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# The role of prion strain diversity in the development of successful therapeutic treatments

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## Abstract

Prions are a self-propagating misfolded conformation of a cellular protein. Prions are found in several eukaryotic organisms with mammalian prion diseases encompassing a wide range of disorders. The first recognized prion disease, the transmissible spongiform encephalopathies (TSEs), affect several species including humans. Alzheimer's disease, synucleinopathies, and tauopathies share a similar mechanism of self-propagation of the prion form of the disease-specific protein reminiscent of the infection process of TSEs. Strain diversity in prion disease is characterized by differences in the phenotype of disease that is hypothesized to be encoded by strain-specific conformations of the prion form of the disease-specific protein. Prion therapeutics that target the prion form of the disease-specific protein can lead to the emergence of drug-resistant strains of prions, consistent with the hypothesis that prion strains exist as a dynamic mixture of a dominant strain in combination with minor substrains. To overcome this obstacle, therapies that reduce or eliminate the template of conversion are efficacious, may reverse neuropathology, and do not result in the emergence of drug resistance. Recent advancements in preclinical diagnosis of prion infection may allow for a combinational approach that treats the prion form and the precursor protein to effectively treat prion diseases.

## 1. Prion diseases

Prions are a self-propagating misfolded conformation of a cellular protein. The conversion of the normal isoform to the misfolded prion form occurs in a stepwise manner, but the exact mechanism is unknown.[1–4] Prions are found in several eukaryotic organisms. Yeast prions are a dominant, non-Mendelian form of epigenetic inheritance that causes a detectable phenotype. For example, the [URE3], [SWI+], and [GAR+] prions in *Saccharomyces cerevisiae* enhance growth on nutrient-poor sources, while the [PSI+] prion enables translational readthrough.[5–9] The prion state in yeast is dependent on a chromosomal gene and transient overproduction of the normal cellular protein promotes the prion state which can be reversibly cured.[8,10,11] The [Het-s] prion of *Podospora anserina* is infectious and has a necessary role in heterokaryon incompatibility, a process that restricts fusion of hyphae

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to genetically similar partners.[12–14] Prions found in yeast, fungi, and mammals can have advantageous roles. Functional prion-like proteins involved in memory, RNA metabolism, and immunity have been identified.[15–19] However, prions in mammals are most notably associated with neurodegenerative diseases.

Mammalian prion diseases encompass a wide range of disorders. The first recognized prion disease, the transmissible spongiform encephalopathies (TSEs), are characterized by long incubation periods followed by a relatively short duration of clinical signs that ultimately leads to death of the host.[1,20] TSEs occur in both domesticated populations of sheep and goats (scrapie), cattle (bovine spongiform encephalopathy, BSE), and mink (transmissible mink encephalopathy, TME). Chronic wasting disease (CWD) is unique among TSEs as it can affect both wild and domestic populations of cervids.[21–23] Camel prion disease (CPD) in domesticated dromedary camels was recently identified, but the geographic distribution and potential of transmission to wild populations is unknown.[24] Human TSEs can have infectious (Kuru), inherited (Gerstmann-Sträussler-Scheinker, GSS), or sporadic (Creutzfeldt-Jakob disease, CJD) etiologies, with sporadic being the most prevalent.[25] Iatrogenic transmission of PrP prions can occur via cadaveric human growth hormone (c-hGH), dura mater grafts, or corneal transplants.[26–29] TSE agent replication involves the misfolded infectious prion conformation of the prion protein, $PrP^{Sc}$, binding to the normal, cellular conformation, $PrP^{C}$, initiating further seeded conversion.

Alzheimer's disease, synucleinopathies, and tauopathies are characterized by the misfolding and aggregation of amyloid-β (Aβ; a peptide derived from the host cellular amyloid precursor protein (APP)), α-synuclein protein (α-syn), and tau protein, respectively. Similar to TSEs, these protein misfolding diseases share a similar mechanism of self-propagation of the prion form of the disease-specific protein and cell to cell spreading of the host protein in the prion state.[30–39] Experimental injection of the prion form of the protein into animals that express the respective precursor protein can accelerate the pathogenesis of disease, reminiscent of the infection process of TSEs (or PrP prions).[34,40–46] Although these non-prion protein disorders have both inherited and sporadic etiologies, conflicting evidence for infectious transmission has been reported. Epidemiological studies concluded that a history of one or more blood transfusions was not associated with a higher risk of developing AD compared to age- and gender-matched controls for each patient case.[47,48] Injection of nonhuman primates with over 600 patient samples from individuals diagnosed with nonspongiform diseases (i.e., AD, PD, etc.) failed to transmit disease.[49] No cases of AD or PD were identified among recipients of c-hGH in the United States despite finding mild amounts of pathological Aβ, tau, and α-synuclein by immunohistochemical analysis of pituitary glands from neurodegenerative disease and control patients.[50] However, a recent study reported that recipients of c-hGH contaminated with PrP prions subsequently developed CJD with concurrent Aβ deposition in the brain, suggesting iatrogenic transmission of Aβ.[51] Retrospective studies on the archived c-hGH samples identified significant amounts of tau, $Aβ_{42}$ and $Aβ_{40}$.[52] Intracerebral injection of these c-hGH samples to transgenic mice expressing a human mutant of the amyloid precursor protein resulted in the development of brain pathology similar to the patients injected with the same material.[52] This finding suggests, under certain conditions, iatrogenic

transmission of AD is possible. Overall, the aforementioned neurodegenerative disorders share profound similarities at the biochemical and cellular level.

## 2. Prion strain diversity

Prion strains are operationally defined by heritable differences in the biological properties of disease under controlled agent and host parameters.[53–56] In yeast, prion strains are classified based on several traits that differ depending on the prion state (i.e., [PSI+] or [URE3]) but were initially distinguished by strength of phenotype (i.e., “strong” versus “weak”).[57–59] TSE strains are classified by differences in incubation period and neuropathology but can differ in their clinical signs and biochemical features of $PrP^{Sc}$.[60,61] Similar to TSEs, Aβ, tau, and α-synuclein strains are defined by differences in abnormal protein deposition and pathology in the brain as well as protein conformation or seeding activity of the prion form of the respective protein.[38,44,62–64] The relationship between the strain-specific biochemical features of the prion form of the protein and the phenotype of disease is poorly understood.

### 2.1 Prion strain diversity in yeast

Studies of yeast prion strain diversity have added much to the knowledge of prion strain diversity. Yeast prion strains differ in strength of phenotype, stability of propagation, toxicity/lethality, ability to overcome inter- or intraspecies transmission barriers, and biochemical and physical properties.[57–59,65–67] Nuclear magnetic resonance (NMR) spectroscopy identified conformational differences between [*PSI*+] yeast prion strains, and atomic force microscopy (AFM) reveals strain-specific differences in yeast prion fibril morphology.[68] There are strong correlations between biochemical properties of the prion form of the protein and strain phenotype in yeast. The strength of the yeast prion phenotype corresponds with an increased fragility of protein aggregates.[69] Mechanistically, increased fragility of prion aggregates results in rapid generation of new free ends, accelerating prion formation (Fig. 1, step 5).[69]

### 2.2 TSE strain diversity

Strain diversity is hypothesized to be encoded by strain-specific conformations of $PrP^{Sc}$.[63,64,68,70–72] Strain-specific differences in the biochemical properties of $PrP^{Sc}$ were first identified in mice.[73] These differences were later observed in hamsters infected with the hyper (HY) or drowsy (DY) strains of hamster-adapted transmissible mink encephalopathy (TME).[74] Detergent extraction and protease digestion of HY and DY $PrP^{Sc}$ revealed strain-specific Western blot migration profiles suggesting strain-specific differences in the PK digestion site on $PrP^{Sc}$.[75] Edman protein sequencing of $PrP^{Sc}$ from HY or DY TME-infected hamsters confirmed that $PrP^{Sc}$ from these two strains had different PK cleavage sites.[70] Based on these observations, the authors proposed the hypothesis that strain-specific conformations of $PrP^{Sc}$ encoded prion strain diversity.[70,75,76] Consistent with this hypothesis, the molecular weight of the proteinase K-resistant fragment of $PrP^{Sc}$ after deglycosylation in fatal familial insomnia is 19 kDa while the same fragment was 21 kDa in sporadic and familial Creutzfeldt Jakob disease.[72] When inoculated into mice, the strain-specific migration pattern of $PrP^{Sc}$ from human prion strains was preserved supporting the hypothesis that strain-specific conformations of $PrP^{Sc}$ is the basis of strain diversity.[72]

The presence of cellular cofactors during prion conversion may be responsible for the maintenance of strain-specific, infectious conformations of $PrP^{Sc}$. Development of synthetic, *in vitro*-generated prions able to successfully infect wild-type rodents relied on cofactors such as polyanions and lipids.[77,78] Propagation of a recombinant prion strain in PMCA in the presence of both recombinant murine PrP and the lipid phosphatidylethanolamine (PE) retained infectivity.[79] Withdrawal of the cofactor (PE) led to the emergence of a protein-only strain, named OSU, that did not cause disease in mice suggesting cellular cofactors are required for infectivity. Additionally, *in vitro* PMCA propagation of three distinct prion strains with PE as the sole cofactor resulted in the three strains evolving into a single strain.[79] This work on the directed evolution of prions suggests that cellular cofactors are involved in prion strain diversity. Recent work has clarified the role of prion cofactors. Using a novel *in vitro* system where protein only $PrP^{Sc}$ preparations, in the absence of PMCA adaptation, are directly infectious to animals, but only in the presence of co-factors.[80] Importantly, protein-only and cofactor generated $PrP^{Sc}$ had identical strain properties indicating that the conformation of $PrP^{Sc}$ is sufficient to encode prion strain information and that cellular co-factors are needed for prion propagation.[80] Overall, these data provide a unified model of prion infectivity where strain information is encoded by the structure of $PrP^{Sc}$ and host cellular cofactors are necessary for prion conversion and subsequent infectivity.

Lesion profiles, cellular assays, and the biochemical properties of $PrP^{Sc}$ are used to differentiate prion strains. The scrapie cell assay (SCA) and the cell panel assay (CPA) assess the relative susceptibility or resistance of murine neuroblastoma cell lines to infection by different prion strains.[81–83] The Western blot migration profile, conformational stability, and sensitivity to proteolytic digestion of $PrP^{Sc}$ can be strain specific. It is unclear which, if any, of the strain-specific biochemical differences of $PrP^{Sc}$ directly contributes the strain-specific phenotype of disease. For example, in murine PrP prions, decreased $PrP^{Sc}$ conformational stability is positively correlated with a shorter incubation period, however, in humans and hamsters the inverse relationship between $PrP^{Sc}$ conformational stability and incubation period was identified.[71,84–87]

### 2.3 Aβ strain diversity

The heterogeneous clinical presentation of AD may be attributed to strain diversity.[88] The Arctic and Swedish APP mutations in humans result in distinct AD pathologies.[89–92] It was hypothesized that the two AD clinical disease outcomes were a result of two distinct Aβ strains. Conformational stability studies of Aβ from patient brains with Arctic AD and Swedish AD mutations established that aggregates of Aβ found in the Arctic AD patients were significantly more sensitive to denaturation with guanidine hydrochloride compared to Swedish AD.[44] Further, injecting transgenic mice expressing human APP containing the Swedish mutation with the Arctic or Swedish AD brain material resulted in two distinct phenotypes of disease that were maintained upon serial passage consistent with the predicted behavior of prion strains.[44] Luminescent conjugated oligothiophenes, a class of amyloid binding dyes, to investigate how the Aβ amyloid structure in familial AD differs from sporadic AD.[93] The emission spectra of luminescent conjugated oligothiophenes changes when bound to amyloid of differing structure. The emission spectra of luminescent

conjugated oligothiophenes bound to Aβ some of the subtypes of familial and sporadic AD varied significantly, suggesting a variety of Aβ structures exist in different AD cases.[93] Solid state nuclear magnetic resonance imaging analysis of Aβ from patient tissue samples from PCA-AD or rp-AD indicated that Aβ from PCA-AD resembled typical AD with predominant Aβ40 fibril structure while rp-AD samples were composed of a cloud of variant Aβ40 structures.[94] Overall, this data is consistent with the hypothesis that Aβ structural variance contribute to the clinical and pathological heterogeneity of AD.

### 2.4 Tau strain diversity

Alzheimer's disease (AD), corticobasal degeneration (CBD), progressive supranuclear palsy (PSP), Pick's disease (PiD), and argyrophilic grain disease (AGD) are unique tauopathies defined by the pattern of tau deposition in the brain along with distinct clinical phenotypes.[95] Transmission studies in wild-type and transgenic mouse models have established that tau extracts from various tauopathies transmit distinct tau neuropathology.[38,64,96–98] For example, AD-tau variants can differentially seed tau pathology after intracranial injection of wild-type mice; the tau variants were determined to be structurally different by trypsin digests that resulted in three distinct trypsin resistant fragments.[38] Injecting wild-type mice with tau from AD, CBD, or PSP resulted in differences in tau conversion efficiency and cellular tropism in the CNS suggesting the three tau isolates are distinct prion strains.[64] Consistent with this hypothesis, tau fibrils from the tau strains showed varying resistance to Gdn-HCl denaturation with CBD-tau, AD-tau, and PSP-tau having the lowest to highest stability, respectively.[64] Two recombinant tau strains in HEK293 cells expressing the tau repeat domain that propagated conformationally distinct forms of tau that could be serially passaged in PS19 transgenic tau mice that retained the strain-specific conformation of tau.[99] Further, the HEK293 cell model was used to examine AD, CBD, and AGD patient samples, and each tauopathy induced unique tau inclusion morphology.[99] Consistent with this finding, 18 tau strains were identified based on differences in limited proteolysis and *in vitro* seeding activity of tau in combination with hippocampal tau pathology when transmitted to PS19 transgenic tau mice.[100] Taken together, these data support that tau structural variance may explain the distinct pathologies and clinical presentation of the tauopathies.

### 2.5 α-synuclein strain diversity

Strain-specific prion forms of α-synuclein have been identified in synucleinopathies including Parkinson's disease (PD), dementia with Lewy bodies, and multiple system atrophy (MSA). Synthetic α-synuclein fibrils generated under varying salt concentrations form distinct conformations with differences in seeding ability and toxicity *in vivo* and *in vitro*.[62,101,102] Recombinant fibrillar α-synuclein created under different laboratory conditions varied in ability to cross-seed tau aggregation in cell culture and *in vivo*.[63] Consistent with this observation, proteinase K digestion revealed differences in protein cleavage between the synthetic α-synuclein as well as two distinct α-synuclein digestion products isolated from Parkinson's disease with dementia (PDD) patient brains.[63] Distinct digestion products are suggestive of two α-synuclein conformations reminiscent of prior observations in PrP prions.[72,75,103–105] *In vitro* seeding assays utilizing a cell line that readily propagates MSA α-synuclein was not susceptible to α-synuclein from PD patient

brains suggesting that PD contains a different strain of α-synuclein.[106] Brain material from PD and MSA patients has distinct seeding activity and cellular inclusion pathology in HEK293T cells, suggestive of distinct strain of α-synuclein.[107] Overall, α-synuclein strains share many features of PrP prion strains that include differences in biochemical features, neuropathology, incubation period, and clinical manifestation of disease.[38,62,63]

## 3. Prion strains exist as a mixture

Multiple prion strains can co-exist in an individual host. In cases of sporadic Creutzfeldt-Jakob disease (sCJD), the polymorphism at codon 129 and different proteinase K cleavage patterns via Western blot classify disease as Type 1 (21 kDa) or 2 sCJD (19 kDa).[103] Type 1 and Type 2 sCJD $PrP^{Sc}$ can co-exist in the CNS of the same patient.[108–116] It is suggested that up to 40% of sCJD cases contain more than one strain, however, the prevalence rate is controversial since methodological differences in strain typing and the region of the brain that is evaluated can both affect the outcome.[108–116] It is not well understood if strains of Aβ can co-exist in a single host, however, distinct strains of Aβ have been identified raising the possibility that sporadic AD may be a mixture of strains.[44] Mapping phospho-tau patterns of deposition in the hippocampal regions of individuals with various tauopathies established that the pattern of pathology is disease-specific with regional selectivity reminiscent of strain targeting in TSE's.[117] This same study determined that AD-related tau hippocampal deposition is greatly influenced by simultaneous argyrophilic grain disease (AGD), another tauopathy, suggesting that coexisting tau strains can create heterogeneous brain pathologies and overlapping clinical features highlighting the need for discriminatory methods of post-mortem diagnosis.[117] Antibodies that distinguish AD tau from non-AD tau deposits of CBD, PSP, and Pick's Disease (PiD) can discern the differential deposition in co-occurring pathologies.[118] While ample experimental evidence suggests that different α-synucleinopathy strains exist, the coexistence of α-synucleinopathy strains has not been described.

Coexisting prion strains can interfere with each other. Strain interference is a phenomenon in which a slowly-converting blocking strain can delay or prevent the emergence of a quickly-converting strain. A number of parameters govern prion strain interference. Increasing the titer of the blocking strain results in a corresponding increase in the interference effect.[119–122] Importantly, prion conversion in a common population of cells is required for interference to occur as the strains compete for a limiting cellular resource, $PrP^{C}$.[123,124] Consistent with this hypothesis, co-infection of two low conversion efficiency prion strains results in each strain converting independently without interference that is due to $PrP^{C}$ not becoming limiting.[125] Overall, strain interference is an important parameter in the emergence of a dominant strain from a mixture.

Currently, strain interference has not been reported for mammalian non-PrP prions, and further investigation is required to determine if this phenomenon is a common property of mammalian prions. In yeast, there is evidence that strain variants compete within a cell during and after mating, with "strong" variants outcompeting "weak" variants for the template of conversion.[126] Mechanistically, "weak" variants are characterized by quick growth but low frangibility, whereas "strong" variants grow slowly but are more easily

fragmented, leading to increased free ends for growth and therefore a competitive advantage over “weak” variants.[69]

## 4. Prion therapeutics

Therapeutics that target prion conversion can work via several mechanisms. Drugs can target $PrP^{C}$ prior to its interaction with $PrP^{Sc}$ by affecting the post-translational processing, metabolism, cellular trafficking, or localization of $PrP^{C}$ (Fig. 1, step 1). Drugs that bind to or induce a conformational change in $PrP^{C}$ can hinder the interaction between $PrP^{C}$ and $PrP^{Sc}$ (Fig. 1, step 2). Drugs can target $PrP^{Sc}$ by stabilizing or redistributing $PrP^{Sc}$, interfering with the interaction between $PrP^{C}$ and $PrP^{Sc}$ (Fig. 1, step 3). The conversion of $PrP^{C}$ to $PrP^{Sc}$ can be targeted by drugs that block the $PrP^{Sc}$ binding site on $PrP^{C}$, cap $PrP^{Sc}$ aggregate growth, or inhibit cofactors that enable conversion (Fig. 1, step 4). Therapeutics that target clearance of $PrP^{Sc}$ either increase fragmentation of $PrP^{Sc}$ aggregates (Fig. 1, step 5) or promote clearance of $PrP^{Sc}$ from cells (Fig. 1, step 6).

### 4.1 Treatments that target $PrP^{Sc}$

Several drugs that directly interact with $PrP^{Sc}$ have anti-prion effects (Table 1 and Fig. 1). Congo red, an amyloid binding dye, was one of the first compounds identified to extend the incubation period of prion-infected animals and can reduce the formation of $PrP^{Sc}$ in scrapie-infected mouse neuroblastoma cells ($Sc^{+}$ MNB).[127] Congo red is hypothesized to directly bind and over-stabilize $PrP^{Sc}$, preventing further prion formation.[127–129] Oral administration of Compound B, an amyloidophilic compound, extends the incubation period of TSEs and is hypothesized to inhibit formation of $PrP^{Sc}$ by directly binding $PrP^{Sc}$ or by interacting with cofactors/chaperones necessary for prion conversion.[134,135] The diphenyl-pyrazole anle138b inhibits $PrP^{Sc}$ amplification and reduces neurotoxicity by shifting $PrP^{Sc}$ oligomers to a smaller size by reducing intermolecular hydrogen bonding and obstructing the formation of higher order oligomers, modulating the formation of toxic $PrP^{Sc}$ aggregates.[136,137] Anle138b has anti-prion activity both *in vitro* and *in vivo* and substantially prolonged survival even when treatment began after onset of signs of prion disease.[137]

Cellular redistribution of $PrP^{Sc}$ can inhibit prion formation. Chlorpromazine (CPZ), an antipsychotic, redistributes $PrP^{Sc}$ from the early endosomal/endocytic recycling pathway to the late endosomal/lysosomal pathway resulting in an inhibition of prion conversion in prion-infected N2a cells; however, CPZ was ineffective *in vivo*.[139] A cholesterol synthesis inhibitor, U18666A, can redistribute $PrP^{Sc}$ from the early endosomal/endocytic recycling pathway to the late endosomal/lysosomal pathway, causing an increase in $PrP^{Sc}$ degradation that corresponds to a decrease in total $PrP^{Sc}$ in prion-infected N2a cells.[139]

Increasing degradation of $PrP^{Sc}$ can ameliorate prion infection (Fig. 1, step 6). The tyrosine kinase inhibitor STI571 decreases the half-life of $PrP^{Sc}$ in prion-infected ScN2a cells via interaction with the tyrosine kinase c-Abl without affecting the cellular location or trafficking of $PrP^{Sc}$.[208] Several drugs increase degradation of $PrP^{Sc}$ by inducing autophagy. A-12 and A-14, derivatives of the antitumor drug celecoxib, can reduce or eliminate $PrP^{Sc}$ in prion-infected neuronal cells lines by stimulating autophagy.[209] Lithium, which is used to

treat depression, reduces $PrP^{Sc}$ in prion-infected neuronal and non-neuronal cells cultures by inducing autophagy.[210]

Pentosan polysulfate (PPS), a cysteine protease inhibitor, induces a reduction in prion conversion by causing fragmentation of $PrP^{Sc}$ at the cell surface or utilizing endogenous glycosaminoglycans (GAGs) to competitively interfere with the binding of $PrP^{C}$ to $PrP^{Sc}$.[139,140] Treatment of prion-infected mice with PPS decreased $PrP^{Sc}$ accumulation and prolonged survival compared to untreated controls even when administered after the onset of $PrP^{Sc}$ deposition in the CNS.[139,140] While effective in rodents, treatment of human prion diseases with PPS has varied results. In some patients treated with intravenous PPS, there is a significant increase in survival compared with untreated patients[141–143]; however, there are several cases indicating treatment is not effective at extending survival or ameliorating clinical features of disease.[144–146]

Treatment of prion disease with heterologous prion proteins (HetPrP) can interfere with the formation of $PrP^{Sc}$ in both $Sc^{+}$MNB cells and *in vivo*.[148–151] Treatment of RML-infected mice with hamster HetPrP significantly delayed onset of clinical symptoms and significantly decreased $PrP^{Sc}$ accumulation in brain and spleen.[150] Mechanistically, it is hypothesized that HetPrP either incorporates itself into a growing $PrP^{Sc}$ aggregate and, unable to be converted to $PrP^{Sc}$ by the host species $PrP^{Sc}$, hinders conversion or binds directly to the conversion site on $PrP^{Sc}$ blocking the $PrP^{C}$ conversion site.[148] Overall, drugs that target $PrP^{Sc}$ that are effective in prion-infected cell cultures and animals are ineffective in human clinical trials. The lack of efficacy in human clinical trials could be partly due to timing of treatment initiation. In prion-infected rodents, anti-prion drugs administered prior to or shortly after prion infection have the highest efficacy, with administration at the onset of clinical disease being less effective. In human prion disease, treatment is typically not begun until after onset of clinical signs, decreasing treatment efficacy.[211] In an attempt to overcome this, an ongoing study is treating subjects at risk for developing fatal familial insomnia with doxycycline.[212]

### 4.2 Strain-specific efficacy of anti-prion treatments

Treatment of prion-infected animals or cell cultures with anti-prion drugs can lead to the emergence of drug resistant prion strains. Swainsonine inhibits the processing of asparagine-linked glycoproteins by impeding the action of Golgi α-mannosidase II as well as lysosomal mannosidase.[213–215] Treatment of prion-infected neuroblastoma-derived R33 cells with swainsonine can result in the emergence of swainsonine-resistant prions.[216] Importantly, $PrP^{Sc}$ from swainsonine-resistant and swainsonine-sensitive prions from cell culture have different $PrP^{Sc}$ conformational stabilities suggesting they are distinct prion strains.[216,217] Treatment of prion-infected cells with swainsonine selected for a drug resistant substrain, but when the inhibitor is removed, the susceptible substrain reverted to a drug sensitive population in cell culture.[218] Additionally, passage of swainsonine-resistant RML prions from AMO10 to PK1 cells in the presence of swainsonine resulted in the selection of swainsonine-dependent prion variants with an increased efficiency to propagate in the presence of the drug.[219] However, passaging the swainsonine-dependent prions in the absence of drug resulted in reversion to a swainsonine-sensitive prion population in cells.[219]

RML-infected ScN2a cells treated with the 2-aminothiazole IND24 resulted in the emergence of an IND24-resistant prion strain (RML [IND24]) that had a different host cell range, biochemical and neurological features compared to RML-infected mice treated with vehicle (RML [V]).[152] In addition to IND24, this drug-resistant prion strain was also resistant to the anti-prion drugs Compound B and quinacrine.[152] This suggests that the mechanism of resistance between the three anti-prion compounds is similar. Susceptibility of the RML[IND24] strain to IND24 could be restored when passaged in the absence of IND24 resulting in the reversion of the RML[IND24] phenotype to the RML[V] phenotype in mice.[152]

The mechanism underlying the emergence of drug-resistant prion strains is not known. Anti-prion treatments can be strain specific.[152,157] One hypothesis is that compounds effective against the most predominant, but not all, strains in a mixture can allow for the emergence of a drug resistant strain. Prion strains are thought to exist as a dynamic mixture of substrains, or mutant spectra.[220,221] In this scenario, the dominant strain suppresses the emergence of substrains and, once the dominant strain is removed by the anti-prion drug, the suppressive effect is diminished allowing for emergence of a preexisting drug resistant strain. Studies of strain interference have established the conditions and mechanism of how a dominant strain can suppress the emergence of a minor strain consistent with this hypothesis.[121,123,221–223] Selective removal of a dominant strain from a mixture using strain-specific differences in the stability of $PrP^{Sc}$ can allow for the emergence of a highly pathogenic substrain from a mixture, providing additional support for this hypothesis.[224] Alternatively, it is possible that anti-prion compounds directly interact with $PrP^{Sc}$ altering its conformation (i.e., mutation) and therefore strain properties. Anti-prion compounds can bind to $PrP^{Sc}$ consistent with this hypothesis, however, RML[IND24] remained resistant to IND24 for up to 20 passages in CAD5 cells in the absence of IND24 suggesting that IND24 interaction with $PrP^{Sc}$ is not required for the resistant phenotype.[152] Overall, it is unclear if the emergence of drug resistant prion strains is due to selection of a preexisting mutant strain from the mutant spectra and/or if the anti-prion drug acts as a prion mutagen to generate the drug resistant strain.

Maintenance of a drug resistant phenotype may have a high fitness cost. Passage of drug resistant prion strains in the absence of the anti-prion drug can result in rapid reversion to the original drug sensitive strain. This suggests that the drug resistant prion strain has relatively poor fitness for the host. In the absence of drug, the mutant spectra that arises from prion conversion produces mutants that have increased fitness for the host. This observation raises several questions. First, reversion of the drug resistant strain in the drug-free environment leads to the emergence of the original drug sensitive strain. It is unclear why the drug resistant strain would revert to the parent strain as opposed to another strain if generation of the mutant spectra is random. This suggests that the drug resistant strain may contain a “memory” that favors reversion to the original strain. Second, treatment of a host with an anti-prion drug can extend the incubation period of disease without the emergence of a drug resistant strain. The mechanism behind this observation is unknown. It is possible that certain strains can still retain sufficient suppressive effect of substrains during anti-prion treatment. The relative distribution of the mutant spectra in any given isolate may differ depending on its passage history. For example, biologically cloned strains may have a more

limited mutant spectra compared to uncloned or field isolates. Finally, highly stable class I strains may have a more limited mutant spectra compared to unstable class III strains.[225] Overall, very little is known about how the interaction between the host and the strain influences prion strain fitness.

The emergence of drug resistant prions is a significant barrier to the development of effective treatments of prion diseases that target the prion form of the protein. Treatments that reduce or eliminate the abundance or the ability of the conversion template to form the prion form of the protein may offer an alternative strategy for anti-prion therapies.

### 4.3 Treatments that target $PrP^C$

Prion conversion is dependent on the presence of the template of conversion (Fig. 1, step 2). Genetic ablation of PrP in mice results in complete resistance to prion infection with little known detriment to development or function.[226,227] Similarly, ablation of $PrP^C$ in cattle does not result in major functional or immunological deficits and tissue from these animals does not support prion conversion.[228] In a conditional $PrP^C$ knockout model, transgenic mice express $PrP^C$ until approximately 12 weeks of age before undergoing gene-directed depletion of $PrP^C$.[229] When these mice are challenged with RML prions at 3–4 weeks of age, prion infection was established, but the depletion of $PrP^C$ at 12 weeks halted progression to the clinical phase of prion disease and reversed spongiosis.[229] Overall, these studies provide evidence that elimination of $PrP^C$, even after the establishment of prion infection, has potential as a therapeutic strategy.

Antisense oligonucleotides (ASOs) can modulate expression of $PrP^C$. Sequence-specific ASOs lower levels of $PrP^C$ and $PrP^{Sc}$ in ScN2a cells.[165] When sequence specific ASOs were administered to mice via either i.p. or intracerebroventricular (ICV) routes, ASOs were well tolerated and resulted in a decrease in *Prnp* mRNA levels in the brain compared to PBS negative controls.[165,166] When ASOs were administered prophylactically or at time of inoculation with the RML prion strain, there was diminished $PrP^C$ expression and a corresponding reduction in $PrP^{Sc}$ in the brains of ASO-treated mice compared to PBS controls. Reduction of $PrP^{Sc}$ levels correlated to a prolongation of the incubation period of disease, but animals ultimately succumbed to prion disease.[165,166] When ASOs are administered just prior to the onset of clinical signs of disease, this resulted in many of the animals rapidly succumbing to the toxic effect of ASO administration.[166] Encouragingly, the mice that survived the ASO treatment had a delayed onset of clinical disease as well as an extended clinical duration of disease that was three times longer than saline treated controls.[166] Overall, ASOs can delay the onset of prion disease whether given prophylactically or after the establishment of infection and, one issues of toxicity are addressed, may be an attractive method of depressing $PrP^C$ expression to treat prion diseases.

Treatments that alter the expression of $PrP^C$ on the cell surface can reduce prion conversion. For example, a group of chalcones and oxadiazoles can directly bind to $PrP^C$ and decrease its cell surface expression arresting $PrP^C$ trafficking to the endoplasmic reticulum resulting in a decrease in $PrP^{Sc}$ abundance and aggregation in prion-infected N2a cells.[167,168]

Alternatively, some of these compounds prevent conversion of $PrP^C$ to $PrP^{Sc}$ by binding to and stabilizing $PrP^C$ or directly binding to $PrP^C$ at the seeding surface.[168,184,185]

Alteration of post translational processing of $PrP^C$ can inhibit prion formation. $PrP^C$ can undergo either α- or β-endoproteolytic cleavage resulting in C1 and C2 fragments, respectively.[230] The C2 amino acid sequence approximately aligns with that of the $PrP^{Sc}$ protease resistant core, and has been found in abundance in prion infected brains.[231–237] The production of proteolytic cleavage products with varying pathogenic propensities is similar to what is observed of amyloid precursor protein, where APP cleavage is central to the development of AD.[238] Processing of APP by the enzymes β-secretase and γ-secretase leads to production of the peptides Aβ-40 and Aβ-42. While Aβ-40 has neuroprotective qualities,[239,240] Aβ-42 has been implicated in AD pathogenesis.[241,242] Endopeptidase cleavage of α-synuclein results in aggregation and toxicity that ultimately causes PD.[243] Pharmacological alteration of cellular proteolytic cleavage of the precursor proteins is an attractive druggable target. For example, a drug that preferentially inhibits the production of the C2 fragment would be a viable anti-prion treatment as the unfavorable $PrP^C$ cleavage product (C2) would be sequestered while the C1 fragment would be preserved and able to continue to perform a protective function.[244] The metalloprotease, ADAM10, plays a role $PrP^C$ shedding from the cell surface, but has also been suggested to contribute the α-cleavage of $PrP^C$.[232,245–249] Inhibition of ADAM10 resulted in an earlier onset of prion disease and slower spread to other brain regions in mice as a result of $PrP^C$ sequestration, suggesting ADAM10 may cleave newly formed $PrP^{Sc}$ from the surface of infected cells.[247,250] These data suggest that a therapeutic that increases $PrP^C$ shedding, instead of inhibiting post-translational processing of $PrP^C$, may be more effective at dampening disease progression.

Monoclonal antibodies (mAb) directed against $PrP^C$ can inhibit prion conversion. Several mAbs (D18, 6H4, ICSM 18, 31C6, etc.) are able to abrogate prion conversion and cure prion-infected N2a cells.[171,172,176–180,251] In prion-infected N2a cells, the inhibition of prion conversion depends on the binding affinity between the mAb and $PrP^C$ at the cell surface, with the most potent inhibitors often binding the α-helical domain of PrP.[174,176,177] Inhibition of $PrP^{Sc}$ formation by antibodies in cell culture has been proposed to occur via several mechanisms. The antibodies D18 and 6D11 can abolish prion conversion by binding to $PrP^C$ at the cell surface and hindering $PrP^{Sc}$-templated conversion.[171,177] Similarly, treatment of prion-infected N2a cells with mAb 44B1 retains $PrP^C$ at the cell surface in an antigen–antibody complex rendering it unavailable for conversion resulting in a significant decrease of $PrP^{Sc}$ levels.[139] A group of N-terminal monoclonal antibodies (DE10, DC2, EB8, and EF2) are hypothesized to directly bind to a region on $PrP^C$ that has been shown to tightly bind $PrP^{Sc}$, effectively blocking the $PrP^C$-$PrP^{Sc}$ interaction.[186] Antibodies that disrupt $PrP^C$ metabolism or trafficking either by preventing internalization of $PrP^C$ or reducing the half-life of $PrP^C$, result in a decrease in $PrP^{Sc}$ in cell culture models.[176,178,251] Although the aforementioned antibodies were reported to have no effect on cell growth or induce cellular toxicity, there are reports of anti-PrP antibodies (e.g., POM1) that are neurotoxic.[181,252–255] Anti-PrP antibodies that are toxic tend to bind epitopes in the globular domain of PrP and may trigger similar pathogenic cell signaling pathways as prion disease.[181,254]

Monoclonal antibodies directed against $PrP^{C}$ can alter prion pathogenesis and delay the onset of clinical signs of prion infection. ICSM 18, when administered to mice either intraperitoneal (i.p.) or i.c. 7 or 30 days after i.p. inoculation with RML was found to decrease $PrP^{Sc}$ levels in the spleen and delay the onset of clinical signs of prion infection compared to untreated controls.[175] However, treatment was ineffective when administered at the onset of clinical signs or if mice were i.c. inoculated with RML.[175] Treatment of mice infected with the Chandler strain of murine-adapted scrapie with mAb 31C6 120 dpi led to an increase in the incubation period, a decrease in $PrP^{Sc}$ levels, and delayed progression of neuropathological lesions in the cerebellum.[201] Creating $PrP^{0/0}$ mice expressing the 6H4 antibody as a transgene (*Prnp*$^{0/0}$-6H4μ) led to these mice producing anti-PrP titers in sera.[173] Crossbreeding of *Prnp*$^{0/0}$-6H4μ mice with C57BL/6 mice led to re-introduction of one or two PrP alleles, generating *Prnp*$^{+/0}$-6H4μ and *Prnp*$^{+/+}$-6H4μ mice, respectively, which developed anti-PrP serum levels.[173] Following intraperitoneal inoculation of the RML strain of scrapie, *Prnp*$^{+/0}$ 6H4μ mice lacked deposition of $PrP^{Sc}$ in the spleen or the brain, whereas *Prnp*$^{+/0}$ mice had deposition in both tissues.[173] Expression of the 6H4 antibody as a transgene drastically delayed prion pathogenesis in mice.[173] Overall, anti-PrP antibodies can reduce or eliminate $PrP^{Sc}$ formation in cells and animals and provides proof of principle of the efficacy of this approach.

Vaccines that result in host induced production of antibodies that recognize $PrP^{Sc}$ or $PrP^{C}$ have been attempted. Vaccination of mice or white-tailed deer with attenuated *Salmonella* expressing PrP resulted in significant prolongation of onset of clinical disease in the vaccinated group with complete protection in a portion of the animals as determined by the absence of $PrP^{Sc}$ deposition; however, these studies involved a relatively small sample size.[256,257] Vaccines consisting of peptides derived from the primary amino acid sequence of the prion protein resulted in a humoral immune response to all peptides and delayed the onset of clinical signs of prion infection compared to control prion-infected mice.[258] Other studies have focused on protein epitopes specific to $PrP^{Sc}$ with strong induction of antibody responses after vaccination of mice, sheep, and white-tailed deer, strengthening the possibility of an effective $PrP^{Sc}$-specific vaccine.[259–262] In contrast to the previous studies, vaccinating elk to induce a antibody response against $PrP^{Sc}$ resulted in a significant shortening of incubation period of CWD.[263] The acceleration in disease onset could be attributed to antibody-mediated misfolding of $PrP^{C}$ or by promoting the uptake of $PrP^{Sc}$ in the gut.[263] Since $PrP^{C}$ is a host-encoded protein, development of an effective vaccine directed immune response is impeded by host tolerance. While many approaches have aimed to overcome tolerance, there are negative consequences of inducing an antibody response to a host-encoded cellular protein. The effects of antibodies directed at the globular domain of $PrP^{C}$ results in varying degrees of neurotoxicity while other $PrP^{C}$-specific antibodies lead to apoptosis and improper signaling cascades.[181,253,264] Vaccination strategies have successfully induced strong humoral and mucosal immune responses in multiple animal models; however, more work is required to evaluate the most effective vaccination strategy to prevent disease onset while avoiding toxicity.

## 5. Future prospects

Development of ultra-sensitive methods of $PrP^{Sc}$ detection have greatly improved and may lead to routine detection of prion infection in asymptomatic individuals. Protein misfolding cyclic amplification (PMCA) and real time quaking induced conversion (RT-QuIC) can detect a single infectious prion particle.[265,266] Both *in vitro* methods can detect $PrP^{Sc}$ in urine, feces, blood, saliva, and cerebrospinal fluid (CSF).[267–272] PMCA detects $PrP^{Sc}$ from vCJD patient blood and urine samples, while RT-QuIC is currently being used for the diagnosis of sCJD from CSF and nasal mucosa swab samples.[267,269–271,273] Advancements in *in vitro* techniques have enabled reliable preclinical detection in experimental prion disease. PMCA can detect prions in the blood or skin from pre-clinical prion-infected mice and hamsters.[274–276] The sensitivity of RT-QuIC can detect prions in the blood of prion-infected preclinical hamsters and deer at timepoints as early as 5 days and 1 month post inoculation, respectively.[277,278] Like $PrP^{Sc}$, Aβ, tau, and α-synuclein are present in biological fluids making these *in vitro* techniques promising for preclinical diagnosis.[279–283] Overall, preclinical diagnosis of prion diseases will provide a larger window for therapeutic treatments and consequently a greater likelihood of success. Challenging this is the presence of multiple prion strains. Strain-specific efficacy of anti-prion drugs or vaccines can result in emergence of resistant strains that may be more pathogenic or have a different host range compared to the original dominant strain. These challenges are reminiscent of those faced by bacterial, fungal, and viral drug development and a successful anti-prion therapy may require a multidrug approach. The unique nature of prions, however, provides an additional therapeutic target, namely the precursor protein. Treatments that target the prion precursor protein can inhibit prion formation and may reverse pathological changes. A combinational approach that treats the prion form and the precursor protein will likely be needed to effectively treat the broad spectrum of prion diseases.

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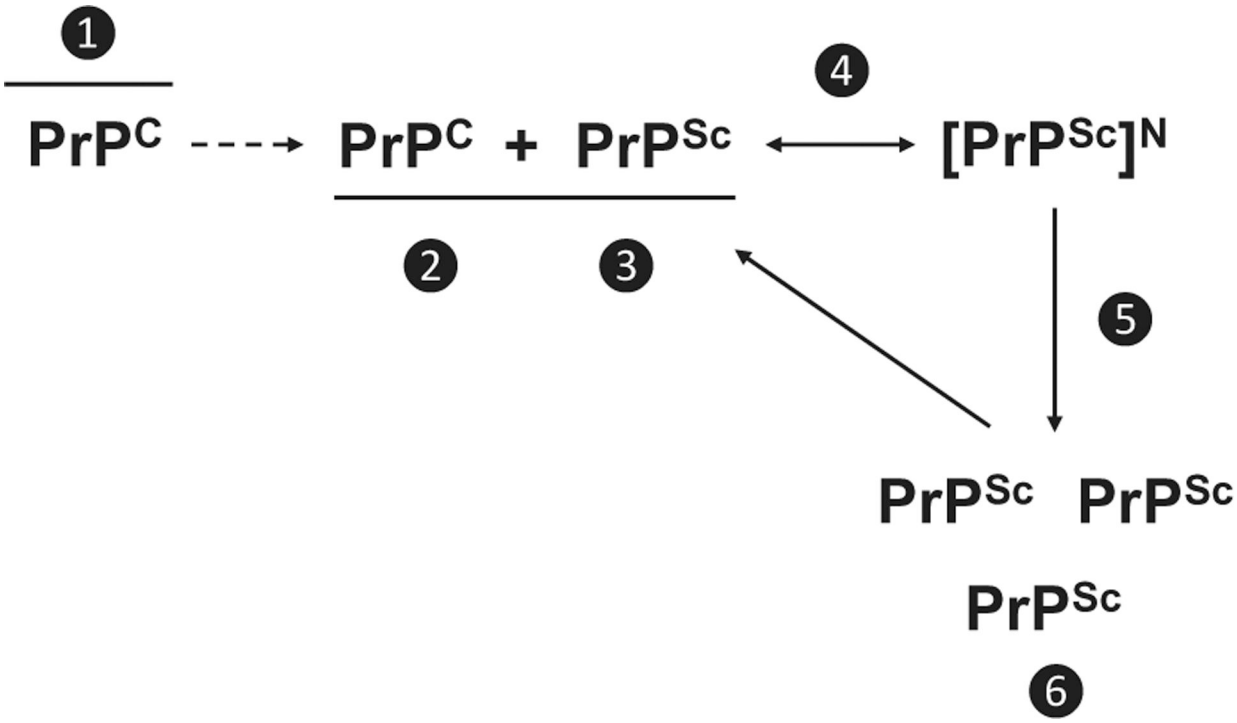


**Fig. 1.**

Mechanistic location of therapeutic targets for PrP prion diseases. Therapeutic targets: ❶ $PrP^{C}$ post-translational processing, metabolism, cellular trafficking, or localization; ❷ $PrP^{C}$ by binding or inducing conformational change that prevents $PrP^{C}$ interaction; ❸ $PrP^{Sc}$ by stabilization, redistribution; ❹ conversion process by blocking binding site on $PrP^{C}$, capping growth, inhibition of cofactor interactions, or ❺ promoting fragmentation or ❻ clearance from the host.

**Table 1**

Anti-prion therapeutics.

| Drug | Type | Model | Efficacy | Suggested mechanism | Strain Specificity | References |
|---|---|---|---|---|---|---|
| *$PrP^{Sc}$ target* | | | | | | |
| Congo Red | Sulfonated amyloid dye | Neuronal cell culture, hamster | Decreases $PrP^{Sc}$ accumulation and inhibits propagation in culture, prolongs mean incubation period *in vivo* | Stabilizes $PrP^{Sc}$, inhibits GAG-PrP binding and potentially $PrP^{C}$-$PrP^{Sc}$ binding | 127S, Sc237, PrP (27–30), 263K, 139H | 127–131 |
| LIN5001, LIN7002, LIN5044 | Luminescent conjugated polythiophenes (LCPs) | Mouse | IV infusion prolongs survival, reduces infectivity | Aggregate hyperstabilization | RML6, 263K | 132,133 |
| Compound B | 4-pyridinecarboxaldehyde, 2-[4-(5-oxazolyl)phenyl] hydrazone | Neuronal cell culture, mouse | Eliminates $PrP^{Sc}$ in culture; prolongs incubation period/life, reduces PrP deposition, large quantity needed for efficacy | Binds to abnormal PrP, inhibits new formation of abnormal PrP | RML, 22L (marginally), Fukuoka-1 GSS, 263K (barely) | 134,135 |
| anle138b | 3,5-diphenyl-pyrazole (DPP) | Neuronal cell culture, mouse | Antiprion activity *in vitro* and *in vivo*, substantial prolongation of survival (txt after clinical onset), good bioavailability, BBB penetration | Directly blocks $PrP^{Sc}$ amplification, causes shift towards smaller $PrP^{Sc}$ oligomer size, inhibits pathological aggregation ($PrP^{Sc}$ specific) | RML, ME7, 301c, sCJD, vCJD, a-syn | 136–138 |
| U18666A | Cholesterol synthesis inhibitor | Neuronal cell culture | Significantly decreased PrP-res levels | Redistributes $PrP^{Sc}$ to late endosome-lysosome, degradation of $PrP^{Sc}$ in secondary lysosomes | 22L | 139 |
| Pentosan Polysulfate (PPS) | Cysteine protease inhibitor | Neuronal cell culture, mouse, human | IV infusion prolonged survival even when administered after abnormal PrP deposition, rapidly and significantly decreased $PrP^{Sc}$ levels, various efficacy in humans | May act as a competitive coreceptor (with endogenous GAG or other proteoglycans) for PrP at cell surface or causes fragmentation of $PrP^{Sc}$ at the cell surface | 263K, RML, Fukuoka-1 GSS, 22L, iCJD, vCJD | 139–147 |
| Heterologous protein | Hamster PrP | Neuronal cell culture, mouse | Decreases PrP-res in culture, slows disease progression, increases survival, significantly delays onset of clinical signs, decreases $PrP^{Sc}$ accumulation in brain/spleen | Either binds to $PrP^{Sc}$ creating a functionally impotent aggregate unable to produce additional $PrP^{Sc}$ or binds and blocks the conversion site from $PrP^{C}$ | RML-Chandler | 148–151 |
| IND24, IND81, IND114338, IND125, IND126461 | 2-aminothiazoles | Neuronal cell culture, mouse | Strain-specific and administration time-dependent extension in survival, decreased & altered distribution of $PrP^{Sc}$ in brain, can lead to drug resistance (e.g. IND24-resistant RML) | Inhibits formation of new $PrP^{Sc}$, reduces $PrP^{Sc}$ load via drug-like mechanism, might promote clearance of $PrP^{Sc}$ | RML, ME7, 22L, CWD | 138,152–156 |
| Amphotericin B | Fungizone (polyene antibiotic) | Neuronal cell culture, mouse, hamster, human | Administration time-dependent prolongation of incubation period, delays onset of clinical signs without improvement of neurological symptoms or prolongation of clinical disease, delays accumulation of$PrP^{Sc}$ in the brain | Potentially could: interact with $PrP^{C}$/$PrP^{Sc}$, interfere with uptake of $PrP^{Sc}$ into cells, target detergent-resistant microdomains (modify cell surface distribution and/or trafficking of PrP), regulate microglial/glial activation, | 263K, CJD | 140,157–164 |

| Drug | Type | Model | Efficacy | Suggested mechanism | Strain Specificity | References |
|---|---|---|---|---|---|---|
| | | | | directly bind and either “cap” amyloid growth or overstabilize $PrP^{Sc}$ | | |
| *$PrP^{C}$ Target* | | | | | | |
| 771, active ASO 1, active ASO 2 | Antisense oligonucleotide | Neuronal cell culture, mouse | Reduces $PrP^{C}$/$PrP^{Sc}$ in cell culture and in vivo, delays onset of clinical signs, slows disease progression, toxicity issues when administered after prion inoculation | Decreased $PrP^{C}$ levels slow accumulation of $PrP^{Sc}$ | RML, potentially more since $PrP^{C}$ is the target | 165,166 |
| J1, J8, J20, J35 | Chalcones | Neuronal cell culture | Decreases $PrP^{Sc}$ levels, completely blocks PrP109–149 aggregation | Directly binds to $PrP^{C}$ (C-terminal), decreases amount of $PrP^{C}$ on cell surface (arrest in ER), may stabilize $PrP^{C}$ or bind seeding surface | RML, 22L, CWD, 263K, CJD | 167,168 |
| Y13, Y17 | Oxadiazole | Neuronal cell culture | Could not totally block conversion but Increased lag phase for conversion; minor decrease in PrP-res formed in RT-QuIC | Direct binding to $PrP^{C}$ (stabilize $PrP^{C}$ or bind at seeding surface), potentially interacts with $PrP^{C}$/$PrP^{Sc}$ N-terminal region, decrease amount of $PrP^{C}$ on cell surface causing arrest in ER | RML, 263K, CJD | 167,168 |
| Fe(III)-TMPyP | Cationic tetrapyrrole | Neuronal cell culture, mouse | Inhibits replication in vitro/cell culture, prolongs survival, inhibits amplification of $PrP^{Sc}$, inhibits cytotoxic effects of delta CR PrP | Interacts with C-terminal domain of $PrP^{C}$ (Helix-3), acts as pharmacological chaperone for $PrP^{C}$ (decreases prion-induced misfolding) | RML, 22L, bank vole strains (Italian, UK (SCR1), and CH1641) | 169,170 |
| D18 | Anti-recombinant PrP (29–231) antibody (residues 132–156; helix A) Fabs | Neuronal cell culture, mouse | Abolishes prion replication and clears existing $PrP^{Sc}$ in cell culture (2 week txt), prolongs incubation period and decreased titer in mice | Binds to $PrP^{C}$ on cell surface and hinders docking of $PrP^{Sc}$/a cofactor needed for conversion | Scrapie | 171 |
| 6H4 | Anti-murine $PrP^{C}$ antibody (Mab and expressed as transgene) | Neuronal cell | Prevents infection of susceptible cells, cures chronically infected cultures, conferred anti-PrP titers ($Prnp^{0/0}$ mice) w/o induction of autoimmune disease, no infectivity detected ($Prnp^{+/0}$-6H4u mice) when challenged, prevents or drastically delays pathogenesis | Occludes $PrP^{C}$ at prion replication sites, captures and degrades (immune-mediated) incoming $PrP^{Sc}$ inoculum, steric competition with template-directed refolding, interference with seeded $PrP^{Sc}$ nucleation reaction | RML | 172,173 |
| ICSM 18 | Isotype IgG1, anti-murine alpha-PrP antibody (residues 146–159) | Neuronal cell culture, mouse | Reduces splenic PrPSc levels, increases survival, passive transfer of Abs had no effect late in incubation period | Direct inhibition of $PrP^{Sc}$ production, binding may bury “active residues” and stabilize helix 1 | RML | 174,175 |
| 110 | Monoclonal anti-PrP antibody (PHGGGWG at aa 59–65 and aa 83–89; octarepeat region) | Neuronal cell culture | Reduced $PrP^{Sc}$ accumulation (dose-dependent) in cell culture, long term txt increased $PrP^{C}$ levels | Retains $PrP^{C}$ on cell surface (may interfere with $PrP^{C}$ metabolism), mAb-$PrP^{C}$ binding inhibits $PrP^{C}$-$PrP^{Sc}$ interaction by occupying binding domain or through steric interference | Chandler (139A), RML | 176 |
| 6D11 | Monoclonal antibody, recognizes $PrP^{C}$, $PrP^{Sc}$, and recPrP (epitope between 93–109) | Neuronal cell culture | Complete abrogation of $PrP^{Sc}$, no re-emergence for at least 14 days, (following removal of txt), pre-incubation prevents infection | May prevent $PrP^{C}$-$PrP^{Sc}$ interaction or interfere with binding auxiliary molecules needed for prion propagation | 22L | 177 |

| Drug | Type | Model | Efficacy | Suggested mechanism | Strain Specificity | References |
|---|---|---|---|---|---|---|
| 7H6 | Monoclonal Ab, recognizes residues 130–140 | Neuronal cell culture | Complete abrogation of $PrP^{Sc}$, no re-emergence for at least 14 days, (following removal of txt) | Similar to 6D11? | 22L | 177 |
| 7A12 | Monoclonal Ab, recognizes residues 143–155 (alpha-helix A) | Neuronal cell culture | Complete abrogation of $PrP^{Sc}$, no re-emergence for at least 14 days, (following removal of txt), weakest preventative effect | Similar to 6D11? | 22L | 177 |
| SAF34 | Monoclonal Ab, recognizes residues 59–89 (HuPrP) | Neuronal cell culture | Dose-dependent decrease of $PrP^{Sc}$ levels, increased inhibition when used in conjunction with SAF61, re-emergence of $PrP^{Sc}$ following removal of txt | Decreases half-life of $PrP^{C}$, antibodies act on $PrP^{C}$ or $PrP^{Sc}$ isoforms, antibodies prevent $PrP^{C}$-$PrP^{Sc}$ interaction | 22L | 178 |
| SAF61 | Monoclonal Ab, recognizes residues 144–152 (HuPrP) | Neuronal cell culture | Dose-dependent decrease of $PrP^{Sc}$ levels, increased inhibition of $PrP^{Sc}$ when used in conjunction with SAF34, no re-emergence of$PrP^{Sc}$ following removal oftxt | Decreases half-life of $PrP^{C}$, antibodies act on $PrP^{C}$ or $PrP^{Sc}$ isoforms, antibodies prevent $PrP^{C}$-$PrP^{Sc}$ interaction, increase clearance/degradation of $PrP^{C}$, modulate cellular trafficking of PrP, induce conformational change exposing PrP to protease attacks | 22L | 178 |
| PrioV3 | Camelid anti-PrP antibody (residues between 171 and 190, YYR motif) | Neuronal cell culture, *endo*-thelial cells, mouse | Crosses BBB *in vitro and in vivo*, abrogates $PrP^{Sc}$ replication in cells, permanent depletion of $PrP^{Sc}$ in cells, marked inhibition of$PrP^{Sc}$ accumulation in spleen | Alters $PrP^{C}$ expression (direct neutralizing effect on $PrP^{C}$/$PrP^{Sc}$), could block $PrP^{C}$ incorporation into infectious $PrP^{Sc}$ | RML | 179,180 |
| 44B1 | Monoclonal antibody | Neuronal cell culture | Rapidly and significantly decreased $PrP^{Sc}$ levels | Retains $PrP^{C}$ on cell surface as an antigen-antibody complex, interferes with internalization and trafficking of $PrP^{C}$ to endocytic compartments | 22L | 139 |
| POM2 | Anti-PrP antibody to flexible tail of $PrP^{C}$ | Mouse COCS | Prevented prion-mediated neurodegeneration but prion titer was not decreased | Induces shift in distribution of$PrP^{Sc}$ moieties without affecting overall quantity | RML | 181 |
| LD7, JZ107 | Phenethyl piperidine | Neuronal cell culture | Reduced $PrP^{Sc}$ by 50%, permanently cured RML-infected cells (1 month exposure), protects against dendritic spine loss in presence of $PrP^{Sc}$ | Might interact with $PrP^{C}$ in a cellular context, perhaps in conjunction with other cell-surface receptors, may interact with $PrP^{C}$ substrate | RML, 22L | 182 |
| Alprenolol hydrochloride | B-adrenergic blocker | Neuronal cell culture, mouse | Reduced levels of $PrP^{Sc}$ in cells and brains of infected mice, inhibited $PrP^{Sc}$ accumulation and spongiform changes but did not prolong survival | May inhibit via interaction with $PrP^{C}$ | Fukuoka-1 | 183 |
| R12, R24, R12-A-R12 | RNA aptamer | Neuronal cell culture | Significantly reduces $PrP^{Sc}$ levels in cell culture | Tightly binds to and stabilizes $PrP^{C}$, blocking conversion to $PrP^{Sc}$ | Fukuoka-1 | 184,185 |
| DE10, DC2, EB8, EF2 | Monoclonal antibodies to N-terminal region of $PrP^{Sc}$ | Neuronal cell culture | Promoted complete clearance of$PrP^{Sc}$ in cell culture | Binds to a region on $PrP^{C}$ that $PrP^{Sc}$ also binds, blocking $PrP^{C}$-$PrP^{Sc}$ interaction | RML | 186 |
| *$PrP^{C}$/$PrP^{Sc}$* | | | | | | |

| Drug | Type | Model | Efficacy | Suggested mechanism | Strain Specificity | References |
|---|---|---|---|---|---|---|
| Chlorpromazine (CPZ) | Phenothiazine | Neuronal cell culture | No to modest effect on $PrP^{Sc}$ accumulation, inhibits prion replication in cells but not *in vitro* | Decreases $PrP^{C}$ at cell surface by inhibiting clathrin-mediated endocytosis, redistributes $PrP^{Sc}$ to late endosome-lysosome | sheep scrapie 127S, Sc237, Type 1 CJD, RML, 22L | 129,139,187 |
| Quinacrine | Lysosomotropic agent | Neuronal cell culture, mouse, humans | Inhibits cell growth at concentrations greater than 2.0 μM, no prolongation effect in mice and humans, may lead to drug resistance | Competitive inhibition of GAG-PrP interaction, unfolding of PrP-res, destablilization of PrP-res conformation, induces conformational change that disfavors $PrP^{Sc}$ conformation | Scrapie, 263K, vCJD, sCJD, iatrogenic CJD, genetic CJD | 140,188–193 |
| E-64d | Cysteine protease inhibitor | Neuronal cell culture, mouse | No toxicity to cell growth at [ ] up to 100 μM, no prolongation effect | Competitive inhibition of GAG-PrP interaction, unfolding of PrP-res, destabilization of PrP-res conformation | Scrapie, 263K | 140,190 |
| MS-8209 | Amphotericin B derivative | Neuronal cell culture, mouse, hamster | Prolongs incubation period (dose- and timing dependent), delays onset of PrP-res/GFAP accumulation, vacuolation, spongiosis, and astrogliosis in brain, variable efficacy across prion species | Potentially affects conversion, may interact with astrocyte lysosomal system and limit propagation of PrP at inoculation site | Sheep scrapie 127S, 139A, Sc237 (weaker with 139H), type 1 CJD, C506M3 (similar to ME7), 263K | 129,194–200 |
| 31C6 | Monoclonal anti-PrP antibody (residues 143–149), IgG1 | Neuronal cell culture, mouse | Reduces $PrP^{Sc}$ in cell culture (dose-dependent), no re-emergence following txt, mAb txt at 120 dpi increased survival (not statistically significant), slowed weight loss, disease progression, and accumulation of$PrP^{Sc}$ in brain | Direct inhibition of $PrP^{C}$-$PrP^{Sc}$ interaction by occupying binding domains, could interfere with $PrP^{C}$ metabolism by retaining $PrP^{C}$ on cell surface | Chandler (139A), RML | 176,201 |
| Doxycycline | Antibiotic | Neuronal cell culture, human | Variably affected PrP-res accumulation in culture, variable prolongation of survival, reduction in widespread & severe lesions (early txt) | Destabilize abnormal PrP, could operate at cell level by modulating formation of PrP aggregates | CJD (sporadic and genetic–E200K or V210I), sCJDMM1, sCJDVV2a, vCJDMM2b, iCJDMM1 | 202–206 |
| *Other* | | | | | | |
| GSK2606414 | Protein kinase RNA-like ER kinase (PERK) inhibitor | Mouse | Oral treatment reversed cognitive deficits and prevented clinical disease, effective pre-and post-symptomatic, halted progression in spongiform degeneration, protected from neuronal loss | Prevents activation of UPR branch that mediates prion neurotoxicity by inhibiting PERK | RML | 207 |