE		TRUE
X26MQB_22_sa	X26MQB	THLE2	PRNP183	8000	R6	19882982	19689875	18148539	1541336	178411	99.03	11082668	10184873	897795	55.74	8990578	71.17	FALSE		FALSE		TRUE
X26MQB_23_sa	X26MQB	THLE2	PRNP173	0	R2	19554092	19388570	17807516	1581054	141737	99.15	11405142	10448787	956355	58.33	9691027	74.14	FALSE		FALSE		TRUE
X26MQB_24_sa	X26MQB	THLE2	PRNP173	32000	R5	19740724	19510765	17911872	1598893	217069	98.84	12532156	11486637	1045519	63.48	10379504	71.35	FALSE		FALSE		TRUE
X26MQB_25_sa	X26MQB	THLE2	PRNP173	32000	R3	19038098	18873599	17382428	1491171	141799	99.14	10667134	9796751	870383	56.03	8915993	74.16	FALSE		FALSE		TRUE
X26MQB_26_sa	X26MQB	THLE2	PRNP173	32000	R1	20514404	20347791	18645706	1702085	153942	99.19	10800983	9868147	932836	52.65	9310224	75.6	FALSE		FALSE		TRUE
X26MQB_27_sa	X26MQB	THLE2	PRNP174	500	R1	20204297	20025129	18397697	1627432	154497	99.11	11726623	10752933	973690	58.04	9815141	72.93	FALSE		FALSE		TRUE
X26MQB_28_sa	X26MQB	THLE2	PRNP173	2000	R5	18873805	18696465	17266357	1430108	153730	99.06	9913000	9123905	789095	52.52	7911397	71.03	FALSE		FALSE		TRUE
X26MQB_29_sa	X26MQB	THLE2	PRNP173	0	R7	19458059	19273629	17790847	1482782	161482	99.05	9994881	9192101	802780	51.37	8111232	72.35	FALSE		FALSE		TRUE
X26MQB_30_sa	X26MQB	THLE2	PRNP174	2000	R5	20469320	20259261	18643908	1615353	185288	98.97	12522462	11501907	1020555	61.18	10460327	73.24	FALSE		FALSE		TRUE
X26MQB_31_sa	X26MQB	THLE2	PRNP174	128000	R6	18909092	18728147	17288757	1439390	156473	99.04	12358922	11388618	970304	65.36	9784061	69.02	FALSE		FALSE		TRUE
X26MQB_32_sa	X26MQB	THLE2	PRNP173	8000	R2	20561404	20335021	18593745	1741276	202007	98.9	9520297	8657614	862683	46.3	7843202	70.08	FALSE		FALSE		TRUE
X26MQB_33_sa	X26MQB	THLE2	PRNP173	500	R6	19206888	19021277	17535677	1485600	162871	99.03	10860306	9986633	873673	56.54	9135067	74.35	FALSE		FALSE		TRUE
X26MQB_34_sa	X26MQB	THLE2	PRNP183	2000	R6	20362858	20181352	18565534	1615818	158114	99.11	11022711	10111943	910768	54.13	9351723	74.76	FALSE		FALSE		TRUE
X26MQB_35_sa	X26MQB	THLE2	PRNP173	128000	R7	20353444	20155001	18542797	1612204	173899	99.03	12056842	11065809	991033	59.24	9890090	70.79	FALSE		FALSE		TRUE
X26MQB_36_sa	X26MQB	THLE2	PRNP173	32000	R6	19608372	19443181	17862396	1580785	141991	99.16	11893611	10903653	989958	60.66	10008780	73.12	FALSE		FALSE		TRUE
X26MQB_37_sa	X26MQB	THLE2	PRNP183	0	R3	20064139	19877786	18376029	1501757	163079	99.07	12120764	11181628	939136	60.41	10128200	74.22	FALSE		FALSE		TRUE
X26MQB_38_sa	X26MQB	THLE2	PRNP174	32000	R7	19391856	19182819	17765983	1416836	186886	98.92	9356996	8628680	728316	48.25	7495627	71.92	FALSE		FALSE		TRUE
X26MQB_39_sa	X26MQB	THLE2	PRNP173	500	R1	15540266	15391938	14124383	1267555	130989	99.05	7189023	6567741	621282	46.26	6063967	74.52	FALSE		FALSE		TRUE
X26MQB_40_sa	X26MQB	THLE2	PRNP173	500	R2	19664268	19448448	17892102	1556346	194167	98.9	9262248	8484153	778095	47.1	7765407	74.25	FALSE		FALSE		TRUE
X26MQB_41_sa	X26MQB	THLE2	PRNP173	2000	R1	18900837	18727235	17206960	1520275	151970	99.08	11937834	10950167	987667	63.16	10332214	76	FALSE		FALSE		TRUE

sample_id	batch	cell_line	construct	moi	replicate	input_reads	mapped_reads	unique_mapped_	multi_mapped_r	unmapped_reads	mapping_rate_pc	dedup_mapped_	dedup_unique_r	dedup_multi_rea	dedup_rate_pct	assigned_reads	assigned_pct	outlier_flag	outlier_reason	failure_flag	failure_reasons	included_in_analysis
X26MQB_42_sai	X26MQB	THLE2	PRNP174	2000	R2	19800920	19621158	18048714	1572444	165895	99.09	11431915	10490186	941729	57.73	9634981	74.78	FALSE		FALSE		TRUE
X26MQB_43_sai	X26MQB	THLE2	PRNP174	0	R2	19845417	19679892	18014612	1665280	143734	99.17	11047835	10088531	959304	55.67	9676490	76.6	FALSE		FALSE		TRUE
X26MQB_44_sai	X26MQB	THLE2	PRNP174	8000	R6	19353304	19160417	17618897	1541520	170938	99	11678618	10719466	959152	60.34	9953274	74.91	FALSE		FALSE		TRUE
X26MQB_45_sai	X26MQB	THLE2	PRNP174	2000	R7	20199978	19983896	18428425	1555471	201405	98.93	10291612	9456683	834929	50.95	8576112	74.39	FALSE		FALSE		TRUE
X26MQB_46_sai	X26MQB	THLE2	PRNP173	500	R5	19525726	19342243	17834486	1507757	157803	99.06	11393028	10480327	912701	58.35	9273244	71.82	FALSE		FALSE		TRUE
X26MQB_47_sai	X26MQB	THLE2	PRNP183	32000	R2	19909518	19740389	18167586	1572803	146044	99.15	12596491	11571768	1024723	63.27	10755906	74.92	FALSE		FALSE		TRUE
X26MQB_48_sai	X26MQB	THLE2	PRNP183	32000	R6	20805340	20630991	19020820	1610171	149659	99.16	11610446	10675671	934775	55.81	9692284	74.04	FALSE		FALSE		TRUE
X26MQB_49_sai	X26MQB	THLE2	PRNP183	2000	R5	11939117	11812210	10849607	962603	113738	98.94	5424462	4959591	464871	45.43	4600121	75.07	FALSE		FALSE		TRUE
X26MQB_50_sai	X26MQB	THLE2	PRNP174	8000	R2	19920580	19728301	18142779	1585522	169155	99.03	11541172	10588484	952688	57.94	9773038	74.61	FALSE		FALSE		TRUE
X26MQB_51_sai	X26MQB	THLE2	PRNP173	500	R3	14944920	14792383	13574712	1217671	136378	98.98	6735737	6151570	584167	45.07	5565416	72.82	FALSE		FALSE		TRUE
X26MQB_52_sai	X26MQB	THLE2	PRNP173	2000	R2	16166855	15986176	14682999	1303177	162979	98.88	7340117	6707791	632326	45.4	6094406	73.28	FALSE		FALSE		TRUE
X26MQB_53_sai	X26MQB	THLE2	PRNP183	128000	R5	15479734	15304171	14153160	1151011	160374	98.87	7102096	6535345	566751	45.88	5753803	72.67	FALSE		FALSE		TRUE
X26MQB_54_sai	X26MQB	THLE2	PRNP173	8000	R1	19329274	19163869	17639594	1524275	141785	99.14	11268668	10349973	918695	58.3	9480083	74.38	FALSE		FALSE		TRUE
X26MQB_55_sai	X26MQB	THLE2	PRNP183	32000	R7	15221681	15071760	13845928	1225832	140672	99.02	7015847	6417973	597874	46.09	5969526	75.55	FALSE		FALSE		TRUE
X26MQB_56_sai	X26MQB	THLE2	PRNP174	2000	R1	20149335	19975221	18370782	1604439	161023	99.14	12205776	11201800	1003976	60.58	10420769	75.32	FALSE		FALSE		TRUE
X26MQB_57_sai	X26MQB	THLE2	PRNP173	0	R3	18868088	18682046	17187913	1494133	163794	99.01	11224876	10303616	921260	59.49	9507728	74.85	FALSE		FALSE		TRUE
X26MQB_58_sai	X26MQB	THLE2	PRNP173	8000	R5	19617365	19428449	17876742	1551707	166208	99.04	10536789	9664904	871885	53.71	8820782	74.03	FALSE		FALSE		TRUE
X26MQB_59_sai	X26MQB	THLE2	PRNP183	8000	R3	18369145	18191841	16755787	1436054	164584	99.03	8519670	7811867	707803	46.38	7042488	73.8	FALSE		FALSE		TRUE
X26MQB_60_sai	X26MQB	THLE2	PRNP174	0	R7	17927450	17742913	16402833	1340080	172033	98.97	8213465	7555580	657885	45.82	6585304	71.98	FALSE		FALSE		TRUE
X26MQB_61_sai	X26MQB	THLE2	PRNP174	500	R5	20615062	20383397	18893422	1489975	214848	98.88	10963337	10123458	839879	53.18	8618892	70.76	FALSE		FALSE		TRUE
X26MQB_62_sai	X26MQB	THLE2	PRNP183	500	R5	20746339	20544550	18901852	1642698	188061	99.03	9647227	8838228	808999	46.5	8066433	74.53	FALSE		FALSE		TRUE
X26MQB_63_sai	X26MQB	THLE2	PRNP183	500	R7	20770425	20577467	18902018	1675449	168391	99.07	10908877	9988695	920182	52.52	9204883	73.38	FALSE		FALSE		TRUE
X26MQB_64_sai	X26MQB	THLE2	PRNP174	32000	R2	18848143	18690498	17114823	1575675	136414	99.16	10629229	9709553	919676	56.39	9288162	76.33	FALSE		FALSE		TRUE
X26MQB_65_sai	X26MQB	THLE2	PRNP183	2000	R2	20346367	20152740	18523537	1629203	169846	99.05	12535962	11502206	1033756	61.61	10679003	74.81	FALSE		FALSE		TRUE
X26MQB_66_sai	X26MQB	THLE2	PRNP173	0	R5	19058190	18851015	17387144	1463871	184734	98.91	11629645	10704455	925190	61.02	9553269	71.67	FALSE		FALSE		TRUE
X26MQB_67_sai	X26MQB	THLE2	PRNP173	0	R1	17017721	16856748	15425060	1431688	152226	99.05	7680180	6996974	683206	45.13	6653375	76.59	FALSE		FALSE		TRUE
X26MQB_68_sai	X26MQB	THLE2	PRNP183	32000	R3	19598788	19390146	17873950	1516196	188308	98.94	9542338	8757717	784621	48.69	7941068	74.18	FALSE		FALSE		TRUE
X26MQB_69_sai	X26MQB	THLE2	PRNP173	128000	R1	18612665	18435358	16963771	1471587	165512	99.05	8696681	7962985	733696	46.72	7228641	74.08	FALSE		FALSE		TRUE
X26MQB_70_sai	X26MQB	THLE2	PRNP174	128000	R7	20638727	20455327	18789926	1665401	171725	99.11	12732913	11674991	1057922	61.69	10880178	75.02	FALSE		FALSE		TRUE
X26MQB_71_sai	X26MQB	THLE2	PRNP174	32000	R1	19802565	19607082	18026826	1580256	183950	99.01	12225372	11223863	1001509	61.74	10241416	73.1	FALSE		FALSE		TRUE
X26MQB_72_sai	X26MQB	THLE2	PRNP173	0	R6	20159342	19973145	18392058	1581087	162890	99.08	12304734	11305551	999183	61.04	10424257	74.59	FALSE		FALSE		TRUE
X26MQB_73_sai	X26MQB	THLE2	PRNP174	0	R5	19371241	19210840	17682299	1528541	139079	99.17	11492249	10554195	938054	59.33	9925426	76.43	FALSE		FALSE		TRUE
X26MQB_74_sai	X26MQB	THLE2	PRNP174	32000	R5	19426641	19264049	17668437	1595612	153018	99.16	12619805	11559467	1060338	64.96	10881885	75.01	FALSE		FALSE		TRUE
X26MQB_75_sai	X26MQB	THLE2	PRNP173	32000	R2	19015166	18863551	17217601	1645950	141660	99.2	8932566	8124171	808395	46.98	7841164	77.3	FALSE		FALSE		TRUE
X26MQB_76_sai	X26MQB	THLE2	PRNP174	500	R2	11460246	11324132	10438357	885775	129156	98.81	5399032	4954802	444230	47.11	4438900	73.75	FALSE		FALSE		TRUE
X26MQB_77_sai	X26MQB	THLE2	PRNP183	0	R5	20661502	20478028	18829798	1648230	159983	99.11	12243564	11233820	1009744	59.26	10364456	74.28	FALSE		FALSE		TRUE
X26MQB_78_sai	X26MQB	THLE2	PRNP174	500	R7	377363	366128	338864	27264	10887	97.02	148352	136640	11712	39.31	52120	30.16	TRUE		TRUE	fc_assigned_pct;	FALSE
X26MQB_79_sai	X26MQB	THLE2	PRNP173	128000	R5	19204995	19022754	17505709	1517045	159421	99.05	12032863	11052248	980615	62.65	9957945	72.09	FALSE		FALSE		TRUE
X26MQB_80_sai	X26MQB	THLE2	PRNP183	500	R1	19426206	19228564	17679475	1549089	174699	98.98	11705730	10740375	965355	60.26	9800330	72.99	FALSE		FALSE		TRUE
X26MQB_81_sai	X26MQB	THLE2	PRNP183	128000	R6	19276016	19114156	17582898	1531258	149760	99.16	10845703	9949467	896236	56.27	9246750	75.88	FALSE		FALSE		TRUE
X26MQB_82_sai	X26MQB	THLE2	PRNP174	128000	R5	19397554	19256010	17630292	1625718	132093	99.27	12609873	11530514	1079359	65.01	10923309	76.41	FALSE		FALSE		TRUE
X26MQB_83_sai	X26MQB	THLE2	PRNP174	128000	R2	19863152	19663229	18049367	1613862	177232	98.99	11642546	10666345	976201	58.61	9949846	74.45	FALSE		FALSE		TRUE
X26MQB_84_sai	X26MQB	THLE2	PRNP183	8000	R2	20585322	20400672	18717288	1683384	161127	99.1	11744460	10751575	992885	57.05	10239243	76.78	FALSE		FALSE		TRUE
X26MQB_85_sai	X26MQB	THLE2	PRNP183	8000	R5	19484396	19289722	17678040	1611682	184429	99	9712319	8872350	839969	49.85	8505038	77.48	FALSE		FALSE		TRUE
X26MQB_86_sai	X26MQB	THLE2	PRNP183	0	R6	18774197	18625743	17142984	1482759	135696	99.21	12321267	11324623	996644	65.63	10336879	73.59	FALSE		FALSE		TRUE
X26MQB_87_sai	X26MQB	THLE2	PRNP174	128000	R3	19503314	19323029	17730833	1592196	158902	99.08	12236824	11207927	1028897	62.74	10674835	76.43	FALSE		FALSE		TRUE
X26MQB_88_sai	X26MQB	THLE2	PRNP174	8000	R7	20605088	20416112	18816030	1600082	165264	99.08	13330670	12265497	1065173	64.7	11311171	74.74	FALSE		FALSE		TRUE
X26MQB_89_sai	X26MQB	THLE2	PRNP173	2000	R6	19378811	19212243	17604084	1608159	155340	99.14	9556649	8723641	833008	49.31	8205366	76.1	FALSE		FALSE		TRUE
X26MQB_90_sai	X26MQB	THLE2	PRNP183	500	R6	20590923	20419832	18780670	1639162	147679	99.17	12452316	11429153	1023163	60.47	10676527	75.85	FALSE		FALSE		TRUE
X26MQB_91_sai	X26MQB	THLE2	PRNP174	2000	R6	19511119	19349770	17776135	1573635	149982	99.17	11489657	10534009	955648	58.89	9898650	76.3	FALSE		FALSE		TRUE
X26MQB_92_sai	X26MQB	THLE2	PRNP173	2000	R7	19647645	19475720	17887637	1588083	149538	99.12	12799289	11735861	1063428	65.14	11053455	76.03	FALSE		FALSE		TRUE
X26MQB_93_sai	X26MQB	THLE2	PRNP183	128000	R7	18909835	18732637	17236361	1496276	155183	99.06	12165811	11177826	987985	64.34	10236015	73.53	FALSE		FALSE		TRUE
X26MQB_94_sai	X26MQB	THLE2	PRNP183	8000	R7	20431379	20239434	18608583	1630851	167963	99.06	12608013	11574290	1033723	61.71	10676428	74.13	FALSE		FALSE		TRUE
X26MQB_95_sai	X26MQB	THLE2	PRNP173	500	R7	19886068	19717160	18076072	1641088	157309	99.15	11971351	10949918	1021433	60.2	10211247	74.9	FALSE		FALSE		TRUE
X26MQB_96_sai	X26MQB	THLE2	PRNP174	500	R3	19780962	19604235	17998814	1605421	154995	99.11	12503915	11465167	1038748	63.21	10773188	75.93	FALSE		FALSE		TRUE
X26MQB_97_sai	X26MQB	THLE2	PRNP174	8000	R5	20205309	19976275	18377928	1598347	204473	98.87	12659221	11627135	1032086	62.65	10482788	72.4	FALSE		FALSE		TRUE
X26MQB_98_sai	X26MQB	THLE2	PRNP183	2000	R3	19166932	18896245	17361958	1534287	247847	98.59	11953653	10964521	989132	62.37	9947802	72.9	FALSE		FALSE		TRUE
X26MQB_99_sai	X26MQB	THLE2	PRNP173	128000	R2	12714342	12449328	11357245	1092083	250684	97.92	6656279	6058871	597408	52.35	5521610	71.41	FALSE		FALSE		TRUE
X26MQB_100_si	X26MQB	THLE2	PRNP174	500	R6	19904339	19677563	18057343	1620220	213598	98.86	10615640	9713648	901992	53.33	8976203	74.81	FALSE		FALSE		TRUE
X26MQB_101_si	X26MQB	THLE2	PRNP173	128000	R3	18973325	18691843	17171685	1520158	266821	98.52	11222096	10286242	935854	59.15	9370099	73.7	FALSE		FALSE		TRUE
X26MQB_102_si	X26MQB	THLE2	PRNP183	500	R3	19018730	18750134	17238224	1511910	245272	98.59	13569006	12457444	1111562	71.35	11082807	70.65	FALSE		FALSE		TRUE
X26MQB_103_si	X26MQB	THLE2	PRNP183	128000	R2	19111287	18879195	17506531	1372664	208743	98.79	11732377	10856437	875940	61.39	9372866	71.06	FALSE		FALSE		TRUE
X26MQB_104_si	X26MQB	THLE2	PRNP183	32000	R1	19632580	19402700	17822922	1579778	214363	98.83	13236839	12141388	1095451	67.42	10858192	71.12	FALSE		FALSE		TRUE
X26MQB_105_si	X26MQB	THLE2	PRNP183	32000	R5	19612245	19360371	17775022	1585349	230317	98.72	10197963	9342173	855790	52	8528956	74.01	FALSE		FALSE		TRUE
X26MQB_106_si	X26MQB	THLE2	PRNP183	500	R2	19076194	18821967	17340363	1481604	239963	98.67	10609293	9746865	862428	55.62	8664448	72.79	FALSE		FALSE		TRUE
X26MQB_107_si	X26MQB	THLE2	PRNP174	0	R3	11396526	11104632	10208563	896069	283185	97.44	5948481	5451142	497339	52.2	4707001	70.1	FALSE		FALSE		TRUE
X26MQB_108_si	X26MQB	THLE2	PRNP174	8000	R3	18981075	18779816	17228885	1550931	187460	98.94	12491312	11443526	1047786	65.81	10487173	73.69	FALSE		FALSE		TRUE