U.S. FOOD & DRUG ADMINISTRATION

Preliminary Meeting Responses

Our Reference: PTS #PS009720 / Meeting ID #20961

DATE: February 5, 2025 **PAGES:** 9

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SUBJECT: INTERACT meeting to receive feedback on the questions provided, which are critical for the advancement of PRNP-CHARM-001, a novel genetically targeted therapy with a well-established mechanism of action being developed for a rapidly fatal neurodegenerative disease with no standard of care. Above all, this meeting will be a success if a feasible path forward is identified for both the symptomatic and presymptomatic patient populations.

PRODUCT: Recombinant Adeno-Associated Viral Vector-9 containing a single-stranded AAV2 genome encoding an epigenetic editor targeted to the PRNP promoter by a zinc finger (ZF) binding domain and an engineered AAV9-derived capsid BI-hTFR1v2, that has been designed to enter the human CNS via its ability to bind to human Transferrin receptor (TfR1)/Product Name: PRNP-CHARM-001

PROPOSED INDICATION: Treatment of Prion disease

FDA Participants:
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This material consists of our preliminary meeting responses to your questions and any additional comments in preparation for the discussion at the meeting scheduled for February 10, 2025. We are sharing this material to promote a collaborative and successful discussion at the meeting.

Although we continue to reserve February 10, 2025 from 1:00PM – 2:00PM EST, with you regarding this product, if you find that our attached responses and advice are sufficiently clear and complete to obviate the need for further discussion, please inform us in writing as soon as possible, and 3 calendar days from the date of receipt of FDA's Preliminary Responses, so that we may clear the meeting time. These responses would then become the official FDA responses to your questions.

If you determine that discussion is needed for only some of the original questions, you have the option of reducing the agenda and/or changing the format of the meeting from Face-to-Face (virtual) to teleconference. If you have questions regarding specific responses or advice included in this preliminary response, please inform the RPM so that the appropriate members of the Review Committee can provide clarification during the reserved meeting time. Please refer to the *Respond to Meeting Request-Granted* communication you received for details about your scheduled meeting.

Please be aware that your future submission should include all components for a complete submission and should be in compliance with all appropriate statutes and regulations. For input on additional issues that were not posed in your meeting package or addressed in our preliminary meeting responses, you may submit a new meeting or a WRO request, as we may not be prepared to discuss or reach agreement on new topics at the meeting.

Please include a reference to PTS #PS009720 and/or Meeting ID #20961 in your future submissions related to this product.

Preliminary Meeting Responses

Question 1:
We propose to perform parallel trials in symptomatic prion disease patients and pre-symptomatic carriers of high penetrance pathogenic PRNP mutations. Does the Agency concur?

FDA Response to Question 1:
Yes, we agree with your proposal to enroll symptomatic patients and pre-symptomatic carriers in the same study.

Question 2:
In both symptomatic and presymptomatic trials we propose to measure cerebrospinal fluid (CSF) prion protein levels as a pharmacodynamic biomarker of drug activity in the brain. Does the Agency concur?

FDA Response to Question 2:
Yes, we agree. In your future Investigational New Drug (IND) submission, please provide details of the assay you plan to use to measure prion protein levels in CSF. Please also provide justification for a threshold of CSF prion protein level reduction that may be considered reasonably like to predict clinical benefit.

Question 3:
Our novel engineered AAV9-derived viral vector, BI-hTfR1v2, crosses the blood brain barrier by binding the human transferrin receptor. Because this interaction depends on the human-specific amino acid [redacted]*, BI-hTfR1v2 will not achieve pharmacologically relevant biodistribution in any common preclinical species such as wild-type mice, rats, dogs, minipigs or monkeys (8.3.1). For this reason, we propose that homozygous humanized TFRC knock-in mice are appropriate and sufficient as the single species for definitive biodistribution and toxicology studies, and that initial human doses could be selected based on these data. Does the Agency concur?*

FDA Response to Question 3:
We agree with the scientific rationale for the selection of your nonclinical animal model for your pivotal toxicology study. We have the following comments which you should address as part of your pre-IND and IND submissions:

1. Please discuss the basis on which you will select a reasonably safe and biologically active dose level of PRNP-CHARM-001. Please discuss relevant study endpoints (i.e., survival, biochemical, histological, physiological/functional), dose-dependent pharmacology, durability of the epigenetic edits to the PRNP locus, and other relevant factors that help inform your dose rationale.

2. Please provide your method of dose level extrapolation from animals to humans and the rationale, with supporting data and publications, for this method.

Question 4:
Given the lack of homology between the human PRNP promoter and that of mouse, the planned human ZF-CHARMs will not have on-target activity in the TFRC mice in which we propose to conduct our definitive biodistribution and toxicology studies (8.3.2). We acknowledge that while this study will capture off-target effects of CHARM this study is not designed to capture on-target toxicity. However prion protein is known to be non-essential and we therefore do not expect on-target toxicity from PrP lowering. In order to assess on-target effects we propose to supplement our definitive biodistribution and toxicology studies with separate biodistribution and safety information collected in a non-GLP human PrP-lowering study (9.3.2.2) that will test the human ZF-CHARM candidate in humanized PRNP (huPrP) mice. Does the Agency concur?

FDA Response to Question 4:
We agree that the use of huPrP mice to evaluate the biological activity of human ZF-CHARM is acceptable. In your pre-IND and IND submissions, please provide a discussion of whether co-expression of murine PrP in the huPrP mice will impact the interpretability of your data.

Question 5:
As a Potency Assay for release criteria, we propose to use RT-qPCR-based quantification of PRNP RNA in human HEK293T cells. Should an alternative assay be needed, we propose luminescence-based quantification of tagged endogenous PrP in U251MG cells. Does the Agency concur?

FDA Response to Question 5:
Yes, we agree that the RT-qPCR-based quantification of PRNP RNA in human HEK293T cells is appropriate as the potency assay for product release. Additionally, if there are issues developing the RT-qPCR assay, the luminescence-based quantification of tagged endogenous PrP in human U251MG cells would also be appropriate as the potency assay for product release. Based on the information you provided in the briefing document, we have the following additional comments and advice:

1. Regarding your proposed RT-qPCR-based quantification of PRNP RNA or luminescence-based quantification of tagged endogenous PrP:

    a. In your IND submission, please provide a detailed description of your planned functional potency assay, describing how the assay is performed, assay controls, reference materials, and any other information that is relevant to performing the analytical method.
    b. Though assay qualification is not required for initiation of your study, we encourage you to qualify your functional potency assay that measures your product's biological activity as early in product development as feasible and implement it for lot release testing and stability testing (please note, potency is generally a stability-indicating quality attribute).

2. Potency assays may have substantial variability that can be difficult to eliminate. Therefore, we recommend quantitating your product potency relative to a well-characterized reference material, to improve precision of the reportable value for your potency assay:

    a. We recommend reporting potency for each lot as a percentage of the potency of the reference lot (assessed in parallel with the test lot).

    b. The manufacturing, testing, and characterization of the reference lot should be comparable to that used for clinical lots. A robust qualification and stability plan for the reference material/standard should be put in place. Additionally, you should develop a plan to bridge reference lots prior to exhaustion of the original reference material.

    c. We recommend that you plan to use the same reference material for all the potency assays within your potency matrix (i.e., early-phase transgene expression assays and late-phase biological/functional assay).

3. Should you plan to apply for a marketing application for your product, please note that your potency assay(s) should be qualified prior to studies designed to obtain primary efficacy data for a license and validated prior to submission of a license application. For additional guidance on potency assay development and potency assurance strategy, please refer to the December 2023 Draft Guidance for Industry: Potency Assurance for Cellular and Gene Therapy Products, available at https://www.fda.gov/media/175132/download.

Additional FDA Questions/Comments:

Chemistry, Manufacturing, and Controls (CMC)

1. As you prepare for a future IND submission, please note the following regarding your product strength-determining (vector genome titer) assay:

    a. To ensure that an accurate dose is administered, this assay must be qualified and must be shown to have adequate performance prior to initiating a clinical study.

    b. The qualification study should be performed using a product-specific test material.

    c. Please provide a detailed protocol for the qualification study and the SOP for the assay.

    d. Please provide the full qualification study report with data documenting accuracy, precision (repeatability and intermediate precision), specificity, range, and linearity. The coefficient of variation (CV) for intermediate

precision should be 15%. A precise assay is necessary to ensure that subjects receive the intended doses, to support consistent dosing throughout the clinical study, and to monitor product stability.

e. Please describe any deviations that occurred during the qualification study.

f. To ensure consistent dosing between clinical and nonclinical studies, we recommend using the same qualified assay for measuring the vector genome titer of the nonclinical and clinical lots.

g. The assay to determine strength should be validated prior to the initiation of any clinical study that is intended to provide the primary evidence of effectiveness for a marketing application.

2. Please note that as the IND Sponsor you are responsible for providing all the CMC information necessary to assess product safety for the planned clinical study, either in your IND submission or through submission of an appropriate Letter of Authorization (LoA) to reference an appropriate IND or Master File. For assistance in preparing the CMC section of your IND, please refer to "Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs)" from January 2020 (https://www.fda.gov/media/113760/download).

Pharmacology/Toxicology

3. Please note that we strongly recommend that you refrain from initiating your definitive GLP toxicology study (Section 9.3.2, pages 34-35) until receipt of additional detailed feedback from us in your pre-IND meeting. We recommend that you complete your ongoing and proposed POC studies and provide a detailed summary of each study in your pre-IND meeting package.

4. Please be advised that the definitive GLP nonclinical study (Section 9.3.2, pages 34-35) should use the PRNP-CHARM-001 that is identical to the intended clinical product in terms of the capsid, promoter, payload, manufacturing process, product identity, and formulation.

5. Regarding the biodistribution (BD) analysis described in Section 9.3.2.3 (pages 34-35) of the protocol:

   a. In addition to the listed tissues, please include the spleen, dorsal root ganglion, and lungs. Please note that blood is also a component of the BD analysis.

   b. Please evaluate the BD profile and the kinetics of transgene expression following administration of PRNP-CHARM-001 via the proposed clinical

ROA at multiple time points that coincide with the peak vector/transgene expression levels and decline of these levels.

c. We agree with the strategy to only evaluate BD in animals receiving vehicle control and mid-dose human PRNP-CHARM. However, all tissues, whether analyzed or not, should be archived for possible future analysis including possible future analysis of AAV vector integration.

6. We recommend that you evaluate the pharmacokinetic profile of CHARM to assess persistence of protein expression and discuss how this may impact the rate of off-target silencing over time.

7. Please refer to the Long-Term Follow-Up After Administration of Human Gene Therapy Products: Guidance for Industry (January 2020), available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/long-term-follow-after-administration-human-gene-therapy-products, for further discussion regarding tissue collection and the qPCR assay methodology.

8. In your IND, please provide complete study reports for all nonclinical studies used to support the safety and rationale of your proposed clinical trial. These reports should include, but should not be limited to: a) a prospectively written protocol and all protocol amendments or a detailed methodology; b) a detailed description of the study design [e.g., description of the test system used, animal species/animal models, control and test articles administered, dose levels, detailed procedures for test article administration (including delivery device description), and collection of all study protocol parameters, etc.]; c) results for all parameters evaluated for each animal on study; and d) your analysis and interpretation of the study data.

9. For all planned studies, please use the same assay for vector titer determination for the nonclinical lots that will be used for the clinical lots. The dose levels of PRNP-CHARM-001 should be calculated based on this analysis. We also recommend that you retain adequate material from each nonclinical lot so that it can be retested if the assay for future clinical lot(s) is modified in some manner.

10. Please provide a tabulated summary of the similarities and differences between the vector product used in each nonclinical study and your intended clinical product.

11. For additional guidance regarding the nonclinical assessment of cell and gene therapy products, GLP testing requirements, and the contents of nonclinical study reports, please refer to: a) the document titled, *Guidance for Industry: Preclinical Assessment of Investigational Cellular and Gene Therapy Products* (November 2013), available at: https://www.fda.gov/regulatory-

information/search-fda-guidance-documents/preclinical-assessment-investigational-cellular-and-gene-therapy-products and b) the *OTP Learn* Webinar Series, available at: https://www.fda.gov/vaccines-blood-biologics/news-events-biologics/otp-learn.

12. For a summary regarding the nonclinical assessment of gene therapy products for neurodegenerative disease indications, please refer to the document titled, *Human Gene Therapy for Neurodegenerative Diseases: Guidance for Industry* (October 2022), available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/human-gene-therapy-neurodegenerative-diseases.

Bioinformatics

13. Because your drug product (DP) has a ZFP that is programmed to recognize a specific target sequence in the genome, you should perform an off-target safety assessment. Please address the following in your pre-IND or IND submission:

    a. Please provide the target recognition sequence for the ZFP.

    b. Please perform multiple orthogonal off-target assessment studies to determine whether the ZFP binds only to the target recognition sequence and whether the DP methylates only the promoter region of the PRNP gene.

    c. Please perform confirmatory testing for both the on-target site and off-target nominated sites and provide the on-target editing rate through post methylation editing by your DP.
    d. Please provide a justification for the samples used in off-target assessment and confirmatory testing study and provide information whether the samples have adequate amount of endogenous DNMT3A.

    e. Please provide a tabulated report of potential off-target sites, including annotation information and risk assessment as applicable.

14. You provided a plan for evaluating down-regulated genes using RNA sequencing (RNA-Seq) with human cell and cell lines. However, you did not provide justification for choosing proposed human samples, information on the amount of endogenous DNMT3A that will be recruited by D3L, and a detailed description on the RNA-Seq assay. Regarding RNA-Seq assay, please address the following in your pre-IND or IND submission:

    a. Please provide justification for the samples used in RNA-Seq and comment on whether the samples have an adequate amount of

endogenous DNMT3A.

b. Please provide the number of samples you plan to use for each cell and cell lines when detecting differentially expressed genes (DEGs).

c. Please provide a tabulated report for all DEGs for each cell type, including expression level, amount of fold change, and statistical significance.

d. Please provide an integrated assessment report for significantly downregulated and hypermethylated genes, i.e., potential off-target genes, through post methylation editing by the DP, including risk assessment for those genes as applicable.

15. Please provide detailed information on your next generation sequencing (NGS) based assays used in off-target assessment studies for methylation and RNA-Seq, including library preparation, sequencing platform, sequencing data quality control metrics with acceptance criteria, and a detailed bioinformatics workflow with software tools.

Administrative Information:

16. We recommend that you request a pre-IND meeting with CBER/OTP when ready, to obtain formal nonbinding comments regarding your product development plan from the three CBER/OTP review disciplines, consisting of product manufacturing (CMC), pharmacology/toxicology (P/T), and clinical. Please be advised that you should consider and address all recommendations provided in these INTERACT comments when you submit a pre-IND meeting package.

17. We refer you to *OTP Learn*, a series of online presentations provided by the Office of Therapeutic Products (OTP) which address important topics in the development of products regulated by OTP. You may find some of these presentations useful in your preparation of regulatory submissions and briefing materials for meetings with FDA. *OTP Learn* is available at: https://www.fda.gov/vaccines-blood-biologics/news-events-biologics/otp-learn.

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