# Type C Meeting Request

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| Requestor: | Broad Institute of MIT and Harvard |
| Pre-IND: | 141250 |
| Product Name: | Prion Protein (PrP)-lowering antisense oligonucleotides |
| Proposed Indication: | Genetic prion disease |
| Proposed Format: | Face to face |
| Meeting Materials: | Included in this request (see below) |

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# Proposed Participants

Requestor

- Broad Institute and Massachusetts General Hospital:
  - Eric S. Lander, Founding Director of the Broad Institute
  - Sonia M. Vallabh, Prion Scientist at Broad/MGH
  - Eric V. Minikel, Prion Scientist at Broad/MGH
  - Steven E. Arnold, Principal Investigator of MGH pre-symptomatic genetic prion disease biomarker study
- Ionis Pharmaceuticals
  - Anne Smith, Director, Clinical Development
  - Tiffany Baumann, VP Regulatory Affairs
  - Holly Kordasiewicz, Director, Neuroscience

FDA

- Requested FDA personnel:
  - Billy Dunn, Director, Division of Neurology Products
  - Eric Bastings, Deputy Director, Division of Neurology Products
  - Christopher Leptak, Associate Director, Biomarker Development and Regulatory Science
  - Lois Freed, Nonclinical Supervisor
  - Nick Kozauer, Associate Director, Division of Neurology Products
  - Jackie Ware, Chief Regulatory Project Manager
  - Teresa Buracchio, Clinical Team Lead
  - Heather Bullock, Regulatory Project Manager

We welcome FDA's input as to which personnel are most important for this meeting, given the topics to be discussed. If FDA deems appropriate, we are happy to proceed with scheduling the meeting even if not all individuals listed above can attend.

# Suggested dates and times

Thursday October 31, 2019, 11:00 am

# Background

FDA has generously provided guidance and offered to partner with us as we work to enable clinical trials of PrP-lowering antisense oligonucleotides (ASOs) in pre-symptomatic genetic prion disease mutation carriers. In Critical Path Innovation Meeting on November 14, 2017, FDA expressed support for evaluation of prion protein (PrP) in cerebrospinal fluid (CSF) as a surrogate endpoint under the Accelerated Approval program in this population (see Appendix 1 and 2). Following a subsequent phone call

on January 29, 2019, FDA assigned us a Pre-IND number (141520) and invited us to request a Type C meeting to discuss this proposed surrogate biomarker in greater technical detail. While our Prion team at the Broad Institute is working to characterize this biomarker and is the requestor of this meeting, we also seek to include our collaborators at Ionis Pharmaceuticals. Ionis scientists have partnered with us on the development of the PrP-lowering ASO, and Ionis will be the sponsor that brings an ASO to trials. The current status of product development is that Ionis is in the process of selecting a human ASO candidate for advancement to GLP toxicity studies and ultimately to the clinic. In the requested Type C meeting, we would like to hear FDA's feedback on two key issues related to evaluation of the CSF PrP surrogate endpoint: 1) analytical and biological validation of CSF PrP as measured by a commercially available ELISA kit, and 2) preclinical experiments to determine the relationship between brain PrP and CSF PrP upon administration of a PrP-lowering compound.

## Brief Statement of Purpose

We are seeking FDA's advice on experiments to establish whether quantification of prion protein (PrP) in cerebrospinal fluid (CSF) will be suitable as a surrogate endpoint for Accelerated Approval of PrP-lowering drugs in pre-symptomatic individuals with genetic prion disease mutations.

## Proposed Agenda

90-minute meeting:

- Participant introductions (5 minutes)
- Overview and status update on PrP-lowering ASO project (5 minutes)
- Biomarker assessment (30 minutes)
    - Technical performance of BetaPrion Human ELISA (10 minutes)
    - Pre-analytical factors (5 minutes)
    - Tissue of origin (5 minutes)
    - Test-retest reliability (10 minutes)
    - Suggestions for further studies (10 minutes)
- Preclinical experiments (30 minutes)
    - Development of cross-species ELISA (5 minutes)
    - Target suppression vs. survival (10 minutes)
    - Target suppression vs. brain PrP knockdown (5 minutes)
    - CSF PrP knockdown vs. brain PrP knockdown (10 minutes)
- Overall feedback from FDA – suggestions and expectations for further development of this biomarker prior to trials (20 minutes)

# Questions for FDA

## 1. Questions regarding biomarker assessment

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?
2. Have we adequately controlled pre-analytical variability in our handling of CSF for PrP quantification? What, if any, further experiments are needed?
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?
4. Have we adequately established test-retest reliability of CSF PrP in pre-symptomatic 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?
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?

## 2. Questions regarding preclinical experiments to support use of PrP as a biomarker

1. 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?
2. 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?
3. 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?
4. What further studies in this domain will best help to support this biomarker for use as a surrogate endpoint under the Accelerated Approval program?

# Data and context to support discussion

## 1. Biomarker assessment

**Our goal:** At our CPIM, FDA scientists emphasized that a preventive clinical strategy must rest on a strong biomarker assay, and a strong biological understanding of our proposed biomarker, CSF PrP. We wish to gain a thorough understanding of whether CSF PrP can serve as a technically and biologically appropriate biomarker and surrogate endpoint, for PrP-lowering ASOs.

**Our questions:** Below, we describe key findings from the experiments we have performed to date, and the next steps we have planned. We would value FDA feedback on our results so far. How thoroughly do these results answer our motivating questions, and are there key questions we are missing? What additional experiments would FDA recommend to build confidence in this biomarker?

We have evaluated the technical performance of the BetaPrion Human ELISA® kit and have used this kit to ask whether CSF PrP has the necessary biological properties to serve as a surrogate biomarker. Separately, we have developed a targeted mass spectrometry assay and are currently developing a second ELISA assay with cross-species PrP reactivity, so we hope to have multiple options available for measuring PrP in the event that CSF PrP analyte is deemed suitable but the BetaPrion kit fails for any technical reason.

In performing these experiments, we were motivated by the following questions:

1) **Technical performance of the BetaPrion ELISA assay.** Does the BetaPrion ELISA assay perform in a technically reliable way?
2) **Pre-analytical factors that affect CSF PrP.** Is CSF PrP a well-behaved analyte? Is it susceptible to pre-analytical variability? If so, can we control this variability?
3) **Tissue of origin of CSF PrP.** Is CSF PrP derived from the brain, our tissue of interest?
4) **Within-subject test-retest reliability of CSF PrP.** Is CSF PrP stable enough in pre-symptomatic genetic prion disease mutation carriers over the likely duration of a clinical trial on within-subject test-retest that an ASO-dependent decrease in PrP could be reliably measured?

Below is a list of key findings so far from our studies.

### 1. Technical performance of the BetaPrion ELISA assay

- We evaluated the performance of the BetaPrion Human ELISA kit in our own lab by analyzing *N*=225 CSF samples, including symptomatic prion disease samples, healthy prion disease mutation carrier samples, non-prion dementia samples, and non-dementia control samples.

- PrP was present at measurable levels in all samples, across diagnostic categories.
- We found technical replicate reproducibility of CV = 8% within plate, and CV = 22% between plate (including inter-operator, inter-platereader, and inter-lot variability). We have proposed that samples to be compared experimentally (such as repeat samples from the same individual) should be co-located on the same plate.
- In assessing calibration curve reproducibility, we found CV < 10% at all six non-zero standard curve concentrations, across five replicate standard curves run on the same plate.
- We observed 90% spike-in recovery of recombinant PrP across five concentrations.
- CSF PrP is correlated with CSF total protein. Such a correlation could in principle reflect A) genuine biological correlation, B) pre-analytical factors (see below) or C) contribution to ELISA signal of non-specific binding from other proteins. In a subset of *N*=55 samples analyzed by targeted mass spectrometry, the PrP – total protein correlation was replicated, and moreover, total protein was not correlated with any residual ELISA signal not explained by mass spec-quantified PrP. Together, these findings argued against option C, and suggested that the ELISA is specifically measuring PrP.
- The assay was non-reactive for rat and monkey CSF and recombinant mouse PrP. We are developing an ELISA using cross-species reactive PrP antibodies to support preclinical experiments (see next section).

## 2. Pre-analytical factors that affect CSF PrP

- CSF PrP is susceptible to loss upon plastic exposure, but this loss can be mitigated by early addition of a small amount (0.03%) of CHAPS detergent to samples. For instance, transferring a CSF sample to a new microcentrifuge tube three times eliminated at least 73% of detectable PrP ($P < 1 \times 10^{-6}$, two-sided t test) without CHAPS, but only 7.1% ($P = 0.37$) of PrP was lost in the presence of 0.03% CHAPS. Based on our findings, we have written a handling protocol with the goal of minimizing the impact of pre-analytical variability on measured CSF PrP levels.
- Our retrospective study of *N*=225 re-used CSF samples found a ~100-fold difference in PrP concentration between different samples, consistent with prior literature. However, in *N*=57 samples we collected at Massachusetts General Hospital under our new handling protocol with early addition of CHAPS, there is only a 5-fold difference between the lowest and highest sample. This supports the conclusion that the majority of inter-individual variability reported previously is due to pre-analytical factors.

## 3. CSF PrP tissue of origin

- PrP is abundant in human post-mortem brain tissue (range 211-1,736 ng/mL) but ranges from undetectable to very low levels (<27 ng/mL) in blood fractions.
- Spiking whole blood into CSF samples up to 1% did not alter detected PrP.

- Hemoglobin levels in *N*=128 CSF samples were not correlated with PrP levels as quantified by ELISA (*P*=0.57). We also found no correlation (*P*=0.51) among the *N*=55 samples analyzed by mass spectrometry.

## 4. Within-subject test-retest reliability of CSF PrP

- In a cohort of uniformly handled test-retest CSF samples collected at 8-11 week intervals, from placebo-treated individuals with mild cognitive impairment in an Alzheimer's trial, we found a mean coefficient of variation in CSF PrP levels of 13%.
- In July 2017 we launched a clinical research study at Massachusetts General Hospital to collect test-retest samples at a 2-4 month interval from pre-symptomatic *PRNP* mutation carriers and controls. The CSF is handled according to our protocol with early addition of 0.03% CHAPS before aliquoting and freezing. So far we have found a mean CV of 9.5% among the 14 mutation carriers analyzed, and 5.9% among 6 controls analyzed.
- Three individuals in our study (one control and two carriers) have also had a third visit 10-18 months after the second visit. Among these three individuals, the mean CV over all three visits is 4.4%.
- It is known from prior literature, and we have also replicated, that symptomatic prion disease patients have on average lower CSF PrP than patients with other rapidly progressive dementias. This is most likely because PrP in the symptomatic stage is more in aggregates and is less often shed into CSF. However, CSF PrP appears to be stable in the pre-symptomatic population, because a) we found very tight test-retest reliability in 22 pre-symptomatic mutation carriers, including longitudinally over 10-18 months in three individuals, and b) we did not see a decline in CSF PrP over 2 months even in an individual with biochemical evidence of prion pathology that might suggest the inception of the disease process.

## 5. Conclusions and further reading on biomarker assessment

From these findings, it appears to us that PrP is measurable, brain-derived, and exhibits good test-retest reliability over the duration studied provided that samples are handled uniformly to minimize loss to plastic adsorption.

For the full data and methods behind the above findings, please see Appendices 3-5.

## 2. Preclinical experiments

**Our goal:** At our CPIM, FDA scientists recommended that we design experiments to assess the correlation between brain and CSF PrP knockdown following ASO treatment, and to determine the percentage PrP knockdown required for therapeutic efficacy. These experiments will be important for understanding and properly interpreting CSF PrP lowering as a biomarker of ASO activity in the brain.

**Our questions:** Below, we describe completed, ongoing, and planned experiments in mice and rats to address brain-CSF knockdown correlation and effect size threshold for ASOs. The goal is to use preclinical data to correlate target suppression to disease benefit, in a way that credentials our proposed surrogate biomarker for clinical use. Are these experiments well designed to answer these important questions? Are there further studies, or changes to the proposed studies, that FDA would recommend?

To date we have completed a survival experiment in ASO-treated mice to determine the relationship between target suppression at the brain RNA level, and disease benefit. We have also retained a contract research organization, Cambridge Biomedical Inc., to develop an ELISA with cross-species reactive PrP antibodies to allow to quantify PrP protein levels in brain tissue and CSF from preclinical animal models. Data so far show good reactivity for recombinant mouse, rat, cynomolgus, and human PrP; analytical validation for the rat CSF matrix is underway.

Once this assay is developed, we plan to measure PrP knockdown in ASO dose response in the brains of mice and in the brains and CSF of rats. Our goals are to:

1) Determine the relationship between brain PrP reduction and survival
2) Determine the relationship between brain PrP reduction and CSF PrP reduction

### 1. ASO-mediated target suppression versus survival in prion-infected animals

These experiments are using mice because we are set up to handle mice in our prion vivarium suite, and because mice are the best-established laboratory model for prion disease.

From completed prophylactic treatment survival experiments, we know that PrP-reducing ASOs showed survival efficacy when administered according to the following paradigm. C57BL/6N mice received two injections of active ASO (first injection 14 days before prion infection and second injection 90 days later) of 500 µg ASO delivered intracerebroventricularly (ICV) by stereotactic surgery. Control animals that received no surgery, received saline, or received an equivalent dose of scrambled, non-targeting ASO all appeared to follow a normal prion disease course.

In early 2018, building on this established treatment paradigm, we launched a follow up experiment to relate target suppression to delay of disease (Figure 1). Because these experiments used a preclinical proof-of-concept compound whose potency may not correspond to that of a clinical candidate, we consider the results primarily in terms of

the degree of target suppression achieved, rather than dose of compound per se. In these experiments, *N*=8 C57BL/6N mice per cohort received two bolus ICV injections of ASO (first injection 14 days before prion infection and second injection 90 days later) of 0 (saline), 30, 100, 300, or 500 µg active ASO delivered ICV. We found that time to disease endpoint was target suppression-responsive (Figure 1), and corresponded to a suppression-dependent delay in onset of body weight loss and behavioral changes (Appendix 6).

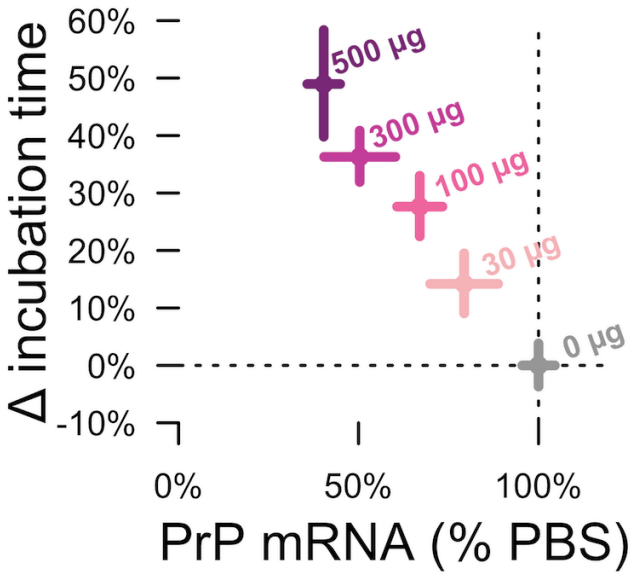


***Figure 1. Impact of level of target suppression on time to endpoint in prion-infected mice.*** *Reduction in PrP mRNA (ipsilateral cortex, 2 weeks post-dose) observed in uninfected mice correlated with the percentage delay in time to disease endpoint (accumulation of five clinical signs of prion disease) observed in prion-infected mice that received two bolus injections of the indicated dose. For further details see Appendix 6.*

The above data consider target suppression in terms of RNA levels quantified by qPCR, because up to now we have lacked a highly quantitative method for PrP protein quantification. Using the rodent PrP ELISA now under development, we plan to measure the degree of PrP protein reduction in mice treated ICV across this same dose range, considering multiple timepoints post-dose. We propose to give cohorts of uninfected *N*=4 C57BL/6N mice a single ICV administration of active ASO at the same 5 doses: 0 (PBS), 30 µg, 100 µg, 300 µg, 500 µg, and harvested at 3 timepoints: 2 weeks, 8 weeks, and 12 weeks, for a total of 60 animals. Upon harvest, whole contralateral (left) hemispheres will be snap frozen for RNA and protein analysis. PrP RNA in brain tissue will be quantified by qPCR to replicate the RNA results already shown above; PrP in brain will be quantified by ELISA.

For each dose level, we propose to plot time of harvest versus percent knockdown of PrP protein to determine the area under the curve, or the average reduction during the 90-day dosing interval. In combination with the survival experiment above, we will also be able to plot that target suppression versus the percent survival extension at each dose. We could then use a log-linear model to estimate the minimum percent knockdown required for any nonzero extension of survival.

### 2. Brain PrP reduction versus CSF PrP reduction in rats

Because mice have small total volumes of CSF, and we have found that plastic adsorption introduces too much pre-analytical variability in small volumes of CSF (Appendix 3), it would be difficult to reliably quantify PrP levels in mouse CSF. Therefore, these experiments will use rats.

We are planning a CSF-brain PrP knockdown correlation experiment in rats with an ASO targeting the rat prion protein gene. We plan to give cohorts of N=4 Sprague-Dawley rats single ICV doses of active ASO at 6 dose levels: 0 (saline), 30 µg, 100 µg, 300 µg, 1 mg, or 3 mg, and harvested at 2 timepoints: 2 weeks and 8 weeks, for a total of 48 animals. Upon harvest, full volume of CSF will be collected from the cisterna magna under a terminal dose of anesthesia, and whole contralateral (left) hemispheres will be snap frozen for RNA and protein analysis. PrP RNA in brain tissue will be quantified by qPCR; PrP in brain and CSF will be quantified by ELISA.

Using these data, we propose to build a pharmacodynamic model relating CSF PrP, brain PrP, and brain *PRNP* RNA knockdown to explore the relationships between these values and establish if/how brain PrP knockdown can be estimated based on observed CSF PrP knockdown.

### 3. Further reading on preclinical studies

For the full data and methods behind the dose-response efficacy, please see Appendix 6.

## Conclusion and next steps

At FDA's convenience, we would like to schedule a Type C meeting to review the above data and proposals in detail. Our overall goal is to honor the advice we received in our CPIM, to discuss how we could improve our plans and experiments, in order to best strategically prepare for trials of PrP-lowering ASOs, and in particular, to lay appropriate groundwork to enable a trial in pre-symptomatic genetic prion disease mutation carriers with a primary outcome of change in CSF PrP for FDA's consideration under the Accelerated Approval pathway. Thank you for your guidance and partnership!

# Appendices

- Appendix 1: Vallabh et al, “Lowering of prion protein load in cerebrospinal fluid is reasonably likely to predict clinical benefit in presymptomatic individuals with prion disease-causing genetic mutations”. White paper submitted to FDA as background reading for Nov 14, 2017 Critical Path Innovation Meeting.
- Appendix 2: FDA, “Memorandum. Critical Path Innovation Meeting: Genetic Prion Disease”. Meeting summary dated Dec 26, 2017.
- Appendix 3: Vallabh et al., “Prion protein quantification in cerebrospinal fluid as a tool for prion disease drug development” (also available online at https://www.pnas.org/content/pnas/116/16/7793.full.pdf)
- Appendix 4: Minikel & Kuhn et al, “Domain-specific quantification of prion protein in cerebrospinal fluid by targeted mass spectrometry” (also available online at https://www.biorxiv.org/content/biorxiv/early/2019/04/05/591487.full.pdf)
- Appendix 5: Vallabh PhD Dissertation Chapter 5: “Preliminary findings from the Massachusetts General Hospital genetic prion disease biomarker study.”
- Appendix 6: Sonia Vallabh, “Dose-responsive efficacy of a prion protein-lowering antisense oligonucleotide in prion-infected mice”, April 2019