Lowering of prion protein load in cerebrospinal fluid is reasonably likely to predict clinical benefit in presymptomatic individuals with prion disease-causing genetic mutations

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Summary: Prion disease is a rare, fatal, untreatable neurodegenerative disease caused by misfolding of the prion protein (PrP). Most human cases arise spontaneously and are not diagnosed until a state of profound dementia. ~15% of cases are genetic, and predictive genetic testing creates an opportunity for early therapeutic intervention to delay or prevent disease. Direct demonstration of clinical benefit in the presymptomatic population would be impractical. Congruent lines of evidence from biochemistry, human genetics, and mouse models agree that PrP is central to prion disease pathophysiology. Preclinical proofs of concept suggest that a reduction in PrP levels in the brain, potentially achievable using antisense oligonucleotides, would delay disease onset in individuals with pathogenic PrP mutations. Here we propose that PrP load in human cerebrospinal fluid (CSF) merits evaluation as a surrogate endpoint, as quantitative demonstration of reduced PrP levels in human CSF is reasonably likely to predict clinical benefit in prion disease. Such an approach could enable, for the first time, rigorously controlled trials in the presymptomatic population in the strongest position to benefit from an anti-prion therapeutic.

Prion disease, though currently untreatable, follows a clear pathogenic mechanism, in which a single gene gives rise to a single protein capable of converting into the sole causal disease agent. This mechanistic clarity will soon yield rational therapies. Disease rarity and tempo pose stark and foreseeable challenges to conducting a clinical trial with a clinical endpoint in this indication. Below, we provide evidence that the biology of prion disease is well suited to use of an on-pathway surrogate endpoint to address these challenges while still enabling rigorous and informative trials. The field's ability to advance life-saving therapeutics will critically depend on the thoughtful deployment of such an alternative approach.

1. The pathogenesis of human prion disease is well understood.

Prion disease is an untreatable, uniformly fatal neurodegenerative disease. Various forms of prion disease in humans and other mammalian natural hosts are noted in Table 1. All cases of prion disease trace to the same molecular event, a misfolding of the native prion protein (PrP), encoded by the prion protein gene (*PRNP*). The misfolded protein, known as a "prion," is capable of autocatalytic conformational templating of other PrP molecules. Through such templated misfolding, prions spread exponentially throughout the brain in a conformational cascade recognized for decades as the molecular mechanism driving PrP's disease-state gain-of-function[1,2].

species	**name**
humans	Creutzfeldt-Jakob disease (CJD) fatal familial insomnia (FFI) Gerstmann-Straussler-Scheinker disease (GSS) variant Creutzfeldt-Jakob disease (vCJD) kuru Huntington disease-like 1 variably protease-sensitive prionopathy PrP cerebral amyloid angiopathy
sheep and goats	scrapie
cattle	bovine spongiform encephalopathy (BSE or "mad cow")
deer and elk	chronic wasting disease (CWD)
any	transmissible spongiform encephalopathy

Table 1. Other names for prion disease. *Several mammalian species besides humans are natural hosts of prion disease. Different species and different clinical presentations of prion disease are associated with historical names, most of which date to before the molecular mechanism of disease was known.*

Human prion disease is rare: the true annual incidence is estimated at 1-2 deaths per million population[3], although due to under-diagnosis only 200-300 cases are diagnosed and reported in the United States each year[4]. Although prion disease is infamous for the small minority (<1%) of cases acquired through infection[5], the majority (~85%) of cases occur spontaneously, with no known environmental or genetic trigger (these cases are referred to as *sporadic*). The remainder (~15%) arise from dominant, gain-of-function, protein-altering variants in *PRNP* (Figure 1A)[6]. Some of these variants are highly penetrant, with lifetime risk approaching 100%, and three such variants account for the majority of genetic cases[6]. Age of onset is highly variable (Figure 1B).

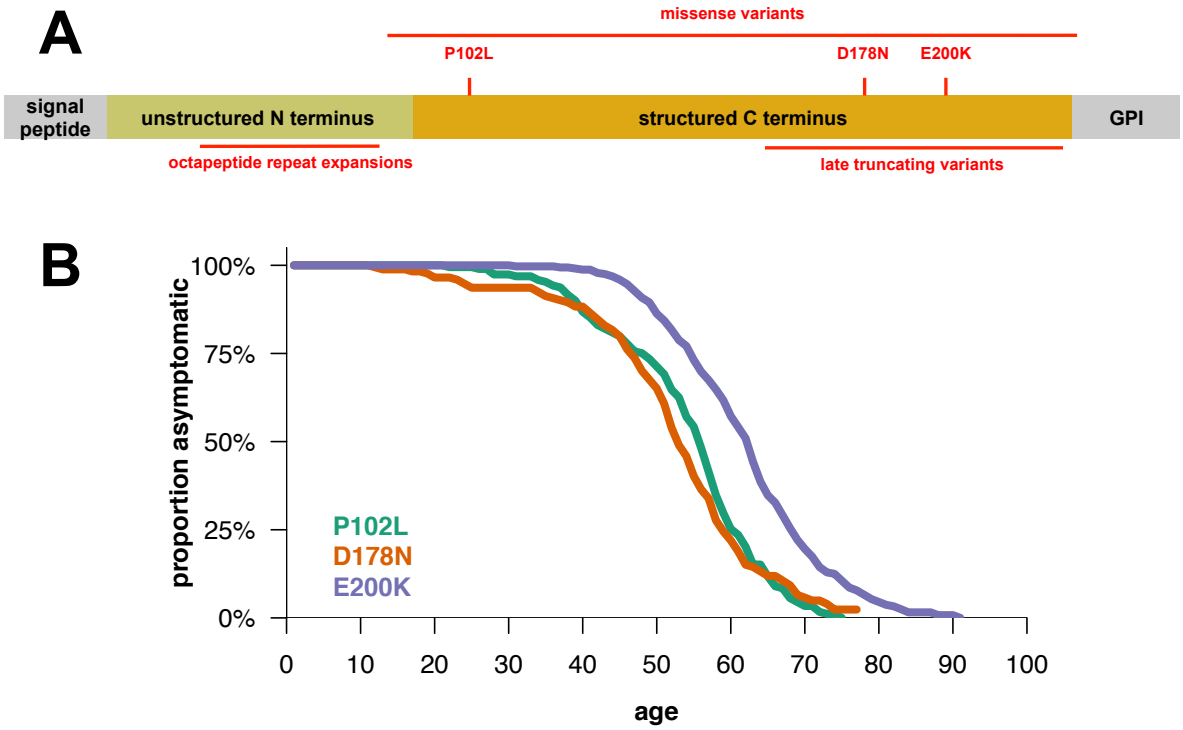

Figure 1. Genetic prion disease. *A)* PRNP *contains a single protein-coding exon, with the mature protein of 208 amino acids comprising an unstructured N terminus and a structured C terminus. Over 60 mutations have been identified in patients with prion disease, though only a subset are highly penetrant; three high-penetrance missense mutations (top) account for >50% of cases*[6]*. In addition to missense, pathogenic variants also include expansions of the N-terminal octapeptide repeat region, and late frameshift or stop variants that leave most of the protein while causing a gain-of-function through change in localization*[6]*. B) Survival curves for the three most common genetic prion disease missense mutations (unpublished data).*

Among neurodegenerative diseases, prion disease is exceptionally rapid. Patients progress from first symptom to death in a median time of only 5 months[7]. In this short time they rapidly descend into profound dementia, losing the ability to perform all activities of daily living, and typically spending the last weeks of life in a state of akinetic mutism. Throughout, the brain is the epicenter of destruction and the only tissue with a known phenotype. Compounding the rapid course of disease, diagnosis is not reached until, on average, two-thirds of the way through the symptomatic phase[8].

Although rare, prion disease is well understood at the molecular level, with all lines of evidence pointing to the centrality of PrP in prion disease. PrP is unique in all of human biology, as the only protein ever demonstrated to form a naturally transmissible proteinaceous pathogen devoid of nucleic acid[1], and biochemical, human genetic, and model organism approaches are in agreement that PrP is absolutely required for prion disease (Table 2).

category	evidence
biochemical	• Prions, the infectious agent in prion disease, are composed of PrP[9]. • Prion "strains" are encoded in distinct conformations of PrP[10–12]. • Prion infectivity can be generated *in vitro* from bacterially expressed recombinant PrP[13].
human genetics	• All multiplex prion disease families possess protein-altering variants in *PRNP*[14]. • Certain missense variants in *PRNP* confer protection against prion disease[15–17]. • *PRNP* is the only locus to exhibit genome-wide significant association to prion disease risk[18].
animal genetics	• PrP is required for prion propagation[19]. • PrP is required for prion neurotoxicity[20]. • PrP dosage and incubation time are inversely correlated[21,22]. • PrP amino acid sequence governs the "species barrier"[23–25].

Table 2. Evidence that PrP is central to prion disease pathophysiology.

2. Substantially lowering PrP levels is likely to be a safe and effective strategy to prevent or treat prion disease.

The study of prion disease benefits from excellent animal models, where intracerebral inoculation of wild-type animals with prions leads to fatal disease after a highly predictable incubation time. Experiments in these models have shown that (i) PrP knockout confers complete resistance to prion disease[19], (ii) prion neurotoxicity only affects cells expressing PrP[20], and (iii) postnatal suppression of PrP expression can delay or halt the progression of prion disease[26,27]. Moreover, PrP gene dosage is correlated with the pace of disease across a wide range of expression levels[22], with heterozygous PrP knockout mice surviving prion infection 2.5 times as long as wild-type mice[21]. Similarly, in transgenic mouse models expressing PrP with mutations that cause genetic prion disease in humans, PrP dosage is inversely correlated with age of onset of spontaneous illness[28].

PrP knockout mice are viable, fertile, have normal lifespans, and exhibit normal behavior, initially defying efforts to identify a knockout phenotype[29]. It has recently been found that, in the peripheral nervous system, PrP undergoes proteolytic cleavage to release a signaling peptide that promotes myelin maintenance[30]. PrP knockout mice develop a slowly progressing demyelinating polyneuropathy, which leads to mild sensorimotor deficits late in life[31]. Heterozygotes are unaffected[31]. No native function has yet been identified in the central nervous system. Knockout cattle[32] and naturally occurring knockout goats[33] are described as phenotypically normal. The few humans with heterozygous loss-of-function variants identified in *PRNP* are healthy[6], indicating that a reduction in *PRNP* gene dosage is well-tolerated in humans.

The above data suggest that lowering PrP levels would be a safe and effective therapeutic strategy for delaying or preventing prion disease. Multiple therapeutic strategies could, in principle, reduce PrP levels, by targeting the *PRNP* gene, RNA, or the mature protein itself. Despite this strong therapeutic hypothesis, however, little drug development has occurred in this area. The four agents that have been advanced to clinical trials in prion disease to date were all existing drugs with no strong preclinical evidence to support advancement into humans[34–40].

3. Prevention of disease in of pre-symptomatic mutation carriers is likely feasible based on preclinical precedent, and would allow extension of healthy life.

No therapeutic intervention administered after onset of symptoms has ever convincingly extended survival in an animal model. In contrast, there do exist proofs of concept for dramatically delaying prion disease by intervening before symptom onset.

Phenotypic screens in prion-infected cells have identified several small molecules that inhibit prion replication by an unknown mechanism of action, and extend survival in animals intracerebrally infected with prions. None are effective against human prion strains[41–44], precluding their advancement to the clinic. Certain high molecular weight, sulfated sugar polymers are also known to inhibit prion replication, but are limited by the infeasibility of broad delivery to the brain parenchyma[45,46]. Despite their lack of prospects for clinical advancement, all of these molecules have nonetheless provided important insights into the time dependence of antiprion therapeutic efficacy.

Four of these compounds have been tested in mice with treatment beginning at a battery of different timepoints during the disease course[42,45,47,48]. In each case, the compound was less effective the later it was administered. None was effective after the onset of symptoms. For example, the most thoroughly studied molecule, IND24, quadrupled survival time when administered before prion infection, increased survival time by about 60% when administered after infection but before symptom onset, and had no effect on survival when given after symptom onset. More than 100 other candidate therapeutics have been tested in prion-infected mice[43,49,50], and while most have proven ineffective regardless of disease stage, the few that have shown convincing evidence of efficacy did so only when administered before symptom onset. For example, monoclonal antibodies to PrP[51] and certain metallated porphyrins[52,53] poor at crossing the blood-brain barrier have delayed the neuroinvasion of peripherally acquired prions, but have been ineffective after onset. Intracerebral infusion of polythiophenes nearly doubled survival when given prophylactically, but had marginal effects when initiated around the time of symptom onset[54].

These results are consistent with the understanding of prion disease kinetics as established in animal models. From transgenic mice expressing varying levels of PrP, it is known that after prion infection begins, prion titers in the brain rise exponentially during a clinically silent incubation phase. The rate of prion load accumulation during this incubation phase corresponds to the PrP expression level of the animal, with higher expression resulting in more rapid accumulation and a shorter time to maximum titers of prions in the brain. Critically, symptoms emerge only when prion titers have plateaued[1,55,56]. Thus, not only does post-symptom intervention face the challenge that neuronal loss is irreversible, it also faces a disease stage that is fundamentally different at the molecular level, compared to pre-symptomatic treatment.

4. Antisense oligonucleotides represent the most feasible near-term strategy for treating prion disease.

While the vision set forth in this white paper should apply to any molecule capable of lowering PrP levels in the brain, the most realistic near-term therapeutic of this nature is likely to be antisense oligonucleotides (ASOs). ASOs are short (17-20 base) single-stranded

oligonucleotides, chemically modified for pharmacokinetic stability, that specifically bind a complementary target RNA and can trigger its degradation[57–59].

Efforts are currently underway to develop ASOs against the PrP RNA, involving scientists at NIH (led by Dr. Byron Caughey), the Broad Institute, and Ionis Pharmaceuticals. Ionis Pharmaceuticals has to date completed a preliminary *in vivo* study showing that two lead ASOs against mouse *Prnp* are capable of reducing PrP mRNA levels by about 50% in the mouse cortex and spinal cord following a single intraventricular dose. We will soon begin survival studies in prion disease mouse models using these ASO molecules. If onset of prion disease in these mice is delayed as would be predicted by the *in vivo* potency studies, it should be possible to begin development of a human ASO candidate directed against human *PRNP* by early 2018.

Antisense oligonucleotides are uniquely modular drugs. The nucleotide sequences of ASOs specify target binding through Watson-Crick base pairing, yet these sequences are orthogonal to the classes of backbone chemistry that determine many pharmacokinetic and pharmacodynamic parameters[60]. ASOs may modulate their target RNAs by a variety of mechanisms, including RNAse H-mediated degradation of target RNA. At present two ASO drugs – mipomersen for homozygous *LDLR* mutant hypercholesterolemia, and nusinersen for spinal muscular atrophy (SMA) – have full FDA approval of an NDA. Another, eteplirsen for exon 51-skippable Duchenne muscular dystrophy, has Accelerated Approval of an NDA. ASOs for neurological applications have been under intensive study, including the recently successfully completed Phase I, II and III trials of nusinersen in children with SMA[61] and an ongoing Phase I/II trial of an anti-Huntingtin ASO (ASO-HTT Rx) in adults with Huntington's Disease (HD)[60]. These drug development programs have compiled a wealth of knowledge regarding the behavior of intrathecally delivered ASOs in the CNS.

1. **Delivery.** In nonhuman primates, ASOs delivered by intrathecal infusion or intrathecal bolus injection achieved broad distribution across the brain and 25 percent to 67 percent knockdown of target mRNA across brain regions including the cortex, striatum, hippocampus, pons,and spinal cord[62,63]. Both the SMA and HD clinical studies rely on bolus ASO delivery by intrathecal injection.
2. **Safety and tolerability.** Published results from the Phase I escalating dose study of intrathecally delivered nusinersen show no safety or tolerability concerns[61]. Notably, this trial was performed in a population of 2-14 year old symptomatic SMA patients, in whom scoliosis and spinal abnormalities are common, making lumbar punctures more challenging than in healthy adults such as presymptomatic *PRNP* mutation carriers.
3. **Time to effect.** ASO activity is reflected in target mRNA levels within 14 days of treatment in rodents and declines by 4 months[62]. Lag in protein levels depends upon the half-life of the protein in question. PrP has an estimated *in vivo* half-life of 18 hours[27], indicating that ASO-based mRNA depletion could quickly impact PrP at the protein level.
4. **Wash out period.** The *in vivo* half life of nusinersen in CSF was estimated at 132-166 days[61]. The ASO-HTT-Rx Phase I study is currently designed to dose once a month. Such periodic dosing offers the opportunity to discontinue administration should adverse events arise.

Together, these findings suggest that intrathecal ASOs have been sufficiently de-risked that, provided preclinical toxicity studies are favorable, a trial in healthy pre-symptomatic individuals would not expose subjects to unreasonable risk.

5. It is appropriate for trials in pre-symptomatic genetic prion disease mutation carriers to use PrP levels in cerebrospinal fluid as a surrogate endpoint, on the strength of PrP's role as the sole necessary and sufficient precursor of the infectious agent in prion disease.

In some cases, one can test a therapeutic against a neurodegenerative disease by conducting a trial in pre-symptomatic individuals with the goal of demonstrating a direct clinical benefit. For example, the Alzheimer's Prevention Initiative is following 300 randomized participants with the *PSEN1* E280A mutation being treated with crenezumab, a monoclonal antibody against amyloid beta, with a cognitive endpoint after five years[64,65].

However, there is no currently realistic route to conduct a trial to directly demonstrate clinical benefit in pre-symptomatic individuals with *PRNP* mutations. Designing such a trial would be infeasible for several reasons.

1. **Recruitment numbers.** Recruitment for the crenezumab trial was made possible by the existence of a single extended family of more than 5,000 individuals; as of October 2016, 1,065 living mutation carriers from this extended kindred had been genotyped through the Columbian Alzheimer's Prevention Initiative Registry[66,67]. No comparably large family exists for any genetic prion disease mutation. Indeed, for the three most common high-penetrance *PRNP* mutations combined, only 1,001 cases have ever been ascertained in the U.S., Europe, Japan, and Australia in total over the 15-20 years that prion surveillance networks have been in effect[6].
2. **Predictability of age of onset.** Age of onset for *PSEN1* E280A has a standard deviation of ~6.4 to 8.6 years[68,69], whereas for the three highly penetrant *PRNP* mutations presented in Figure 1, the standard deviation of age of onset ranges from 10.0 to 11.8 years[70,71]. The crenezumab trial also relies on a correlation between parent and child age of onset that has been reported in *PSEN1* Alzheimer's disease[69], but such a correlation is not observed in prion disease[70]. Both of these factors mean that a larger or longer trial would be required to assess clinical benefit in genetic prion disease.
3. **Economic incentives for drug development.** The economic return on genetic prion disease is low. The cost of the crenezumab trial in Alzheimer's disease is estimated at $96 million[64]. The sponsor has surely weighed the cost of this trial against the prospects for widespread off-label use and/or eventual expanded labeling in late-onset Alzheimer's disease if crenezumab were approved. A drug to lower PrP would have no prospects for off-label or expanded use outside of prion disease, making such a costly trial untenable.

Some have sought to design smaller or shorter prevention trials in Alzheimer's disease by using biomarkers to selectively enroll individuals whose onset appears imminent[65]. At present, however, no reliable imaging or biochemical biomarkers exist that could serve this purpose for prion disease. Brain abnormalities can be detected after symptom onset using diffusion-weighted magnetic resonance imaging (dwMRI) or 18-fluorodeoxyglucose positron emission topography (FDG-PET), but these changes have been observed no earlier than about 1 year prior to symptom onset[72–76]. Even then, pre-symptomatic pathology was subtle enough to be noticed only in hindsight. Prion "seeds" in cerebrospinal fluid or nasal brushings, operationally defined by their ability to trigger fibrillization of recombinant PrP *in vitro*, are diagnostic of sporadic prion disease[77–79]. The presence of "seeds" before symptom onset has not yet been evaluated, but analytical sensitivity for genetic subtypes of prion disease is relatively limited even at the symptomatic stage[80,81], posing a challenge for the development of a highly sensitive

prodromal test. Even if such a biomarker could be identified, it would take many years of following pre-symptomatic people to onset in order to establish its predictive value.

Most importantly, even if a biomarker to enrich for individuals close to onset were available, preclinical proofs of concept indicate that this approach would specifically **enrich for those individuals least likely to benefit from a drug**, as prion amplification and neuropathology have already begun.

In view of the infeasibility of demonstrating clinical benefit in pre-symptomatic people, we propose that PrP levels in cerebrospinal fluid should be considered as a surrogate endpoint for evaluating the efficacy of PrP-lowering therapeutics.

Since 1992, FDA's Accelerated Approval program has allowed for approval of new drugs on the basis of a surrogate biomarker deemed "reasonably likely" to predict clinical benefit[82]. With the 2012 passage of the FDA Safety and Innovation Act (FDASIA)[83], Congress urged FDA to enhance its expedited review of drugs for deadly diseases, particularly rare ones, leveraging the "unprecedented understanding of the underlying biological mechanism and pathogenesis of disease" gained by modern scientific tools. Congress envisioned a "broad range of surrogate or clinical endpoints" used to conduct "fewer, smaller, or shorter clinical trials" without reducing FDA's standards for safety and efficacy. FDASIA specifies that a lack of viable alternative clinical trial designs should be considered in review of surrogate endpoints: "[FDA] shall consider how to incorporate novel approaches into the review of surrogate endpoints… especially in instances where the low prevalence of a disease renders the existence or collection of other types of data unlikely or impractical"[83]. FDA has indicated[84] that its criteria for increased flexibility on acceptance of surrogate biomarker endpoints will include the "severity [and] rarity" of the disease, as well as "the extent to which the pathophysiology of a disease is understood" and how the biomarker fits into that disease pathway.

PrP lies directly on the sole pathway of prion disease pathophysiology (Figure 2), with all available lines of evidence agreeing that PrP is required for disease initiation and progression, in a dose-dependent manner. Our understanding of prion disease biology is sufficiently strong that a reduction in PrP abundance in pre-symptomatic people can be deemed very likely to predict clinical benefit.

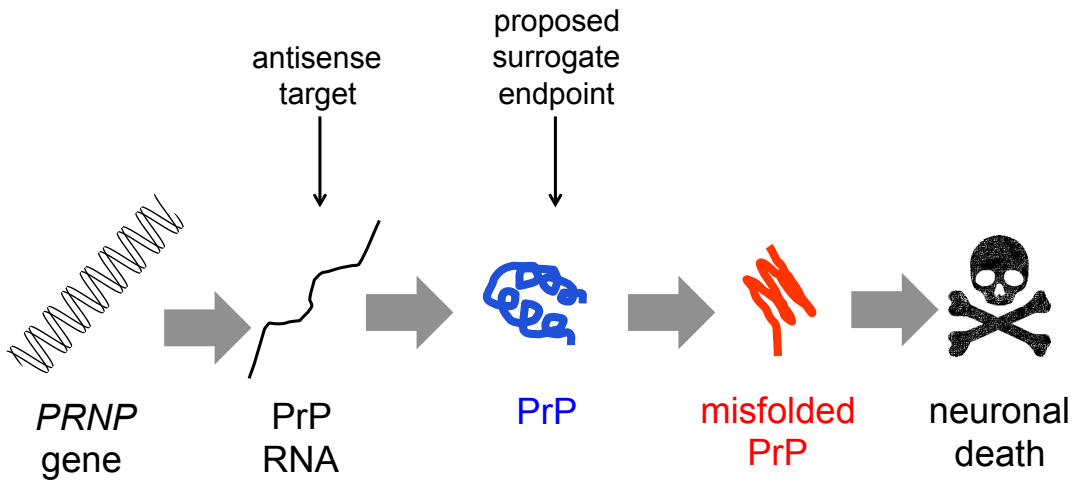

Figure 2. Drug target and proposed surrogate endpoint within the pathophysiological pathway of prion disease. *PrP lies directly on the sole pathway of prion disease.*

Prions are, to date, the only known protein-only pathogen. Conceptually, prion disease resembles an infectious disease, driven by the formation and replication of this single, well-defined infectious agent. The native prion protein, PrP, is the pivotal molecule on this disease pathway, as the sole necessary and sufficient precursor for pathogen formation. PrP levels dose-dependently predict time to prion disease onset, as documented by decades of genetic studies in mammalian hosts of prion disease. “PrP load” as a surrogate endpoint for prion disease is therefore analogous to “viral load” in HIV. Prion disease is no less deadly today than HIV in the early 1990s, and its causality is no less clear.

Direct sampling of brain, the tissue of interest for prion therapeutics, is impractical, but PrP is abundant in human cerebrospinal fluid (CSF), with a concentration on the order of tens to hundreds of nanograms per milliliter detected using commercially available ELISA kits[85–88]. Levels vary by as much as an order of magnitude between individuals[88], but intra-individual variability appears lower. We have found a mean coefficient of variation of only 13% across nine 8-week test-retest samples (Figure 3A). PrP is much less abundant in blood than in cerebrospinal fluid (Figure 3B), suggesting that the detected PrP is primarily brain- rather than blood-derived, and therefore more likely to reflect any therapeutic reduction in brain PrP.

CSF PrP levels are reduced in individuals with symptomatic prion disease compared to controls or patients with other dementias[88], perhaps reflecting the sequestration of normally extracellular PrP within the endosomal-lysosomal pathway where prions are formed[89–91]. For this reason, a reduction in CSF PrP could be difficult to interpret in symptomatic patients, as it could reflect either an intended effect of treatment or simple progression of disease. This provides another motivation for initiating treatment in pre-symptomatic individuals who are, on expectation, years from onset of disease. It is possible that despite being probabilistically far from onset as a group, some of these individuals may convert to symptomatic during the course of a biomarker-based preventative trial. The clinical onset of prion disease is a rapid event in which individuals progress from first symptoms to dementia on a timescale of weeks[7]. Therefore, symptomatic individuals can be readily identified and excluded from analyses of treatment-based PrP reduction, to ensure that changes in PrP levels are attributable to treatment as opposed to symptom onset.

We are currently undertaking efforts to collect samples from pre-symptomatic individuals with *PRNP* mutations in order to characterize CSF PrP levels in this population. We are also working to develop a mass spectrometry-based method for quantification of PrP in CSF, in the hopes that this will offer greater precision than ELISA, as well as the ability to characterize the abundance of PrP proteolytic fragments in addition to the full-length PrP detected by ELISA.

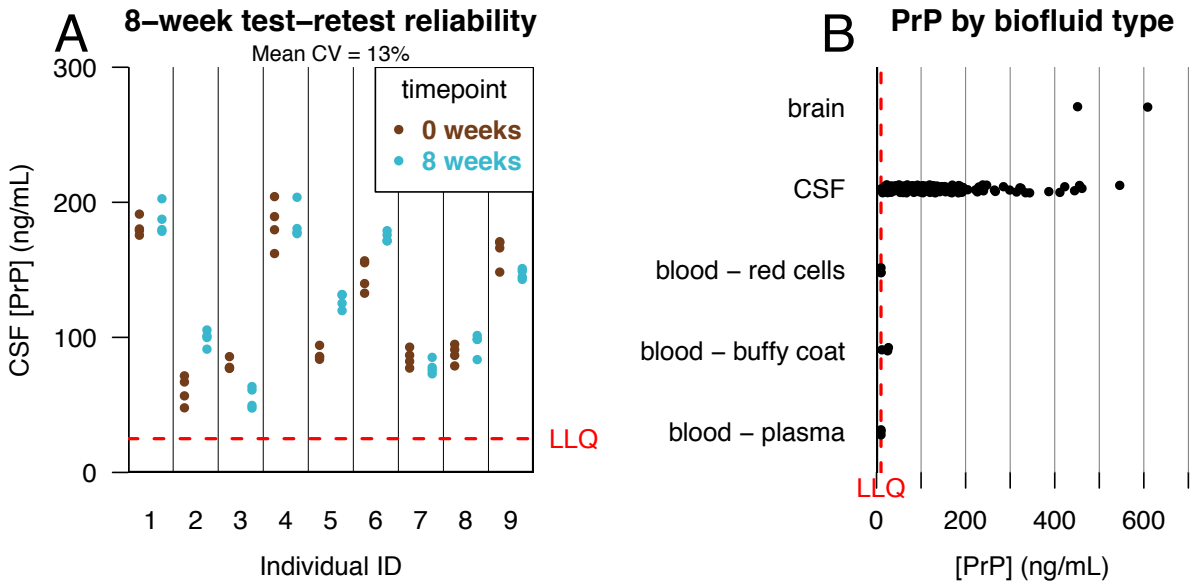

Figure 3. Preliminary data regarding PrP quantification in human CSF. *A) Analysis of CSF PrP in 18 residual samples from 9 individuals with Alzheimer's disease treated with placebo during a clinical trial, performed in quadruplicate using the BetaPrion Human ELISA kit (Analytik Jena). Each point is one technical replicate. Although PrP levels vary ~4-fold between these individuals, the mean coefficient of variation (CV) for 8-week test-retest is only 13%. B) Analysis of ELISA-detectable PrP in CSF from 127 individuals compared with ELISA-detectable PrP in two human brain samples and three distinct human blood fractions from three individuals. PrP levels in plasma and red cells are below the lower limit of quantification in plasma while PrP levels in buffy coat (<1% of blood volume) are comparable to the lowest seen in any CSF sample. This low level of total blood PrP means that blood protein contamination is unlikely to drive the level of PrP detected in CSF. Brain PrP is at or above the highest level seen in CSF, suggesting brain as the likely source of CSF PrP.*

Pending the results of these further experiments, it is our hope that a meaningful reduction in CSF PrP in pre-symptomatic *PRNP* mutation carriers, demonstrated through an adequate and well-controlled trial (such as a blinded, placebo-controlled double cross-over study) could constitute a basis for Accelerated Approval. Confirmatory post-marketing studies could then further assess the clinical benefit of a PrP-lowering therapeutic.

Goals

We are interested in learning from FDA what criteria would be considered in evaluating CSF PrP load as a potential surrogate endpoint for PrP-lowering therapeutics, and in brainstorming with FDA what experiments we could conduct in the coming few years that would assist with evaluation of such an endpoint.

We are aware that FDA considers sampling compartment, timing, effect size, and measurement method as important criteria in surrogate endpoint evaluation. With this in mind, a few preliminary subjects for discussion include:

- **Sampling compartment.** What experiments could be done to establish whether CSF obtained via lumbar puncture is a sampling compartment reflective of overall brain PrP levels?

- **Timing.** Because prion replication is exponential and prion disease is rapidly progressive, we speculate that most pre-symptomatic people enrolled in a trial would be receiving the drug before any prions have even formed, and certainly before any tissue damage has occurred. What could be done to substantiate this claim?
- **Effect size.** In humans, there are no genetic variants known to control overall brain PrP expression, so the ideal "experiment of nature" quantifying the relationship between PrP dosage and disease susceptibility *in humans* may not exist. Could additional data from animal studies help to establish an effect size threshold sufficient for approval? For instance, if we could use a dose-response of ASOs and/or a doxycycline-inducible mouse model to query the relationship between PrP dosage and survival time at intermediate levels of knockdown, in between 50% and 100% of wild-type levels, would these data be considered in evaluating a surrogate endpoint? Alternatively, survival studies in prion-infected, intrathecally ASO-treated macaques could assess extension of survival relative to PrP mRNA and protein knockdown in an animal model of closer to human proportions.
- **Measurement method.** How would an ELISA or mass spectrometry assay need to be validated in order to serve as a measurement of a surrogate endpoint?

We hope to engage FDA early on, during the preclinical stage of therapeutic development, to discuss these questions and chart a productive course to success in the clinic.

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