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# Past, Present and Potential Future Prion Disease Treatment Strategies

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Additional information is available at the end of the chapter

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## Abstract

The prion diseases are rare and invariably fatal neurodegenerative diseases characterized by a unique, protein-only pathogenesis. Mechanistically, the prion diseases result from the coerced conversion of a protease-sensitive form of the cellular prion protein (PrP<sup>C</sup>) into a protease-resistant infectious form (PrP<sup>res</sup>). This chapter reviews the past, present, and potentially future prion disease treatment strategies. This chapter begins with an introduction to prion diseases, the misfolding of prion proteins and what is known about this process, and then proceeds to discuss approaches for treatments. Regarding approaches to treat prion diseases, we discuss (1) small molecule inhibitors, (2) antiprion protein antibodies, (3) prion gene disruption, (4) targeting of the unfolded protein response, and (5) heterologous prion proteins. We elaborate on using heterologous prion proteins to treat prion diseases, as this is an area that we are pursuing. The chapter ends with thoughts on the future direction of prion disease treatment strategies and how these strategies might be applicable to other neurodegenerative diseases involving protein misfolding. The increasing awareness of the role of protein misfolding in many neurodegenerative processes makes the development of an effective treatment strategy for prion diseases a high priority.

**Keywords:** prion, treatment, CJD, GSS, PrP<sup>res</sup>, PrP<sup>C</sup>, heterologous prion proteins, protein misfolding diseases, neurodegenerative disorders

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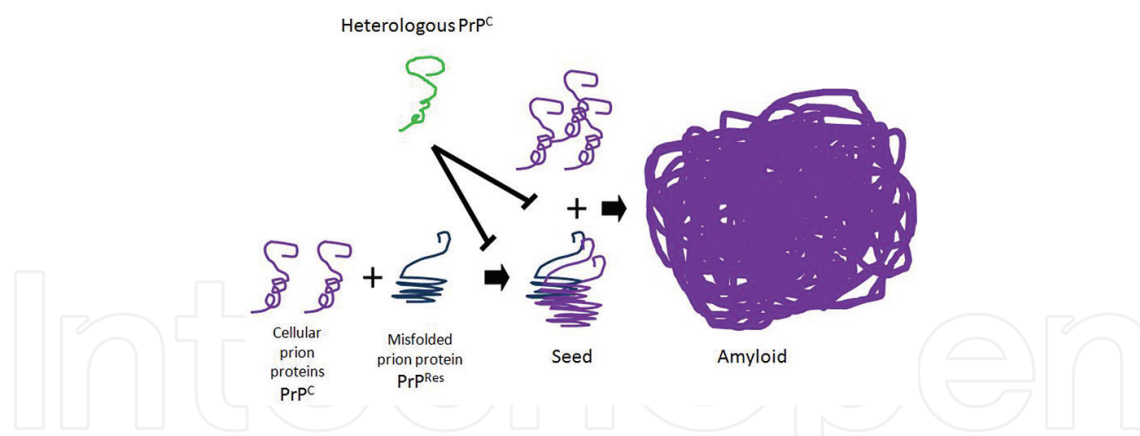
## 1. Introduction

Prion diseases, or transmissible spongiform encephalopathies (TSEs), are unique, fatal neurodegenerative diseases with infectious, genetic, or sporadic causes. Prion diseases affect humans (e.g. Creutzfeldt-Jakob disease [CJD], Gerstmann-Straussler-Scheinker syndrome [GSS], and fatal familial insomnia [FFI]) and nonhumans (e.g. bovine spongiform encephalopathy [BSE] of cattle, chronic wasting disease [CWD] of cervids, and scrapie of sheep and goats.

Irrespective of affected species, prion diseases result in progressive neurocognitive decline following a long incubation period. No effective prion disease treatments exist and most human patients die within 14 months following diagnosis [1]. Notably, many of the fundamental characteristics of prion diseases, including the molecular and biochemical mechanisms underlying the formation, accumulation, and cell-to-cell infectivity of misfolded protein and the role of glial-mediated neuroinflammation aligns prion diseases with more common human neurodegenerative conditions, including Alzheimer's, Parkinson's, and Huntington's diseases.

In the mouse, the prion protein is encoded by the *Prnp* gene. The nascent 254 amino acid long peptide is then posttranslationally cleaved at its N and C terminus to produce the final 210 amino acid long protein [2–4]. Structurally, the prion protein is characterized by a disordered aminoterminal tail and a globular C-terminal domain consisting of three  $\alpha$ -helices and two anti-parallel  $\beta$ -sheets [5, 6]. It is anchored to the outer cell surface membrane via a glycosylphosphatidylinositol (GPI) anchor, which helps tether the protein to the outer cell surface membrane [7].

The hallmark event in the prion disorders is the misfolding of the normal cellular prion protein (denoted  $\text{PrP}^{\text{C}}$ ) into a misfolded isoform (commonly denoted as  $\text{PrP}^{\text{res}}$  or  $\text{PrP}^{\text{Sc}}$ ). In its normal form,  $\text{PrP}^{\text{C}}$  is a monomeric or dimeric protein with abundant alpha helical content, whereas the misfolded variant  $\text{PrP}^{\text{res}}$  is aggregated with a  $\beta$ -pleated sheet rich conformation [8, 9]. In addition to its structural differences,  $\text{PrP}^{\text{res}}$  is characterized by resistance to protease and chemical disinfection [10]. Although the entirety of the process has not been described, it is widely believed that  $\text{PrP}^{\text{res}}$  replication results from the induced misfolding of  $\text{PrP}^{\text{C}}$  through a nucleation-dependent polymerization mechanism [11]. This process is included in the model presented in **Figure 1**.



**Figure 1.** A proposed model of heterologous prion protein treatment in the misfolding, nucleation, and formation of amyloid. In the presence of misfolded  $\text{PrP}^{\text{res}}$ , the normal cellular prion protein ( $\text{PrP}^{\text{C}}$ ) is induced to misfold. This cycle of misfolding repeats leading to seeds of misfolded oligomers and amyloid deposits. Our studies demonstrate that heterologous prion proteins inhibit this process. We propose that heterologous prion proteins bind directly to  $\text{PrP}^{\text{C}}$  and  $\text{PrP}^{\text{res}}$  to block seed and amyloid formation.

In the pathogenesis of prion diseases, the formation of  $\text{PrP}^{\text{res}}$  is generally believed to be a key event in the disease initiation. Owing to its specificity as a marker of tissue infectivity,  $\text{PrP}^{\text{res}}$  is the most commonly used prion disease diagnostic marker. Although the inciting cause (i.e.  $\text{PrP}^{\text{res}}$  formation) and neuropathologic consequences of prion disease (i.e. gliosis, synaptic dysfunction, spongiosis, and neuronal loss) are well characterized, the mechanism(s) by

which the former results in the latter remain unknown. However, it is likely that misfolded PrP<sup>C</sup> has direct/indirect toxic properties as, as PrP<sup>C</sup> does not appear to be detrimental [12].

The conversion of PrP<sup>C</sup> to PrP<sup>res</sup> is a highly specific process of templated conversion requiring direct interaction between the normal and abnormal forms of the protein [13]. The efficiency of this conversion is predicated upon a number of specific reaction conditions, including the secondary structure of PrP<sup>res</sup>, homology of the primary and secondary structures between PrP<sup>C</sup> and PrP<sup>res</sup>, and the architecture of the PrP<sup>C</sup>-PrP<sup>res</sup> complex [14, 15]. Increased contact between PrP<sup>C</sup>-PrP<sup>res</sup> at residue 129 and the relative rigidity of the  $\beta$ 2- $\alpha$ 2 loops in PrP<sup>C</sup> are two important factors in mediating the efficiency of PrP<sup>res</sup> formation and TSE susceptibility [15–17]. In addition to steric factors, the formation of PrP<sup>res</sup> is favored by destabilization of PrP<sup>C</sup> as a number of destabilizing pathogenic mutations in PrP are linked with increasing misfolding rates [18–20].

The presence and primary structure of host PrP<sup>C</sup> are major determinants in conferring susceptibility to prion disease infection. This is most expressly evident by work demonstrating that transgenic mice lacking PrP<sup>C</sup> are conferred resistance to prion infection [21]. Beyond simple PrP<sup>C</sup> expression, the degree of sequence homology between infecting prion and host PrP<sup>C</sup> plays a significant role in determining the efficiency of prion infection and prion replication [22]. Moreover, differences in primary sequence between host PrP<sup>C</sup> and infectious PrP<sup>res</sup> have been proposed to underlie the species barrier that mitigates cross species prion infection as well as prion strains [23–25]. The importance of prion structure extends beyond simple amino acid homology and is also dependent upon secondary structural variations, including differences in loop/turn structures [23, 26]. In light of complementary *in vitro* and *in vivo* work, it appears as though the middle third region of the prion protein is particularly important for the autocatalytic conversion of PrP<sup>C</sup> to PrP<sup>res</sup> [27]. The potential clinical relevance of PrP<sup>C</sup> sequence is demonstrated by work revealing that polymorphisms in this area of the protein can confer prion disease resistance, as mice expressing a variant PrP containing amino acid substitutions in the  $\beta$ 2- $\alpha$ 2 loop were resistant to prion infection [28].

Given studies that have revealed the pathogenic importance of a precisely formed PrP<sup>C</sup>-PrP<sup>res</sup> complex, it seems reasonable to investigate whether interference with this complex might have therapeutic potential. This approach is best described by Singh and Udgaonkar in their comprehensive review on PrP misfolding, namely to test whether or not “...any ligand, whether small or large, that binds to the native conformation of the [PrP<sup>C</sup>] protein would stabilize that state and can therefore be expected to decrease the native-state dynamics that drive misfolding [29].” Support for such an approach has been validated by antibody-based studies, which have stabilized the  $\alpha$ 1 region of PrP<sup>C</sup> and prevented prion disease in animals [30, 31].

In this chapter, we review past, present, and potential future strategies to treat prion diseases.

## 2. Small molecule inhibitors to treat prion diseases

There are a number of small molecules that have proposed as prion therapeutics, including that either inhibit the misfolding of PrP<sup>C</sup> or promote the clearance of PrP<sup>res</sup>. In general, small molecule compounds can be segregated according to their method of action into compounds

that either inhibit the misfolding of PrP<sup>C</sup> (potentially through stabilization) promote the clearance of PrP<sup>res</sup>. Over the past two decades, many small molecules have been evaluated for their in vitro or in vivo antiprion efficacy.

Of the most commonly examined small molecule candidate therapies, many have not stood up to scrutiny when their in vitro efficacy was tested in vivo. This includes quinacrine, pentosan polysulfate, Congo red, amphotericin B, anthracyclines, and memantine [32–39]. Moreover, a subset of these compounds has been shown to extend the lives of prion-infected animals [40–42]. However, as noted by Caughey et al., the “clinical applicability of these compounds is severely limited by a lack of activity when administered after the onset of clinical signs of disease, poor bioavailability to the brain, and/or high toxicity [42–46].” Despite the incremental progress in the field, efforts to more efficiently identify and screen test compounds for antiprion activity are ongoing. Early work by Pruisner et al. searched the Available Chemicals Directory for molecules that inhibit prion replication based upon prior studies, identified a number of a family of compounds (pyridine dicarbonitriles) that showed in vitro efficacy in inhibiting prion replication [47, 48]. Follow-up studies by Reddy et al., who, through the design, synthesis, and screening of a series of related compounds, identified an additional compound that demonstrated efficacy at mitigating PrP<sup>res</sup> formation [49]. Most recently, Ferriera et al. describe the in silico and in vitro identification and screening of new small organic antiscrapie compounds that decreased PrP<sup>res</sup> accumulation and inhibit PrP aggregation [46]. Mechanistically, one of the most intriguing families of antiprion compounds is chemical chaperones. Chaperones are cellular constituents that interact with, stabilize, and assist in the proper folding of nonfolded proteins [50]. When used pharmacologically, chaperones are small compounds that bind to proteins and either induce their refolding or stabilize their structure. Specific chaperones demonstrating in vitro and/or in vivo efficacy in prion disease systems including (along with their mechanism of action): trimethylamine N-oxide, glycerol, dimethyl sulfoxide (protein stabilization by altering solvent properties), and bile salts [51, 52].

### 3. Antiprion antibodies to treat prion diseases

Other treatment strategies for prion diseases have been attempted including vaccination and immunotherapy, but these strategies have had limited success [53]. Nonetheless, there have been several promising studies gaining insights into this approach and its potential. To reduce redundancies, we refer the interested reader to the chapter entitled “Immunobiology of Prion Diseases” for more information on this topic.

### 4. Prion gene disruption to treat prion diseases

Since the cellular prion protein is not essential for life but required for prion disease [54, 55], several groups have worked to develop and test strategies that disrupt normal cellular prion proteins, PrP<sup>C</sup>. With this in mind, a recent treatment strategy used lentivirus vectors that expressed silencing RNAs directed against the cellular form of the prion protein [56]. These



lentiviral vectors were employed to transduce mouse embryonic stem cells and the resultant transduced embryonic stem cells used to create chimeric mice expressing various levels of the silencing RNAs. After infection of these mice with scrapie, mice that were highly chimeric for the transgene and that showed reduced PrP<sup>C</sup> expression in the brain showed increased survival times. Similarly, Mallucci et al. generated an adult-onset PrP knockout mouse model with delayed, neuron-specific deletion of PrP<sup>C</sup>, which mitigated the clinical and neuropathologic consequences of prion disease [57, 58]. In another study, the same group used RNAi-driven gene silencing to reduce PrP<sup>C</sup> expression. Using lenti-shRNA directed against PrP<sup>C</sup>, treated mice experienced a significant downregulation of PrP<sup>C</sup> expression and a delay in prion disease progression [59]. Thus, strategies that reduce or eliminate PrP<sup>C</sup> using inhibitory RNAs show promise as a treatment for prion diseases.

An alternative approach to reducing PrP<sup>C</sup> expression is to edit the gene using Zn-finger nucleases (ZFN), transcription activator-like effector nucleases (TALEN), or clustered regularly interspaced short palindromic repeats (CRISPR) gene editing systems. Indeed, mice, bovine and goat prion genes have been targeted using these approaches [60–62] to produce disease resistant animals, and at least one patent has been filed for gene editing of prion genes in animals [63].

## 5. Target the unfolded protein response to treat prion diseases

The pivotal event in prion disease pathogenesis is the formation and accumulation of misfolded PrP<sup>res</sup> in the brain as it initiates a pathologic cascade of glial activation, neuronal hypometabolism, and apoptotic neuronal loss. An increasing body of work indicates that PrP<sup>res</sup> triggers this pathology, in part, through the activation of the unfolded protein response (UPR) [64]. The UPR is a two-phase, cytoprotective cascade of the endoplasmic reticulum (ER) that is initiated by misfolded or aggregated protein, and it seeks to resolve cellular and ER stress. In the initial adaptive phase of the UPR, misfolded protein stimulates one (or more) of three sensing proteins: (1) PERK (protein kinase RNA-like ER kinase), (2) IRE1 $\alpha$  (inositol-requiring protein 1), and/or (3) ATF6 (activating transcription factor-6). Subsequent homodimerization of two of these proteins (PERK and IRE1 $\alpha$ ) results in the phosphorylation and activation of intermediate messengers, including eIF2 $\alpha$  (eukaryotic initiation factor 2  $\alpha$ ), ATF4 (activating transcription factor), and XBP1 (X-box folding protein). The end result of the adaptive phase of the UPR is an attenuation of protein synthesis, an increased synthesis of ER chaperones, and a mitigation of ER protein processing [65, 66]. However, if these initial adaptive efforts fail, the UPR transitions to a second, apoptotic phase involving the activation of caspases 3, 6, 7, and 8.

Previous work has demonstrated involvement of both phases of the UPR in human and rodent prion disease [67, 68]. In addition to triggering apoptosis, it is increasingly clear that the UPR is able to induce the deleterious, glial-mediated inflammatory response that is characteristic of both prion and other neurodegenerative diseases [69]. Specifically, Moreno et al. have shown that prion replications results in unchecked eIF2 $\alpha$  activation that contributes to synaptic failure, neuronal loss, and clinical deficits in prion-infected mice [70]. However, the role

of the UPR in human prion disease is less clear. Although Hetz et al. demonstrated increased levels of ER stress associated with misfolded protein in the brains of human patients with sporadic or variant CJD [71], subsequent immunohistochemical studies examining the brains of human patients with CJD for activated forms of PERK and eIF2 $\alpha$  have failed to confirm consistent involvement of the UPR [72].

Despite the inconclusive mechanistic data linking the UPR with prion disease pathogenesis, a small number of groups have examined the efficacy of therapeutic strategies directed at mitigating its activation. The therapeutic potential of targeting the UPR pathway is best demonstrated by work performed by Mallucci and Moreno. In their initial studies, they report that genetic mitigation of eIF2 $\alpha$  activation decreases synaptic loss and neuronal loss in prion-infected mice [70]. Moreover, in follow-up work they demonstrate that upstream blockade of UPR activation through pharmacological inhibition of the activation of PERK reverses cognitive deficits and prevents clinical disease in prion-infected mice [67]. A smaller body of work has revealed that pharmacologic inhibition of the UPR using the neuroprotective, antiapoptotic bile acids tauroursodeoxycholic acid (TUDCA) and ursodeoxycholic acid (UDCA), results in decreased levels of activated eIF2 $\alpha$  in organotypic cerebellar slices as well as decreased neuroinflammation and prolonged survival in mice. The reported benefits of bile acids result, in part, from their ability to inhibit the UPR activation across all three sensing pathways as reflected by lower levels of phosphorylated eIF2 $\alpha$ , ATF4, PERK, ATF6, and IRE1 $\alpha$  [73–76].

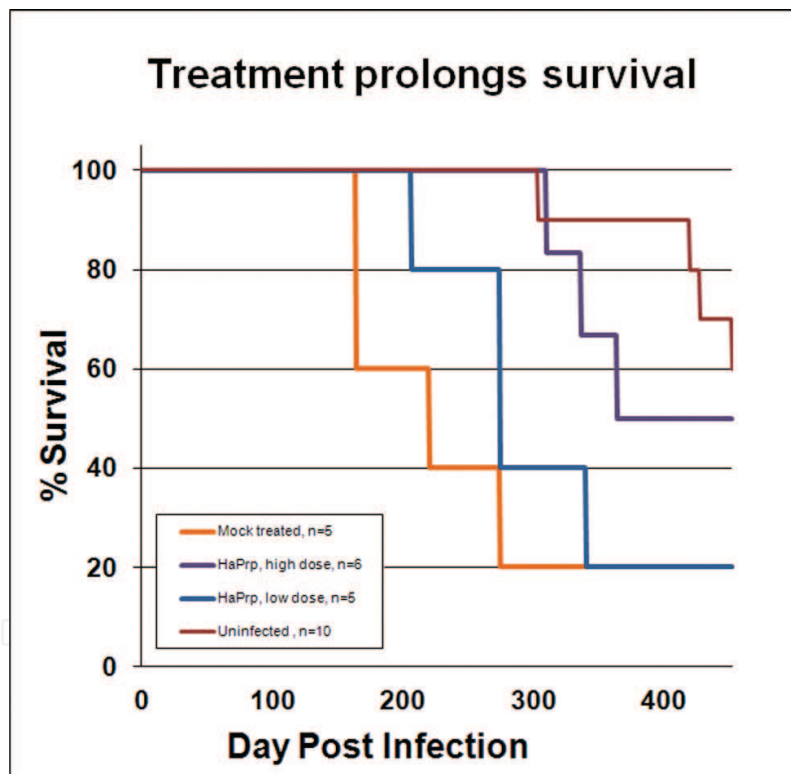
## 6. Heterologous prion proteins to treat prion diseases

The concept of heterologous prion proteins (HetPrP) as potential therapeutics is based on a body of research, including studies performed in cell free, cell culture, and animal models, in which prion proteins from different species were allowed to interact. Horiuchi et al. demonstrate that inclusion of a heterologous species PrP<sup>c</sup> in a cell-free conversion system was capable of interfering with the formation of PrP<sup>res</sup> between two homologous species [24]. When they divide the process of PrP<sup>res</sup> formation into two steps, namely initial binding between PrP<sup>c</sup> and PrP<sup>res</sup> followed by acquisition of protease resistance, the interfering effect of HetPrP appears to occur during the latter [24]. Further, the expression of hamster prion protein (HaPrP) in scrapie-infected mouse cells in vitro lead to near complete elimination of PrP<sup>res</sup> [77] supporting a role for heterologous HaPrP in either inhibiting PrP<sup>res</sup> production or enhancing its clearance. Moreover, the induced expression of rabbit prion proteins in scrapie-infected mouse cells led to substantially less PrP<sup>res</sup> as compared to mouse cells that do not express rabbit prion proteins, supporting a role for rabbit prion proteins interfering with mouse PrP<sup>res</sup> formation [23].

In our work, we extended these in vitro observations into the mouse using the rocky mountain laboratories (RML)-Chandler strain of scrapie and HetPrP therapy using bacterially expressed and purified recombinant HaPrP amino acids 23-231 [78]. For this study, mice were intracerebrally inoculated with an RML-Chandler strain brain homogenate combined

with either recombinant HaPrP or vehicle control. The following day, mice were treated with HaPrP orally. We assessed the effect of HaPrP dosage using two treatment groups, including a high dose of recombinant protein (0.7 mg/ml, high dose) and a low dose (0.35 mg/ml). Lastly, two control groups were included, those being a mock treatment group comprised of mice that were infected and treated with vehicle only, and mice that were not infected and not treated. We assessed the impact of treatment on clinical disease by evaluating mice daily following infection, weekly during the first months and then daily in later months for signs of scrapie-related symptoms including decreased motility, flattened stature, ataxic gait, hind limb paresis, dull eyes, weight loss, and kyphosis.

Treatment with the high dose HaPrP effectively and significantly delayed the onset of clinical symptoms, and prolonged survival compared to the vehicle-treated animals [78]. Moreover, when the study was terminated at 452 days postinfection, half of the high-dose-treated animals were still free of scrapie symptoms. **Figure 2** shows the survival times.



**Figure 2.** Treatment with heterologous recombinant HaPrP prolonged survival. Kaplan-Meier plots showing the survival times of mock-treated (orange,  $n = 5$ ), low-dose-treated (blue,  $n = 5$ ), high-dose-treated mice (purple,  $n = 6$ ) and uninfected (red,  $n = 10$ ). We tested for differences between groups using a modified version of the Gehan-Wilcoxon test and found a statistically significant difference between the mock infected group and the high-dose group ( $p = 0.0348$ ).

In addition to abrogating the clinical signs of prion disease, mice receiving the high-dose of HaPrP, compared to mice treated with a low dose of HaPrP or with vehicle only, accumulated significantly less PrP<sup>res</sup> in both brain and spleen. Furthermore, HaPrP partially mitigated



the neuropathologic consequences of prion infection as high-dose-treated animals showed a trend towards fewer activated astrocytes as revealed by immunohistochemistry for glial fibrillary acidic protein and less severe neuropil spongiosis in total brain and highly significant reductions in the thalamus.

Although we demonstrated that treatment with HetPrP<sup>C</sup> inhibits both the formation of PrP<sup>res</sup> and the clinical consequences of prion infection, the mechanism underlying this phenomenon is not known. We think that HetPrP binds to both PrP<sup>res</sup> and PrP<sup>C</sup> and blocks the production and elongation of PrP<sup>res</sup> chains and amyloid formation. This is modeled in **Figure 1**.

The work of Horiuchi et al. offers two possible mechanistic models for this interference, based upon number and type of binding sites for PrP<sup>C</sup> on PrP<sup>res</sup> [24]. They posit in a “one binding system,” that the binding of HetPrP to a growing PrP<sup>res</sup> oligomer creates an aggregate that is incapable of generating the steric interactions necessary for the continued production of PrP<sup>res</sup>. Alternately, they propose in a “two binding system” that the growing PrP<sup>res</sup> oligomer contains two binding sites, namely a conversion-inducing site and a nonconverting site. In this two-site system, HetPrP interferes with the formation of PrP<sup>res</sup> by binding and blockading conversion site without blocking the nonconverting site.

In addition to biochemical mechanisms described, it is possible that the protective effect of HetPrP in our study resulted from an evoked immune response that impacted PrP<sup>res</sup> formations. However, our data do not support this hypothesis. By western blot analysis of serum from study mice, we did not detect the presence of antihamster PrP antibodies in treated compared to control animals. Lastly, it is important to note that because mice were simultaneously intracerebrally inoculated with both scrapie prions and HaPrP, it is quite likely that the HaPrP in the inoculum served to inactivate the scrapie prion by binding to PrP<sup>res</sup> and forming an inactive complex due to sequence incongruence.

It is increasingly apparent that HetPrP treatment safely inhibits the PrP<sup>C</sup> to PrP<sup>res</sup> conversion process. In vitro and in vivo studies render feasible the prospect of treating human prion diseases with HetPrP. While demonstrating efficacy, in our study the treatment regime used (intracerebral instillation of HetPrP at the time of infection followed by oral ingestion of heterologous PrP<sup>C</sup>) which is not ideal for treating patients with existing prion disease. Delivery via intracerebral injection is certainly not anticipated to allow HetPrP to make contact with and inactivate all PrP<sup>res</sup> in the system. As such, future studies are needed to develop more practical HetPrP delivery modalities as well as to evaluate potentially more effective HetPrP sequences.

While a wide range of mammal species are susceptible to prion infection, the efficiency of interspecies transmission is varied and governed by a “species barrier,” the integrity of which is inversely proportional to the strength of the interaction between host PrP<sup>C</sup> and incoming PrP<sup>res</sup>. Interestingly, rabbits have been shown to be unusually resistant to prion disease inoculation, as attempts to transmit CJD, Kuru, sheep scrapie, TME, and mouse-adapted scrapie to rabbits failed [79, 80]. While subsequent groups have confirmed that the rabbit is not

absolutely resistant or prion infection, there is general agreement that they are only minimally susceptible [81, 82]. The degree of primary sequence homology is important in determining the robustness of the species barrier. The rabbit prion protein shows relatively low sequence homology to other species prion proteins. Based upon this work, we propose that a rabbit PrP-based HetPrP treatment strategy may be more effective than HaPrP at inhibiting prion disease.

While we used IC injection of HetPrP in our study, the clinical evolution of this approach necessitates a more effective and simpler means of delivery. One such approach could be delivery via the bloodstream and use blood vessels to efficiently deliver HetPrP to all areas of the brain. In addition, it may be possible to use peptides derived from HetPrP rather than whole proteins. Indeed Chabry et al. showed in vitro inhibition of PrP conversion with synthetic peptides derived from mouse and hamster PrP [83, 84]. Another such possibility for HetPrP treatment is the adoption of a gene therapy-based approach using lentiviral vectors. Thus, further studies are warranted to optimize both the form of HetPrP as well as its mode of delivery.

In related studies, other groups have found promising therapeutic results as well. Meier et al. engineered PrP<sup>C</sup> fused to immunoglobulin Fcγ<sub>2</sub>, termed PrP-Fc(2) [85]. Wild-type mice expressing PrP-Fc(2) and subsequently infected with scrapie prions showed delayed PrP<sup>res</sup> accumulation and onset of disease [85]. In follow-up studies, they further showed that expression of PrP-Fc(2) transduced by a lentiviral vector at 170 days postinfection was able to reduce prion infectivity by 3–4 logs [86]. Toupet et al. created a recombinant lentiviral vector that transduces expression of a dominant negative mouse prion protein that recapitulates sheep PrP<sup>Q171R</sup> and human PrP<sup>E219K</sup> polymorphisms associated with prion disease resistance [87]. They showed that chronic injection of this vector directly into the brains of prion disease infected mice led to reduced astrocytic gliosis and extended survival [87]. Moreover, Soto et al. designed beta sheet breaker peptides corresponding to the conserved region of PrP 115–122 that is thought to play a central role in conversion of PrP<sup>C</sup> to PrP<sup>res</sup> [88–91]. These beta sheet peptides partly reversed PrP<sup>res</sup> to PrP<sup>C</sup> in vitro, and when mixed with scrapie prions and injected into mice, decreased infectivity by 90–95% [88]. Thus, multiple strategies have been developed and tested in mice that use prion proteins or related peptides to target and reduce prion infectivity and have demonstrated efficacy.

## 7. Potential future strategies to treat prion diseases

Understanding pathogenesis is key to developing new therapies for prion diseases. For example, we [92, 93] and others have gained insights into prion disease pathogenesis by studying in changes in gene expression that occur during the disease process. These expression alterations provide insights to underlying pathological processes, and key mediators of these processes might be targeted in future prion treatment strategies. Another example comes

from Hetz et al., who determined in a scrapie infected cell culture system that PrP<sup>Sc</sup> toxicity and apoptosis induction was associated in an increase in an endoplasmic reticulum resident enzyme caspase-12, and a corresponding increase in caspase-12 was also seen in humans affected by CJD [94]. With this knowledge of a key process in pathogenesis, they were able to inhibit apoptosis by overexpression of a catalytic mutant of caspase-12 [94]. In another set of studies, the 37 kDa/67 kDa laminin receptor LPR/LR was targeted based on knowledge that LPR/LR is a cell surface receptor for PrP<sup>C</sup> [95] and required for PrP<sup>res</sup> propagation in scrapie-infected cells [96]. Zuber et al. created and infused single-chain Fv antibodies directed against LPR/LR into mice just prior to inoculation with scrapie prions and weekly afterwards, and found an ~40% reduction in PrP<sup>res</sup> in spleen [97]. In similar experiments, Pflanz et al. injected lentiviral vectors that transduce small interfering RNAs directed against LPR/LR precursor mRNA into the brains of mice, then infected them with scrapie, and found a 41% reduction in PrP<sup>res</sup> and prolongation of the preclinical phase [98]. Thus, gaining understanding of the molecular event underlying prion disease pathogenesis can identify potential targets for future prion disease therapeutics.

Moving forward to a viable treatment and cure for prion diseases in humans will likely involve a combination of therapies. For example, this might involve a combination of approaches such as gene editing to create disease resistant prion gene alleles, a drug that inhibits apoptosis, a small molecule that stabilizes PrP<sup>C</sup> and regular injections of heterologous prion proteins that bind and clear nascent PrP<sup>res</sup>.

Importantly, strategies that work for treating prion diseases may also be effective when applied to other neurodegenerative diseases that involve protein misfolding, such as Alzheimer's disease and Huntington's chorea. There is increasing evidence of underlying similarities in the pathogenesis of protein misfolding neurodegenerative diseases. Hence, similar cure strategies may be feasible.

## 8. Conclusion

In conclusion, an ever-expanding understanding of basic prion pathogenesis, combined with the rapidly ever-expanding development of new biotechnologies, combined with existing strategies to treat prion diseases, will likely lead to a feasible and effective treatment for prion diseases in the near future. Already, innovations such as genome editing, inhibitory RNAs, and improved gene therapy vectors are being applied to and advancing treatment strategies to create improved treatments. In addition, strategies that show efficacy that target separate components of disease pathogenesis can be combined. Thus, in the coming years, the outlook is very promising for the development of an effective treatment and potential cure for individuals with prion diseases. Furthermore, strategies used to treat prion diseases might be broadly applicable and effective when applied to other protein misfolding diseases. The increasing awareness of the role of protein misfolding in many neurodegenerative processes makes the development of an effective treatment strategy for prion diseases a high priority.

## Abbreviations and acronyms

TSEs	Transmissible spongiform encephalopathies
CJD	Creutzfeldt-Jakob disease
GSS	Gerstmann-Straussler-Scheinker syndrome
FFI	Fatal familial insomnia
BSE	Bovine spongiform encephalopathy
CWD	Chronic wasting disease
Denoted PrPC	Cellular prion protein
Commonly denoted as PrP <sup>Pres</sup> or PrP <sup>Sc</sup>	Misfolded isoform
HetPrP	Heterologous prion proteins
HaPrP	Hamster prion protein

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