FDA U.S. FOOD & DRUG ADMINISTRATION

PIND 141250

MEETING MINUTES

Broad Institute of MIT and Harvard
Attention: Eric Lander, PhD
President and Founding Director
415 Main Street
Cambridge, MA 02142

Dear Dr. Lander:[1]

Please refer to your Pre-Investigational New Drug Application (PIND) file for prion protein (PrP)-lowering antisense oligonucleotides.

We also refer to the meeting between representatives of your firm and the FDA on October 31, 2019. The purpose of the meeting was to discuss a proposed surrogate biomarker for your PrP-lowering antisense oligonucleotides development program.

A copy of the official minutes of the meeting/telecon is enclosed for your information. Please notify us of any significant differences in understanding regarding the meeting outcomes.

If you have any questions, please contact Heather Bullock, Regulatory Project Manager by telephone at [redacted] or by email at [redacted]

Sincerely,

{See appended electronic signature page}

Eric Bastings, MD
Acting Director
Division of Neurology 1
Office of Neuroscience
Office of New Drugs

Enclosure:

- Meeting Minutes

[1] We update guidances periodically. For the most recent version of a guidance, check the FDA Guidance Documents Database https://www.fda.gov/RegulatoryInformation/Guidances/default.htm.

FDA U.S. FOOD & DRUG ADMINISTRATION

MEMORANDUM OF MEETING MINUTES

Meeting Type: C
Meeting Category: Guidance

Meeting Date and Time: October 31, 2019 from 11:00 a.m. to 12:00 p.m. EST
Meeting Location: FDA [redacted]
Silver Spring, MD

Application Number: PIND 141250
Product Name: Prion protein (PrP)-lowering antisense oligonucleotides

Indication: Genetic prion disease
Sponsor Name: Broad Institute of MIT and Harvard

FDA ATTENDEES

Center for Drug Evaluation and Research

Division of Neurology Products
Billy Dunn, MD, Director
Eric Bastings, MD, Deputy Director
Teresa Buracchio, MD, Clinical Team Leader
Rainer Paine, MD, PhD, Clinical Reviewer
Lois Freed, PhD, Supervisory Pharmacologist
Heather Bullock, Regulatory Project Manager
Michael Matthews, Regulatory Project Manger

Office of Clinical Pharmacology
Hobart Rogers, PharmD, PhD, Reviewer, Genomics and Targeted Therapy Group

Office of Biotechnology Products, Division of Biotechnology Review and Research III,
Ashutosh Rao, PhD, Bioassay Reviewer Team Lead
Mari Lehtimaki, PhD, Bioassay Reviewer

Center for Biologics Evaluation and Research

Office of Blood Research and Review (OBRR), Division of Emerging Transfusion-Transmitted Diseases (DETTD), Laboratory of Bacterial & Transmissible Spongiform Encephalopathy Agents
David Asher, MD, Chief
Luisa Gregori, PhD

SPONSOR ATTENDEES

Broad Institute of MIT and Harvard
Eric Lander, PhD, Chief Executive Officer and President
Sonia Vallabh, PhD, Prion Scientist
Eric Minikel, PhD, Prion Scientist
Anne Smith, Executive Director Clinical Development, Ionis Pharmaceuticals
Tiffany Baumann, Executive Director Regulatory Affairs, Ionis Pharmaceuticals
Holly Kordasiewicz, Executive Director of Neuroscience Drug Discovery, Ionis Pharmaceuticals

1.0 BACKGROUND

On November 14, 2017, scientists from the Broad Institute of MIT and Harvard (Broad Institute) had a Critical Path Innovation Meeting (CPIM) with the Agency to discuss their preliminary work to develop a therapeutic strategy for genetic prion disease. On August 19, 2019, the Broad Institute submitted a meeting request to discuss a proposed surrogate biomarker for their PrP-lowering antisense oligonucleotides development program; and the meeting was granted September 11, 2019. The Agency provided preliminary responses to questions contained in the sponsor's background materials on October 25, 2019. On October 29, 2019, Broad Institute provided responses to the preliminary comments in advance of the meeting. The sponsor's response document is attached to these minutes.

2. DISCUSSION

Questions Regarding Biomarker Assessment

Question 1: What, if any, further analytical validation of the technical performance of the BetaPrion Human ELISA kit will be necessary to support its use as an assay for a surrogate biomarker endpoint?

FDA Response to Question 1:

Based on the information you submitted regarding the technical performance of the BetaPrion Human ELISA kit and the targeted mass spectrometry assay, please see below for aspects that we believe either require additional data or clarification. Where possible, we have provided recommendations to address the gaps.

1. In general, please provide detailed standard operating procedures (SOP) for the BetaPrion Human ELISA and mass spectrometry assays as per the modifications and optimizations you have performed leading up to your validation. These modifications should be described in detail with steps specific to your intended

use, samples, instrumentation, and distinct from the commercially available method.

a. Your SOPs should describe steps for any acceptance/rejection criteria for the samples and raw data at each key step, chain of custody of the CSF samples, blinding protocols for the samples, and roles/responsibilities for each analyst or laboratory involved in the procedures.
b. The ELISA method SOP should have sufficient detail on the standard curve material, capture and detection antibodies, dilution steps, detection methods, and steps for calculating the final reported PrP value with appropriate units.
c. Your procedures for rejected samples or deviations from the validated method need to be described with clear instructions on how an analyst must proceed in such cases. For example, is re-testing allowed? What are the limits and objective criteria for re-testing? What are the objective criteria under which a CSF sample might be rejected? Is sample pooling allowed?

2. Regarding your bioassay validation for the ELISA method submitted in Appendix 3:
    a. Clarify whether the kit standard included as part of the commercial kit or the recombinant human PrP standard you generated will be used for the proposed study. Additionally,
        i. Provide data for the source, purity, identity (peptide sequence, tags), and stability of your in-house recombinant human PrP reference standard.
        ii. Justify the representativeness of the in-house recombinant human PrP reference standard to the human PrP found in pre-symptomatic patient CSF.
        iii. We are unclear on what you mean by "A recombinant standard curve was prepared from AAA-quantified recombinant huPrP" in Appendix 3 Supplemental Figure S3. Clarify if the recombinant PrP concentration measurement uses extinction coefficient for the calculations.
        iv. We are concerned that your currently validated range for the standard curve may not capture the range of pre- and post-treated CSF PrP concentrations. In Appendix 3, Supplemental Figure S3B, the linear portion of recombinant human PrP standard curve, measured within the BetaPrion Human ELISA kit's detection range 1-20 ng/ml, has only 4 points in the linear portion of the curve. The two highest concentrations of recombinant human PrP standard resulted in plateauing of the absorbance units and are not part of the linear standard curve drawn. You should ensure that a sufficient number of data points form the linear standard curve within a range that is clinically meaningful in terms of the anticipated drug response you expect from the ASOs you study. We generally recommend at least 6 non-zero data points in the linear range.

Please provide a narrative that justifies your choice of the reference standard and the linear range of the validated reference standard.

v. Clarify the matrix used to dilute the reference standard and clinical samples.

vi. In the Appendix 3 paper and the supplementary discussion, you conclude that "kit standards, while technically reproducible, may most usefully inform relative rather than absolute quantification of PrP." We are unclear on what this statement means. Please clarify and justify your quantitative output given this potential caveat.

b. Clarify the source and epitope specificity of the antibodies used in the ELISA method.

c. The critical reagents in the modified ELISA should be evaluated for their stability at the intended storage or in-use temperature.

d. Appendix 3 provides data on dilution range, spiking, plate to plate variation, and within plate variation produced with different CSF samples. We generally recommend the inclusion of quality control samples, such as a positive and negative control CSF sample. Will quality control samples of known concentrations of PrP be included in the validation runs of the assay?

e. According to submitted information, the LLOQ reported is 3-5x the blank signal. We generally recommend ≥ five times the analyte response of the zero calibrator (see Table 1 in FDA Bioanalytical Method Validation Guidance for Industry (2018)). You state that the variation stems from different plate readers; therefore, consider validating the method using a plate reader of choice or predefine the instrument variables that achieves the recommended LLOQ level in relation to the blank and independent of the plate reader choice.

f. As drug development proceeds, you should plan to include inter-laboratory variability testing (reproducibility), in addition to the intra-laboratory variability (intermediate precision) you have tested.

3. Regarding the mass spectrometry (MS) data submitted in Appendix 4.

   a. Targeted MS is used to show that correlation of total protein to measured PrP in ELISA is not due to ELISA measuring off-target proteins. We are unclear if you are proposing to use the targeted MS for additional assay validation and use with clinical samples. Please clarify your proposed plan for use of the MS method.

   b. If you chose to include this method as part of your proposed clinical study, additional data confirming assay validation would be needed.

   c. We note that the targeted MS method appears to provide greater values for PrP concentration than the ELISA method for comparable samples (Figure 3 in Appendix 4). Please provide your explanation for this difference. If differences might be due to protein conformational and peptide sequence differences as detected by the ELISA method, please consider including both the ELISA and MS methods in your proposed study as orthogonal measures of PrP concentration.

Meeting Discussion:

The sponsor clarified that the validation process will likely be done at a contract laboratory and that laboratory will be involved in testing patient samples for the first-in-human studies. Regarding the reference standard, the sponsor is leaning towards their in-house standard for the ELISA method. The sponsor agreed that more characterization of both the assay and in-house versus kit standards are needed. It was agreed that if the in-house standard is chosen, a new standard curve needs to be provided with sufficient non-zero data points measured within the detection range expected in the intended patient population before and after treatment. It was noted by FDA and the sponsor that the higher concentrations of the reference samples appeared to show a plateaued response compared to the lower concentration reference samples. The sponsor agreed to record both % change and absolute quantity of PrP as starting levels of PrP can vary among patients and the target PrP levels are not known. The sponsor suggested using a single lab to conduct the ELISA and thus avoid having to add inter-lab variance into the validation procedure. Dr. Rao advised that using multiple labs could help identify the possible extent of variability with their bioassay method and having multiple qualified labs would provide the sponsor with multiple choices for their clinical studies, which may be conducted at more than one clinical site. FDA clarified that the inter- and intra-laboratory variability could also help the sponsor determine whether their drug response could truly be attributed to treatment effect versus method variability. Sponsor agreed to validate a single plate reader for their method, as variation in results partly appeared to stem from the use of different plate readers.

FDA recommended that the sponsor keep the MS method they have developed so far in consideration for further optimization as drug development proceeds. This orthogonal method could help the sponsor in case the ELISA method shows unexpected findings and orthogonal methods are needed for confirmation of findings at the peptide sequence level. It was noted that adding the MS-based PrP detection would require a separate validation of the method if the sponsor choses to develop and include this method.

Sponsor and FDA agreed that more characterization is also needed on the antibodies used for the human and animal model bioassays, notable on the epitope and species specificity. FDA offered to provide additional guidance to the sponsor as their method development and validation plans, both for the human and animal model samples, proceed.

Question 2: Have we adequately controlled pre-analytical variability in our handling of CSF for PrP quantification? What, if any, further experiments are needed?

FDA Response to Question 2:

While your overall approach seems reasonable, please provide a detailed SOP that allows a review of the standardized and objective methods you propose to use to control for the pre-analytical variability during the handling of CSF. Also see general comments on SOPs in response to Question 1.

We note that some of the CSF samples tested in the data you submitted were collected almost two years prior to the analysis. In your SOPs, please define limits for storage time and temperature for the samples based on available data on the stability of PrP concentration measured over time.

Meeting Discussion:

The sponsor agreed to provide SOPs when methods have been further developed after incorporating feedback from FDA, and they have defined storage conditions and limits for their samples as method development proceeds.

Question 3: Have we adequately established that the tissue of origin for CSF PrP is brain? Besides the planned preclinical experiments in rats to correlate CSF PrP to brain PrP following ASO treatment (described below), what other experiments would be necessary?

FDA Response to Question 3:

See responses to Questions 6 and 7.

Meeting Discussion:

See discussion under Question 7.

Question 4: Have we adequately established test-retest reliability of CSF PrP in presymptomatic genetic prion disease mutation carriers? Other than continuing to collect a larger cross-sectional cohort and additional longitudinal timepoints, what other data will be necessary?

FDA Response to Question 4:

See responses to Questions 1 and 2.

Meeting Discussion:

See discussion under Questions 1 and 2.

Question 5: What, if any, further studies in this domain will best help to support this biomarker for use as a surrogate endpoint under the Accelerated Approval program?

FDA Response to Question 5:

See responses to Questions 6 and 7.

Meeting Discussion:

See discussion under Question 7

Questions Regarding Preclinical Experiments to Support Use of PrP as a Biomarker

Question 6: Pending results from ongoing analytical validation, will any further validation of the cross-species ELISA be required to support its use in preclinical studies related to this project?

FDA Response to Question 6:

See responses to Questions 1 and 2 for comments regarding ELISA methods validation.

There may be there may be additional considerations for a rodent-specific PrP ELISA bioassay that will need to be reviewed. We ask that you submit data supporting analytical validation of the animal PrP assay to allow our assessment, including species specificity of the antibodies and reference standard, assay linearity, matrix effects, limits of quantitation, and variability of the method. When successfully validated, provide your standard operating protocols for specific use in your rodent studies.

Meeting Discussion:

See discussion under Question 7.

Question 7: Will our proposed experiments relating brain PrP knockdown to survival time in prion-infected mice be adequate to establish the minimum reduction in PrP needed to confer a clinical benefit? What other experiments would be required?

FDA Response to Question 7:

Based on the limited discussion provided in the briefing documents and the proposed use of the nonclinical data, we have the following comments on your nonclinical program:

- To more convincingly demonstrate the relationship between brain PrP knockdown, disease progression, and survival time in animals, we recommend you conduct studies in more than one animal model of prion disease. Justification (with supportive data) should be provided for the animal models selected, as well as a discussion as to the clinical relevance of each. These studies should:
  - Test a wide dose range to fully explore dose-response in vivo, using a sufficient number of animals per group to ensure adequate power to detect a meaningful effect.
  - Be conducted using a clinically relevant route of administration and dosing regimen. In the completed proof-of-concept (PoC) studies in the infected mouse, animals were initially administered the ASO prior to infection, which may be less relevant to the human situation.
- The relevance of data on the tissue of origin of csf PrP and relationship between brain and csf levels of PrP in a species (e.g., rat) other than the one(s) used for animal efficacy studies is uncertain. However, in evaluating the relationship between brain and csf PrP levels, it would be potentially important to assess regional differences in PrP levels in brain as well as total brain levels, prior to and after ASO administration.
- Pivotal nonclinical studies to support the use of csf PrP as a biomarker for humans and to establish a minimum reduction in csf PrP necessary to predict clinical benefit should be conducted in a rigorous manner, with appropriate controls. Study reports should provide a detailed description of the methods used and the results, to include summary and individual animal data.

Meeting Discussion:

The sponsor asked for clarification on the need for and the purpose of animal efficacy studies. The Division stated that animal efficacy studies were critical for establishing a relationship between pharmacological activity and the sponsor's proposed biomarker, CSF levels of PrP. In addition, the Division noted that:

- For this purpose, the animal efficacy studies should be conducted in rigorous manner, similar to what would be expected under the Animal Rule, although the Animal Rule would not apply. That is, the animal efficacy studies should be adequate and well-controlled, with pre-specified endpoints and a statistical analysis plan.
- Unless the sponsor could justify use of a single animal model, the prion-lowering antisense oligonucleotides (ASO) should be tested in at least two

different animal models (not necessarily two different species). The human relevance of the selected animal model(s) would need to be adequately documented.

- The dosing regimen used in the pivotal animal efficacy studies should be the same as that proposed for humans, including the timing in relationship to prion infection or disease progression. For example, the relevance to humans of administering prion-lowering ASOs to animals prior to infection is unclear.

The sponsor presented the study design for an animal efficacy study in prion-infected rat and requested feedback. The Division stated that comments on the design could not be provided at the meeting but recommended that the sponsor provide full protocols, with all supportive data, for review and feedback prior to initiation of the studies. The Division clarified that the animal efficacy data would not be required in order to initiate a clinical study in humans.

Question 8: Will our proposed experiments in rats correlating CSF PrP to brain PrP upon administration of a range of ASO doses be adequate to establish that CSF PrP reflects brain PrP knockdown? Are any other studies required?

FDA Response to Question 8:

See response under Questions 6 and 7.

Meeting Discussion:

See discussion under Question 7.

Question 9: What further studies in this domain will best help to support this biomarker for use as a surrogate endpoint under the Accelerated Approval program?

FDA Response to Question 9:

See response under Questions 6 and 7.

Meeting Discussion:

See discussion under Question 7.

3.0 ADDITIONAL INFORMATION

SECURE EMAIL COMMUNICATIONS

Secure email is required for all email communications from FDA when confidential information (e.g., trade secrets, manufacturing, or patient information) is included in the message. To receive email communications from FDA that include confidential information (e.g., information requests, labeling revisions, courtesy copies of letters), you must establish secure email. To establish secure email with FDA, send an email request to [redacted] Please note that secure email may not be used for formal regulatory submissions to applications (except for 7-day safety reports for INDs not in eCTD format).

4.0 ISSUES REQUIRING FURTHER DISCUSSION

There were no issues requiring further discussion.

5.0 ACTION ITEMS

There were no action items identified during the meeting.

6.0 ATTACHMENTS AND HANDOUTS

- Broad Institute response document to FDA's preliminary comments, received via email on October 29, 2019.

Type C Meeting — Response to FDA Comments

Requestor: Broad Institute of MIT and Harvard

Pre-IND: 141250

Product Name: Prion Protein (PrP)-lowering antisense oligonucleotides

Re: Response to FDA Comments on Type C Meeting Materials

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Overall response to FDA comments

We wish to thank the team for the thoughtful, detailed response to all of the materials we submitted, and for offering us the opportunity to discuss this proposed surrogate endpoint in person.

Below, we provide a point-by-point response to FDA comments. First, we would also like to confirm with FDA whether we have correctly interpreted the following points from the response document:

1. While much work remains to be done in terms of analytical validation and nonclinical studies to support this biomarker, are we correct to infer that there are no other high-level concerns about the fundamental biological validity of the proposed biomarker or its potential to be used as a surrogate endpoint in trials in pre-symptomatic individuals at risk for genetic prion disease, provided that adequate supporting data are provided as suggested in the response?
2. Are we correct to assume that the additional data requested in FDA's response would serve primarily to inform on whether biomarker data from a completed trial can eventually support an Accelerated Approval application, and that the requested experiments are therefore not pre-requisites in order to even begin trials in pre-symptomatic individuals?

Point-by-point response to FDA Comments

1. Analytical validation of BetaPrion Human ELISA kit

Question 1: What, if any, further analytical validation of the technical performance of the BetaPrion Human ELISA kit will be necessary to support its use as an assay for a surrogate biomarker endpoint?

FDA Response to Question 1:

Based on the information you submitted regarding the technical performance of the BetaPrion Human ELISA kit and the targeted mass spectrometry assay, please see below for aspects that we believe either require additional data or clarification. Where possible, we have provided recommendations to address the gaps.

Based on the detailed FDA comments below, we propose that the next steps are for our partners at Ionis Pharmaceuticals to:

1. Determine which assay (BetaPrion Human ELISA or the new cross-species ELISA, see below) will ultimately be used for analysis of clinical trial samples, and in what configuration (e.g. with the kit standard curve versus with an in-house recombinant PrP standard curve). These determinations might be made based in part on advice at this Type C meeting, in conjunction with additional data to be generated.
2. Identify a laboratory that will be responsible for analysis of clinical trial samples.
3. Ask that laboratory to perform a full validation of the desired ELISA assay for the human CSF matrix in order to ensure compliance with the FDA 2018 Guidance on Bioanalytical Method Validation.

The data we submitted in our meeting package (Appendix 3) were generated in-house at the Broad Institute, and while we made every effort to be rigorous in our protocols and data analysis, we are not a GLP/GCP laboratory, so addressing the comments below at a standard acceptable to FDA may not be practical here. Further, Broad is unlikely to be the venue for ultimate analysis of clinical trial samples, and ideally the assay validation should take place in the laboratory that will perform that analysis.

With those caveats, we enclose a new attachment, Appendix 7, providing the SOP information requested by FDA, in order to describe how the data we already submitted were generated. We further provide point-by-point responses to each query below, regarding our in-house assay protocol, in order to enable evaluation of the validity of the data we have already submitted. Note that our responses here are intended as *descriptive* of how the data submitted were generated; we have attempted to note the places where we believe a future re-validation of the assay should differ.

Your SOPs should describe steps for any acceptance/rejection criteria for the samples and raw data at each key step, chain of custody of the CSF

samples, blinding protocols for the samples, and roles/responsibilities for each analyst or laboratory involved in the procedures.

See attached SOPs (Appendix 7). In our initial experiments (Appendix 3) we were limited to re-used residual samples from other studies, so there was no chain of custody, and indeed, a key finding of our studies was the apparently large impact of pre-analytical variables. Blinding was accomplished simply by not asking collaborators for a key to their samples until after we had analyzed them. The analysts were Sonia Vallabh and Eric Minikel, who were responsible for all steps of running the assay and analyzing data after receipt of samples in our lab. We had not formalized any acceptance/rejection criteria; one plate (plate #3) was thrown out due to poor technical replicate agreement (mean CV=59%) and that experiment was repeated.

In future validation studies, we propose that the responsible laboratory would establish all of these steps strictly according to FDA's 2018 Guidance on Bioanalytical Method Validation.

The ELISA method SOP should have sufficient detail on the standard curve material, capture and detection antibodies, dilution steps, detection methods, and steps for calculating the final reported PrP value with appropriate units.

See attached SOPs (Appendix 7). All CSF sample analyses we have reported to date (Appendix 3 and Appendix 5) used the standard curve material, antibodies, and detection reagents provided by the manufacturer. We used our own blocking buffer (5% wt/vol BSA, 0.05% Tween20, 1X PBS) in lieu of the manufacturer's diluent. The reported PrP value was calculated applying a log-linear standard curve fit (`nominal_conc ~ log(A450 - A620)`), flagging any observations outside the kit's nominal dynamic range (1-20 ng/mL), and multiplying by the dilution factor employed.

Your procedures for rejected samples or deviations from the validated method need to be described with clear instructions on how an analyst must proceed in such cases. For example, is re-testing allowed? What are the limits and objective criteria for re-testing? What are the objective criteria under which a CSF sample might be rejected? Is sample pooling allowed?

In the original data submitted (Appendix 3 and 7), all samples analyzed were reported. As noted above during early assay validation experiments (plate #3 in Appendix 3 / Vallabh et al, 2019), one plate was repeated due to poor technical replicate agreement. However, in both Appendix 3 and Appendix 7, all CSF samples analyzed were ultimately reported.

For future analytical validation studies, we would propose that:

- Re-testing of entire plates should be allowed where plate QCs fall outside of a pre-specified acceptable %RE range or where technical replicate coefficients of variation (CVs) fall above a pre-specified cutoff.

- Re-testing of individual samples would be allowed if technical replicate agreement for the sample falls outside of a pre-specified range or if the sample reads out at the upper or lower limit of quantification.
- CSF samples would be excluded, and not analyzed at all, in the event of pre-analytical issues such as: sample at room temperature when received, CSF volume received not equal to that reported in shipping manifest, sample processing SOP not followed properly.
- Sample pooling would not be allowed for human samples, because in humans, one lumbar puncture provides sufficient CSF volume for all necessary analyses. (Pooling may be required in nonclinical biomarker studies described below, where smaller volumes are available).

Regarding your bioassay validation for the ELISA method submitted in Appendix 3:

a. Clarify whether the kit standard included as part of the commercial kit or the recombinant human PrP standard you generated will be used for the proposed study.

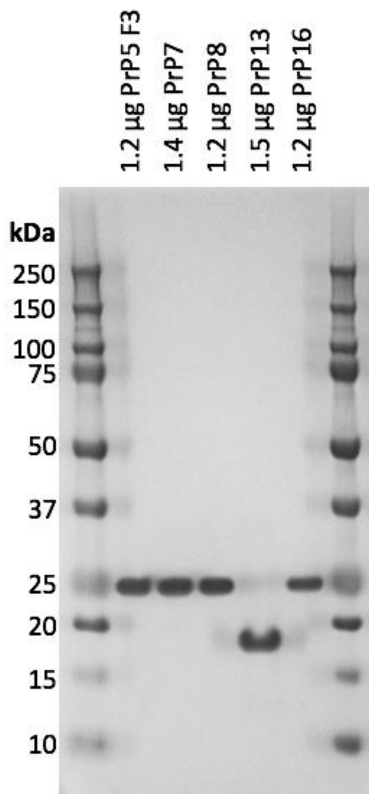
We propose that in principle either standard could be used; see responses to items "i" through "vi" below for more detail on potential considerations.

i. Provide data for the source, purity, identity (peptide sequence, tags), and stability of your in-house recombinant human PrP reference standard.

The recombinant PrP construct was received from Dr. Byron Caughey (NIH Rocky Mountain Labs); the expression and purification procedures have been published[1]. It is untagged (PrP's native sequence is sufficiently histidine-rich that the untagged protein purifies on Ni-NTA columns just like a His-tagged protein). The sequence is:

MKKRPKPGGWNTGGSRYPGQGSPGGNRYPPQGGGGWGQPHGGGWGQPHGGGW
GQPHGGGWGQPHGGGWGQGGGTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLG
GYMLGSAMSRPIIHFGSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITI
KQHTVTTTTKGENFTETDVKMMERVVEQMCITQYERESQAYYQRGS

From a single liquid chromatography fraction (rPrP5_F3), we produced a large number of 40 µL aliquots formulated with 0.03% CHAPS for analysis. We sent two aliquots to New England Peptide for amino acid analysis (AAA) in duplicate and received back individual readings of 0.31 and 0.32 mg/mL. Purity was assessed only informally by Coomassie gel (see below); no bands were visible besides the expected PrP band at 22.9 kDa.



ii. Justify the representativeness of the in-house recombinant human PrP reference standard to the human PrP found in pre-symptomatic patient CSF.

The human PrP open reading frame contains a signal peptide (residues 1-22) and a GPI signal (residues 231-253), both of which are cleaved off before PrP ever reaches the cell surface; the recombinant construct encodes only residues 23-230, representing the mature protein, plus an N-terminal methionine start codon. Recombinant PrP lacks the post-translational modifications (GPI anchor, N-linked glycosylation) found in endogenous PrP, but unlike endogenous PrP, it can be purified in large quantities and readily characterized by biochemical methods, so it is tractable as an assay standard.

In order to establish the analytical validity of the assay, it will be important to perform parallelism experiments to determine whether, or under what conditions, endogenous PrP in CSF dilutes similarly to recombinant PrP in the standard curve (for instance, over what range of dilutions into blocking buffer does one CSF sample yield the same dilution-adjusted PrP concentration calculated from a standard curve fit). We performed such experiments for the BetaPrion Human ELISA kit using kit-supplied standards, with promising preliminary results (Appendix 3 Figure S1A) but they should be repeated in the context of full validation, in the responsible laboratory under the final assay configuration (with the standard curve material that will be used in clinical trial sample analysis), with pre-specified criteria.

iii. We are unclear on what you mean by "A recombinant standard curve was prepared from AAA-quantified recombinant huPrP" in Appendix 3 Supplemental Figure S3. Clarify if the recombinant PrP concentration measurement uses extinction coefficient for the calculations.

We did not use extinction coefficients for this calculation. The only source of concentration information was the duplicate amino acid analysis (AAA) performed by New England Peptide which indicated concentrations of 0.31 or 0.32 mg/mL for the stock 40 μL aliquots, which were subsequently diluted and then plated.

iv. We are concerned that your currently validated range for the standard curve may not capture the range of pre- and post-treated CSF PrP concentrations. In Appendix 3, Supplemental Figure S3B, the linear portion of recombinant human PrP standard curve, measured within the BetaPrion Human ELISA kit's detection range 1-20 ng/ml, has only 4 points in the linear portion of the curve. The two highest concentrations of recombinant human PrP standard resulted in plateauing of the absorbance units and are not part of the linear standard curve drawn. You should ensure that a sufficient number of data points form the linear standard curve within a range that is clinically meaningful in terms of the anticipated drug response you expect from the ASOs you study. We generally recommend at least 6 non-zero data points in the linear range. Please provide a narrative that justifies your choice of the reference standard and the linear range of the validated reference standard.

We agree that the data in Figure S3B contain only 4 recombinant PrP standard points in the assay linear range, and that more are needed.

If it is determined that clinical sample analysis will rely upon the BetaPrion Human ELISA kit with an in-house recombinant standard curve, then the performing laboratory should create a new dilution series to use as a standard curve, and a new curve fit (e.g. 4PL instead of log-linear) might be employed as well. Because we estimate that our recombinant protein reads out at ~4x the concentration of the kit standards, one could begin by testing a range of recombinant PrP concentrations from approximately 0.25 to 5 ng/L, chosen to mirror the 1-20 ng/mL range of the kit standards.

Alternatively, it is possible that the kit standard curve could suffice instead, in which case the 6 standard concentrations provided appear to all fall within a linear range of the assay. Or, it is possible that the cross-species ELISA described further below could be used.

v. Clarify the matrix used to dilute the reference standard and clinical samples.

5% wt/vol BSA, 0.05% Tween20, 1X PBS, filtered.

vi. In the Appendix 3 paper and the supplementary discussion, you conclude that "kit standards, while technically reproducible, may most usefully inform relative rather than absolute quantification of PrP." We are unclear on what this statement means. Please clarify and justify your quantitative output given this potential caveat.

Our goal in a clinical trial would be to determine whether, and by how much, ASOs lower PrP in the CSF of pre-symptomatic mutation carriers. We believe that it is the percent lowering of PrP from each individual's pre-treatment baseline, rather than absolute concentration in ng/mL, that is of biological interest for knowing whether a biomarker readout is reasonably likely to predict clinical benefit. As long as the assay is technically reproducible, we believe it could support a determination that PrP is lowered by (for example) 40% after versus before ASO treatment, or in ASO-treated individuals versus in placebo-treated individuals. For this reason, we believe that the BetaPrion kit standards could be used as the calibration curve in a clinical trial context.

There might be potential advantages to an in-house recombinant PrP standard curve: it might provide quantification that is somewhat more accurate in terms of absolute concentrations, and one could test whether it also offers lower inter-plate variability. A limitation is that recombinant PrP is not commercially available — an academic lab such as ours at the Broad Institute would likely need to produce the recombinant PrP for analysis of clinical trial samples if a recombinant PrP standard curve is required.

If FDA scientists believe that absolute quantification, in ng/mL, should provide biological meaning above and beyond percent change in PrP concentration before/after treatment, we would be interested to understand the motivation for this so that we can better ensure our future data address FDA's requirements.

b. Clarify the source and epitope specificity of the antibodies used in the ELISA method.

The BetaPrion Human ELISA kit uses the 3F3 capture antibody and the HRP-conjugated 15F5 detection antibody. These antibodies are owned by the kit's manufacturer, AJ Roboscreen Gmbh, and are not commercially available to purchase separate from this kit. The antibodies have been described in a previous publication by another group[2]. The antibodies were produced in PrP knockout mice using recombinant human PrP as the immunogen. The detection antibody's epitope is reported to be RYYRENMHRY, while the capture antibody's epitope was reported as being conformational.

c. The critical reagents in the modified ELISA should be evaluated for their stability at the intended storage or in-use temperature.

This should be included as part of a future validation effort.

d. Appendix 3 provides data on dilution range, spiking, plate to plate variation, and within plate variation produced with different CSF samples. We generally recommend the inclusion of quality control samples, such as a positive and negative control CSF sample. Will quality control samples of known concentrations of PrP be included in the validation runs of the assay?

Yes, QCs should be included in a future validation effort. Note, however, that because PrP is an endogenous analyte present in all available CSF samples, the "known" concentration for QCs will simply be the concentration determined through one or more

runs of the assay prior to beginning validation. It is not possible to construct a sample with the endogenous CSF matrix but with a truly “known” PrP concentration.

e. According to submitted information, the LLOQ reported is 3-5x the blank signal. We generally recommend ≥ five times the analyte response of the zero calibrator (see Table 1 in FDA Bioanalytical Method Validation Guidance for Industry (2018)). You state that the variation stems from different plate readers; therefore, consider validating the method using a plate reader of choice or predefine the instrument variables that achieves the recommended LLOQ level in relation to the blank and independent of the plate reader choice.

Noted, a future validation effort should use a single platereader and it should be the same reader that will be used in analysis of clinical trial samples.

f. As drug development proceeds, you should plan to include inter-laboratory variability testing (reproducibility), in addition to the intra-laboratory variability (intermediate precision) you have tested.

We currently envision having a single centralized laboratory be responsible for all analysis of clinical trial CSF samples. (We likewise envision a single laboratory analyzing all preclinical animal CSF samples). Assuming that this is the case, would inter-laboratory variability testing still be recommended?

3. Regarding the mass spectrometry (MS) data submitted in Appendix 4.
a. Targeted MS is used to show that correlation of total protein to measured PrP in ELISA is not due to ELISA measuring off-target proteins. We are unclear if you are proposing to use the targeted MS for additional assay validation and use with clinical samples. Please clarify your proposed plan for use of the MS method.

At this time, we do not see a need for extensive further use of the MS assay. We originally developed the MS assay in order to determine whether it would add any value beyond that offered by ELISA — for example, as one hypothetical outcome, if one peptide had proved to be elevated in the CSF of symptomatic prion disease patients, while other peptides proved to be reduced, then we would know that ELISA was not telling us the full story. Instead, all of our results to date suggest that ELISA and all six peptides detected in MS seem to be measuring the same thing. A caveat is that so far, using this MS assay, we have only measured changes in PrP levels in CSF due to prion disease. We do still intend to do a small experiment to determine whether all peptides also behave similarly in response to treatment with an ASO, in rats. If we indeed determine that the peptides are similarly lowered by ASO treatment, and that the percent lowering observed is similar to that measured by ELISA, then we propose not to do further experiments using the MS method. It is more expensive and time-consuming than ELISA, and the set of CROs that can perform targeted MS assays on peptides is more limited, so we have had difficulty identifying a company that is willing to surmount the prion biosafety challenges in order to scale and perform validation of the assay for use in patient samples. The MS assay can be revisited as a backup option if our ELISA

assays prove impossible to validate analytically, or in the event that new data raises concerns about the interpretability of ELISA data.

b. If you chose to include this method as part of your proposed clinical study, additional data confirming assay validation would be needed.

Agreed — if we do decide to further advance the MS assay, further validation will be undertaken.

c. We note that the targeted MS method appears to provide greater values for PrP concentration than the ELISA method for comparable samples (Figure 3 in Appendix 4). Please provide your explanation for this difference. If differences might be due to protein conformational and peptide sequence differences as detected by the ELISA method, please consider including both the ELISA and MS methods in your proposed study as orthogonal measures of PrP concentration.

In fact, the higher concentration shown for MS in Appendix 4 Figure 3 is an artifact of the normalization procedure used in the analysis of the MS data. The raw MS responses obtained vary by an order of magnitude depending on which peptide is examined, so the mean concentrations of PrP inferred from these responses would range from 39 ng/mL for the lowest-responding peptide to 478 ng/mL for the highest-responding peptide (see Appendix 4 Table S5, p. 26). According to our collaborators in Broad's Proteomics Platform (led by Dr. Steven Carr), variability in response both among peptides of the same protein, and between peptides versus ELISA measurements of the same protein, is typical in tryptic peptide MS assays and should generally be normalized by multiplying the raw MS readings by a "response factor" for each peptide. We applied response factors calibrated to make each peptide respond as highly as the highest-responding peptide, thus, the MS concentrations of PrP appear higher than those from ELISA.

For clinical trial samples, we believe that ELISA should be the sole analytical method for assessing the primary endpoint, because it gives only one readout (the MS assay as currently configured gives six) and because it is suited to validation in an independent lab (see MS limitations noted under 3a above).

2. Pre-analytical variability

Question 2: Have we adequately controlled pre-analytical variability in our handling of CSF for PrP quantification? What, if any, further experiments are needed?

FDA Response to Question 2:

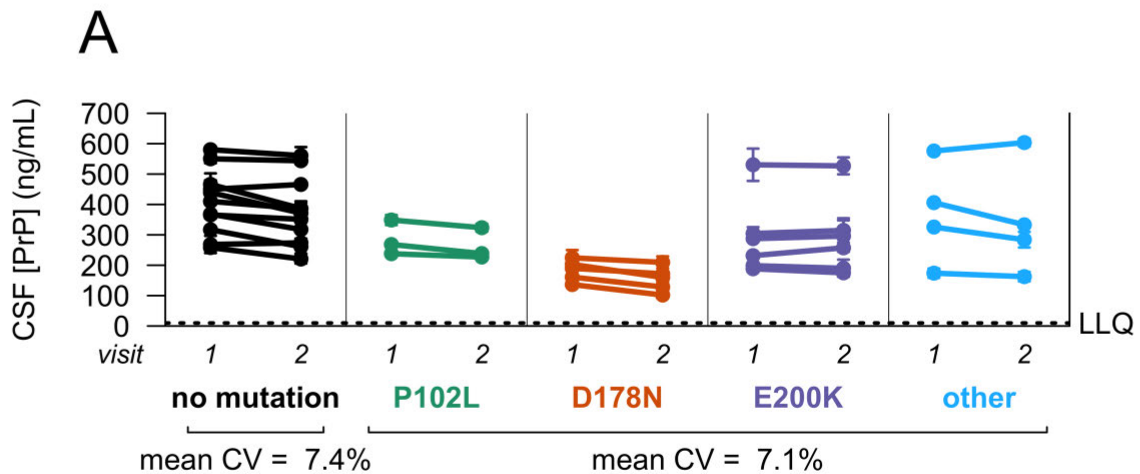
While your overall approach seems reasonable, please provide a detailed SOP that allows a review of the standardized and objective methods you propose to use to control for the pre-analytical variability during the

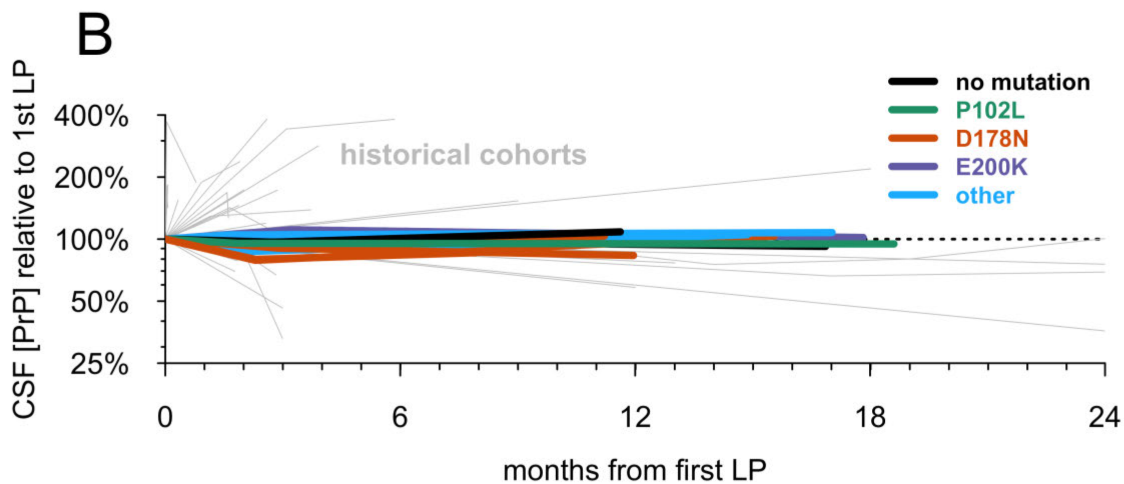
handling of CSF. Also see general comments on SOPs in response to Question 1.

We note that some of the CSF samples tested in the data you submitted were collected almost two years prior to the analysis. In your SOPs, please define limits for storage time and temperature for the samples based on available data on the stability of PrP concentration measured over time.

In a new attachment, Appendix 8, we provide an SOP for how we have been handling CSF samples from the Massachusetts General Hospital clinical study.

All CSF samples in this study have been shipped to our lab on dry ice and stored at -80°C since receipt. While we have not directly compared the PrP concentration in the same sample before and after long-term storage at -80°C, our results suggest that the impact of this storage on PrP concentration is likely to be negligible. That is because the test-retest stability of CSF PrP over a year or more appears comparable to that observed over the short term of 2-4 months (Figure 5-1, Appendix 5). We have now confirmed this result in N=10 individuals who have made a longitudinal follow-up visit 10-20 months after their first visit. Below is an updated version of Figure 5-1 with our current data freeze:





The variation in panel B over 10-20 months is nearly identical (CV=7%) to that observed over 2-4 months in panel A. This suggests to us that storage at -80°C over a year or more is unlikely to substantially change PrP concentration.

This study will continue recruiting participants to make additional longitudinal visits, and we will update this analysis as additional timepoints become available.

3. Tissue of origin

Question 3: Have we adequately established that the tissue of origin for CSF PrP is brain? Besides the planned preclinical experiments in rats to correlate CSF PrP to brain PrP following ASO treatment (described below), what other experiments would be necessary?

FDA Response to Question 3:

See responses to Questions 6 and 7.

4. Test-retest reliability

Question 4: Have we adequately established test-retest reliability of CSF PrP in presymptomatic genetic prion disease mutation carriers? Other than continuing to collect a larger cross-sectional cohort and additional longitudinal timepoints, what other data will be necessary?

FDA Response to Question 4:

See responses to Questions 1 and 2.

5. Further clinical biomarker studies

Question 5: What, if any, further studies in this domain will best help to support this biomarker for use as a surrogate endpoint under the Accelerated Approval program?

FDA Response to Question 5:

See responses to Questions 6 and 7.

6. Analytical validation of cross-species ELISA

Question 6: Pending results from ongoing analytical validation, will any further validation of the cross-species ELISA be required to support its use in preclinical studies related to this project?

FDA Response to Question 6:

See responses to Questions 1 and 2 for comments regarding ELISA methods validation.

There may be there may be additional considerations for a rodent-specific PrP ELISA bioassay that will need to be reviewed. We ask that you submit data supporting analytical validation of the animal PrP assay to allow our

assessment, including species specificity of the antibodies and reference standard, assay linearity, matrix effects, limits of quantitation, and variability of the method. When successfully validated, provide your standard operating protocols for specific use in your rodent studies.

Cambridge Biomedical, Inc., has just completed the assay runs for validation of a new cross-species ELISA assay for the rat CSF matrix as of Oct. 24; the final validation report is still in preparation. In lieu of this final validation report, we provide a new attachment, Appendix 9, showing preliminary data regarding this assay.

We developed this new cross-species ELISA assay in order to use it for the nonclinical studies to which questions 6 and 7 pertain. We would value any input on whether the data collected so far establish that this assay is fit for purpose in this context.

The assay does appear to be cross-reactive for human PrP (Appendix 9), so in the event that this assay proves to have advantages over the BetaPrion Human ELISA assay, it could also be a potential candidate for use in a clinical trial, provided that it can be fully validated for human CSF.

7. Nonclinical experiments regarding minimum knockdown for clinical benefit

Question 7: Will our proposed experiments relating brain PrP knockdown to survival time in prion-infected mice be adequate to establish the minimum reduction in PrP needed to confer a clinical benefit? What other experiments would be required?

FDA Response to Question 7:

Based on the limited discussion provided in the briefing documents and the proposed use of the nonclinical data, we have the following comments on your nonclinical program:

- **To more convincingly demonstrate the relationship between brain PrP knockdown, disease progression, and survival time in animals, we recommend you conduct studies in more than one animal model of prion disease. Justification (with supportive data) should be provided for the animal models selected, as well as a discussion as to the clinical relevance of each. These studies should:**

Test a wide dose range to fully explore dose-response in vivo, using a sufficient number of animals per group to ensure adequate power to detect a meaningful effect.

Be conducted using a clinically relevant route of administration and dosing regimen. In the completed proof-of-concept (PoC) studies in the infected mouse, animals were initially administered the ASO prior to infection, which may be less relevant to the human situation.

To address all of these points, we propose a new dose-response efficacy study in prion-infected rats administered ASOs intrathecally (IT). Justification for each choice of parameter in this study design is described in the table below.

parameter	choice	justification
species	rat	• Rats, like almost all mammals, are susceptible to prion infection, and represent a long-established[3], if not often used, model of prion disease. Recent reports indicate that rats intracerebrally inoculated with prions develop clinical signs in 175-200 days[4,5]. • Because the proposed pharmacodynamic studies to establish the relationship between brain and CSF PrP knockdown after ASO treatment are being conducted in rats, adding an efficacy study in an intracerebrally infected rat model of prion disease would allow us to complete all of these inter-related studies in a single species. • Ionis has extensive experience with IT dosing of ASOs in rats, making this a validated and feasible delivery method in this species.
administration route	intrathecal (IT)	• ASOs would be administered IT in humans. • IT delivery is a validated method for ASO delivery in rats.
treatment timepoint	latest timepoint after prion infection but before rise in plasma neurofilament light chain (NfL)	• In our Massachusetts General Hospital clinical research study, we find that CSF and plasma NfL, markers well-established to rise in neurodegenerative disease generally and in prion disease specifically, are still at normal levels in pre-symptomatic mutation carriers and are indistinguishable from mutation-negative controls. • In mice, NfL levels are reported to rise at 60-75 days after prion infection[6], similar to the time (~55-73 dpi) at which reactive astrocytosis can first be detected by live animal bioluminescence imaging in transgenic mice[7,8]. • We have already completed prophylactic dosing studies, which may be analogous to any pre-symptomatic mutation carriers in whom prion replication has not yet begun. By also adding a timepoint after prion infection but before plasma NfL rises, we can model any hypothetical pre-

		symptomatic mutation carriers in whom prion replication has begun but detectable neurodegeneration has not yet occurred. Between these two paradigms, we can bracket the possible disease stages in which the pre-symptomatic mutation carriers we have so far observed might currently stand. • The exact treatment timepoint, in days post-infection, will be determined in a pilot study in which we collect serial serum samples from prion-infected and uninfected rats at 1-month intervals and measure serum NfL. The latest timepoint at which a significant elevation of serum NfL is not observed will be selected as the treatment timepoint for this study.
dosing regimen	single dose	• Repeated IT dosing in rats is technically challenging, can result in surgical complications and incomplete drug delivery. • In mouse studies, a single dose of PrP-lowering ASO was enough to confer at least some survival benefit (albeit less than chronic dosing).
dose response range	0 (saline), 100 µg, 300 µg, 1 mg, 3 mg	• 3 mg is the maximum dose of ASO routinely administered to rats. • One ASO already identified by Ionis and tested in mice is sequence-matched to rat *Prnp* and appears to be tolerated and active (reducing *Prnp* mRNA) at 3 mg IT in rats. • The lower doses represent approximately half-log increments down from 3 mg. • Doses below 100 µg are not worth testing because, with IT delivery, they may not achieve appreciable target engagement in the brain.
control groups	saline-treated, prion-infected animals; control brain homogenate (CBH)-inoculated animals	• The prion-infected but saline-treated animals (the “0” dose under “dose response range” above) will serve as a control to determine the incubation time to which ASO-treated animals should be compared. • Based on our studies to date[9], we have established that ASOs are effective against prion disease only through sequence-dependent interaction with PrP RNA. A non-targeting control ASO has proven ineffective. Therefore, we propose that saline-injected animals represent an adequate control group for future nonclinical studies.

		• A group of animals inoculated with control (non-infectious) brain homogenate instead of prion brain homogenate, will serve as additional controls not expected to ever develop prion disease, and against which the progression of disease can be compared.
cohort sizes	to be determined	• The number of rats per treatment group will be determined based on the pilot study (see last bullet under "treatment timepoint" above). Based on the variability in time to disease endpoint, we will select a number of rats per group that makes the study well-powered to observe a therapeutic benefit from a single dose of ASO.

We would appreciate feedback on this proposed study design.

In addition, we would like to understand FDA's motivation for requesting these studies and anticipated use of the data. We interpret these requests as required data to inform the use of CSF PrP as a surrogate endpoint, but note that separate GLP toxicology studies will be done by Ionis for the human compound to enable initiation of human studies.

The relevance of data on the tissue of origin of csf PrP and relationship between brain and csf levels of PrP in a species (e.g., rat) other than the one(s) used for animal efficacy studies is uncertain. However, in evaluating the relationship between brain and csf PrP levels, it would be potentially important to assess regional differences in PrP levels in brain as well as total brain levels, prior to and after ASO administration.

If survival studies can be conducted in prion-infected rats, will this address the uncertainty about the relevance of the data?

As for regional differences, we propose that ASO- and saline-treated rats could be compared for PrP concentration in CSF and in five micro-dissected brain regions: cortex, thalamus, hippocampus, brainstem, and thoracic spinal cord.

Pivotal nonclinical studies to support the use of csf PrP as a biomarker for humans and to establish a minimum reduction in csf PrP necessary to predict clinical benefit should be conducted in a rigorous manner, with appropriate controls. Study reports should provide a detailed description of the methods used and the results, to include summary and individual animal data.

Regarding the dose-response data form mice that we have already submitted, we have now amended Appendix 6 to add 1) more detailed methods on procedures used, and 2) a table containing individual-level data on all animals in the completed dose-response study. We will plan to provide similar details for future studies.

Generally, it is worth noting that we are limited by the fact that animal models of prion disease produce infectious material. The shipment of prion-infected animal tissues and the use of prions in animals is regulated by USDA, which poses an additional regulatory hurdle, and the studies require ABSL2 facilities with certain non-standard enhanced practices for isolation and decontamination. Therefore it will not likely be feasible to identify a GLP laboratory capable of conducting nonclinical studies in infected animals — we will be limited to non-GLP studies in academic laboratories at the Broad Institute or elsewhere.

8. Correlation of brain PrP knockdown to CSF PrP knockdown

Question 8: Will our proposed experiments in rats correlating CSF PrP to brain PrP upon administration of a range of ASO doses be adequate to establish that CSF PrP reflects brain PrP knockdown? Are any other studies required?

FDA Response to Question 8:

See response under Questions 6 and 7.

Question 9: What further studies in this domain will best help to support this biomarker for use as a surrogate endpoint under the Accelerated Approval program?

9. Further nonclinical studies

FDA Response to Question 9:

See response under Questions 6 and 7.

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