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**Towards Therapy of Human Prion Diseases**

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## Abstract

Three decades after the discovery of prions as the cause of Creutzfeldt-Jakob disease and other transmissible spongiform encephalopathies, we are still nowhere close to finding an effective therapy. Numerous pharmacological interventions have attempted to target various stages of disease progression, yet none has significantly ameliorated the course of disease. We still lack a mechanistic understanding of how the prions damage the brain, and this situation results in a dearth of validated pharmacological targets. In this review, we discuss the attempts to interfere with the replication of prions and to enhance their clearance. We also trace some of the possibilities to identify novel targets that may arise with increasing insights into prion biology.

## Prions and prion diseases

Prion diseases, or transmissible spongiform encephalopathies (TSEs), are caused by the ordered aggregation of $PrP^{Sc}$, a misfolded version of the cellular prion protein $PrP^{C}$. Because similar mechanisms are operative in many other neurodegenerative and systemic diseases, the protein aggregates causing the latter were termed “prionoids” (1; 2). By our definition, prionoids operate similarly to prions at the molecular level, but have not (yet) been shown to be transmissible from one individual to another. In the case of prion disease, the aggregation is self-sustaining and therefore transmissible between individuals – which renders TSEs infectious (3-6). These diseases comprise Creutzfeldt-Jakob disease, Kuru, Fatal Familial Insomnia and genetic TSEs in humans (7); the latter are caused by mutations in the *PRNP* gene encoding $PrP^{C}$. Neuropathologically, nonspecific signs (astrogliosis, neuronal loss, amyloid deposition) are accompanied by “spongiform” changes; intraneuronal and intraneuritic vacuoles occasionally containing degenerating organelles (8). The spongiform degeneration of neurons is highly specific to prion diseases and typically allows for a definitive diagnosis.

The presence of cellular prion protein is not only necessary for the de novo generation of prions, but also for the host organism to experience prion-related neurotoxicity (9). Mice ablated for $PrP^{C}$ do not acquire the disease after exposure to prions. The availability of $PrP^{C}$ seems to be rate-limiting, as prion-infected mice containing only single allele of the *Prnp* gene encoding $PrP^{C}$ develop the disease much later than wild-type mice.

Prion diseases are rare, with 1.5-2 reported cases per million people per year. Yet they are invariably fatal, and currently there is no effective treatment. Identification of any potential anti prion therapy could also pave way for treatment of misfolding disorders induced by prionoids. Here we focus on the therapeutics that in our opinion may have the potential to succeed and on the challenges awaiting them.

## Targeting prion conversion

The cellular $PrP^{C}$ is expressed on the plasma membrane, where it is sorted into detergent resistant membrane domain (10). The presence of certain mutations may induce $PrP^{C}$ to adopt, through poorly understood mechanisms, a pathological and ultimately infectious conformation leading to disease (11). Ordered $PrP^{Sc}$ aggregates can seed the nucleation of further prions. $PrP^{Sc}$ can assume a broad range of compositions ranging from large insoluble aggregates and plaques to small oligomers (6).

Polyanionic compounds and amyloidotropic dyes can abrogate the conversion of $PrP^{C}$ to $PrP^{Sc}$ *in vitro* (12; 13), but could not be translated into therapies due to toxicity, poor pharmacokinetics and low efficacy (14; 15). Treatment of prion-infected neuroblastoma cells with branched polyamines resulted in clearance of $PrP^{Sc}$ (16). The~~re~~ compounds are protonated at acidic pH and may act on prion conversion in endosomes and lysosomes (16). However, none of these compounds had any beneficial effect in vivo.

Dendrimers are synthetic molecules composed mainly of branched polyamines ~~that~~ with modifiable end groups (17). Phosphorous dendrimers were effective anti-prion agents *in vitro* and significantly cleared $PrP^{Sc}$, yet were not developed further (18). Pentosan polysulfate (PPS) prolonged the survival of prion-infected mice and was thought to interfere with the conversion of $PrP^{C}$ to $PrP^{Sc}$, but did not have any reproducible effect in prion-affected humans (19; 20). Amantadine, originally used prophylactically against influenza virus was suggested to have ameliorated the clinical course of CJD in a variety of reports with anecdotal survival times of up to several years after first symptoms have occurred (21). Other reports however failed to reproduce beneficial effects of amantadine in CJD patients (22; 23). Another antiviral drug, acyclovir, was ineffective in two patients suffering from CJD (24; 25) as was interferon in a case series of two patients (26). Flupirtine, an aminopyridine commonly used as analgetic, was used in a placebo-controlled, double-blind study that suggested amelioration of cognitive deficits in sCJD and gCJD despite of unchanged survival times (27).

Quinacrine is an antiprotozoal drug that was approved in the 1930s as an antimalarial agent and - at the vCJD epidemic at the last turn of the millennial - the lack of efficient anti-prion compounds urged researchers to recruit patients for clinical studies (28). An open compassionate trial of quinacrine in 30 sCJD and two vCJD patients did not significantly prolong the survival or ameliorate the functional impairments and neither did it show beneficial effects on brain pathology (29). PRION-1, a prospective, patient-preference trial of quinacrine also failed to significantly prolong the survival or improve cognitive deficits in the 107 patients enrolled (30). The study conductors hypothesized that low levels of the drug in the cerebrospinal fluid may be responsible for the failure to reach clinical endpoints, although application of the drug showed an overall acceptable safety profile (30). Since

compassionate-use trials cannot detect small effects of a molecule due to the lack of a placebo arm, a randomized, double-blind, placebo-controlled trial of quinacrine in sCJD patients was performed thereafter (31). 51 patients were eventually included for functional and survival analyses. Although patients in the quinacrine arm performed slightly better in terms of functional scores during the early treatment course, no survival benefit was observed upon quinacrine administration, leading to its elimination as a prion disease therapeutic (31).

We have reported the generation of reactive oxygen species to be a downstream effector of prion-induced neurotoxicity and administration of antioxidants such as acetylated hydroxy tyrosol effectively extended the survival of prion-diseased mice. One case report suggested beneficial effects of neurological disease in a CJD patient that received a complex mixture of antioxidants amongst others vitamin E and alpha lipoic acid although he succumbed to disease 22 months after onset of symptoms (32).

A pilot compassionate use trial of doxycycline showed beneficial effects on patient survival independent of age, gender and codon 129 polymorphism of the *PRNP* gene (33). In a first of its kind multicentric, prospective, placebo-controlled and randomized Phase II study doxycycline did not show superiority in the first interim analysis when compared to placebo and the study was stopped (34). Although the latter study provided class 1 evidence that doxycycline does not extend survival in prion disease patients, a subsequently published case report suggested an unusually long survival time (>5 years) of a patient suffering from variably protease-sensitive prionopathy (VPSPr) who was treated with doxycycline (35). These reports indicate the helplessness of clinicians who prescribe anti-prion drugs despite their proven lack of efficiency. Compound B, IND24 and anle138b were amongst other compounds that showed no effect against human prions (34).

These disappointing results reported above raise questions on the viability of strategies to identify prion therapeutics. In particular, it has become evident that cell culture models are poorly predictive of effectiveness in vivo. A plausible reason lies in the fact that it is generally difficult to maintain prion infectivity in immortalized, continuously growing cells. For such cultured cells to remain chronically infected with prions, replication would have to be at least as rapid as cell division: a negative differential would inevitably result in loss of infectivity over time. In addition, prion replication may impose a fitness cost on infected cells, resulting in non-infected cells (which may arise because of inhomogeneously infected cultures or due to acquired resistance) overgrowing the system (36). These characteristics lead us to predict that infected cell cultures are inherently unstable systems – a prediction verified experimentally by the observation that most chronically infected cell lines spontaneously gravitate towards lower infectivity titers over time (our unpublished observations). One could therefore argue that the crucial issue with N2A cells is not how to cure them, but rather how

to maintain them in an infected state. In this interpretative frame, it is not surprising that agents capable of “curing” cultured cells almost always prove entirely ineffective when tested in vivo, where brain-resident cells undergo much slower turnover rates and spontaneous resistance has hardly any chance to develop (Table 1).

Cerebellar organotypic cultures slices (COCS) seem to represent a more realistic system for testing antiprion compounds, and are indeed gathering a much better record than N2A cells in predicting in vivo antiprion efficacy (37-42). The main disadvantage of COCS over N2A cells, however, consists in the laboriousness of their production, which precludes their feasibility for high-throughput screens. Instead, COCS are best suited as secondary screens for intermediate validation of compounds identified in vitro.

One key consideration in designing an effective therapeutic option is to consider the “frangibility” (i.e. the propensity to break) of the pathological protein aggregates. Prion aggregate frangibility is the most important parameter in determining prion infectivity; these predictions from first principles were largely confirmed in animal models (40). By using “β-sheet breaking” compounds that convert large aggregates into a larger number of small oligomers, one might inadvertently create more propagons (43). It follows that maybe an effective therapy should not aspire at breaking down $PrP^{Sc}$ aggregates, but rather at hyperstabilizing said aggregates (Figure 1). Luminescent conjugated polythiophenes (LCPs) appear to act as such prion hyperstabilizers. LCPs bind to cross-β spines in $PrP^{Sc}$ (44; 45). LCPs can detect $PrP^{Sc}$ aggregates with great sensitivity and their emission spectra can differentiate between different amyloids (45) and prion strains (46).

LCPs reduce prion infectivity in samples containing prion aggregates from brains of infected mice (47). The binding of the LCPs to amyloid fibrils was resolved at the atomic level and was found to rely on cooperative electrostatic interactions. However, digestion with proteinase K, which is a proxy for fibril stability, was enhanced by LCPs, whereas infectivity of the same prion preparation was decreased dose-dependently (47). These observations are consistent with the hypothesis that LCPs indeed decrease the infectivity of prions by hyperstabilizing $PrP^{Sc}$ aggregates. The structural elucidation of the interaction between LCPs and amyloid allowed us to design new LCPs with stronger binding. These showed higher efficacy in prolonging the survival of prion-infected mice (48).

## Targeting cellular pathways for prion therapy

The ubiquitin-proteasome system maintains quality control in cells by degrading misfolded or damaged proteins (49). Early studies revealed the presence of ubiquitin within protein aggregates (50; 51). An elevated level of ubiquitinated proteins in the brains of prion-infected mice is associated with a dysfunctional ubiquitin-proteasome system (UPS) (52) which may contribute to neurotoxicity. $PrP^{Sc}$ can bind to the external leaflet of the 20S proteasomal

subunit and may impair its function (53). Other studies have postulated that prion oligomers inhibit the catalytic B subunit or prevent the substrate entry into the proteolytic core (54). These hypotheses may explain the failure of proteasomes in prion infections (52; 55), but it is difficult to conceive how PrP, which resides in the lumen of the endoplasmic reticulum (ER) and in the extracellular space, could be driven to encounter proteasomes in the cytosol. The idea that PrP undergoes conspicuous endoplasmic-reticulum-associated protein degradation (ERAD) is plausible (56; 57) but has been challenged (45).

Inhibition of USP14, a deubiquitinase attached to the 19S proteasome subunit, results in clearance of aggregation-prone proteins (58). A small molecule targeting USP14 accelerates the degradation of proteins associated with neurodegenerative diseases such as TDP43, tau and ataxin. A yet unexplored strategy targeting misfolded proteins in neurodegenerative diseases is generating small molecule compounds, which direct the endogenous E3 ubiquitin ligases to their substrates. PROTACS (proteolysis targeting chimeric molecules) consists of a peptide which recognizes a specific ubiquitin ligase chemically linked to a small molecule that recognizes the target protein (59). Once bound to the target protein, it creates spatial proximity between the substrate and ubiquitin ligase, promoting poly-ubiquitination and enhanced degradation of the target substrate. Extensive studies were carried out to characterize PROTACS for cancer treatment (60) and perhaps they can be utilized to target misfolded prions. A new strategy involves a combination of chaperone proteins and small molecule compounds. The small molecule acts as a guide to the substrate whereas the chaperone engages with the misfolded proteins and renders it amenable to proteasomal degradation (61). Such a strategy was implemented in spinal-bulbar muscular atrophy and amyotrophic lateral sclerosis (62).

### Targeting the unfolded protein response

A common event in protein misfolding disorders is the upregulation of unfolded protein response (UPR), also referred to as endoplasmic reticulum (ER) stress (63). Over 30% of all cellular proteins traverse the ER before being modified and disseminated to their final destinations. The ER controls a complex set of cellular processes by which proteins are synthesized, folded and postranslationally modified (64). Disturbances in the function of the ER affecting may lead to the accumulation of misfolded proteins or alteration in calcium homeostasis, resulting in the induction of stress.

The UPR can restore cellular proteostasis by shutting down global translation and thereby reducing the load of misfolded proteins in the ER (65). Also, the UPR enhances the synthesis of chaperones and other proteins that assist the protein folding to repair the misfolded proteins in the ER (66). The misfolded ER proteins can be retrotranslocated to the cytosol where they are degraded by the ERAD pathway (67). The major transducers of UPR are

Protein kinase RNA-like ER kinase (PERK), inositol-requiring enzyme-1 (IRE1) and activating transcription factor-6 (ATF6). PERK is a transmembrane protein essential for the attenuation of the translation by phosphorylation the eukaryotic translation initiation factor (eIF2α), whereas IRE1 and ATF6 are mainly involved in the synthesis of chaperones necessary for protein folding (68).

Elevated levels of the ER chaperones GRP94, GRP78 and GRP54 were observed in prion-infected humans and mice (69). Prion infection leads to disruption in the calcium homeostasis in the cell affecting the ER (70); cells exposed to purified $PrP^{Sc}$ displayed activation of UPR and calcium release from the ER along with the upregulation of chaperones identified in CJD patients (70). Furthermore, there exists a complex interplay between UPS and ER stress and it is widely believed that inhibition of proteasomal function elicits UPR (71).

Prion-infected mice show sustained activation of PERK and phosphorylation of eIF2α resulting in down-regulation of global protein translation through eIF2α phosphorylation (72; 73), leading to decreased synaptic proteins and neuronal death. Overexpression of the eIF2α specific phosphatase GADD34 rescues synaptic defects and neuronal loss, at least for a while (73). Pharmacological inhibition of PERK restores translation and provides some neuroprotection (73). The ISR inhibitor B (ISRIB), which targets the translational inhibition downstream of eIF2α, was also shown to ameliorate prion pathology (74).

In contrast to PERK inhibitors, Guanabenz and its derivative Sephin 1 prevented neurodegeneration in a mouse ALS model by interfering with GADD34 and enhancing the phosphorylated status of eIF2α (75). This long-term translational arrest may prevent synthesis of new propagons and thereby provide neuroprotection. Guanabenz has previously been shown to enhance prion clearance (76), yet severe side effects have restricted its use so far. This problem illustrates the basic conundrum of UPR-based therapies: interfering with a general control mechanism of translation is inevitably ridden with deleterious, unintended consequences (Figure 1).

### Targeting Lysosomal degradation and autophagy

The conversion of $PrP^{C}$ to $PrP^{Sc}$ occurs at the plasma membrane (77) and in the endocytic pathway, including recycling endosomes and multivesicular bodies (78; 79). The accumulation of misfolded prions in the endocytic compartments may alter the composition of the vesicular compartments and their functioning. Lysosomes are the major sites for degradation of cellular $PrP^{C}$, and $PrP^{Sc}$ can accumulate in lysosomes (80). In cell cultures $PrP^{Sc}$ can be cleared by lysosomes, however other defects arising in the endolysosomal machinery and $PrP^{Sc}$ overload may ultimately render lysosomes nonfunctional. Indeed, prion

infection results in reduced levels of membrane-bound rab7, affecting lysosomal maturation and their capacity to degrade proteins (81).

Another key delivery route of $PrP^{Sc}$ to lysosomes for degradation is autophagy (82). In autophagy the cytosolic constituents are engulfed by a double membrane structure, the autophagosome, which fuses with lysosomes releasing its contents for degradation. Giant multivesicular bodies and autophagy vesicles (AV) are observed in neurons of prion-infected mice, in prion-infected cell cultures, and in genetic prion models (83). Autophagy may play a protective role by scooping up aggregates and delivering them for degradation. It was originally believed that spongiform vacuoles observed in prion diseases are AVs, yet they do not have the membrane characteristics of AVs, nor do they display any autophagy markers. Impairing autophagy pharmacologically or by siRNA inhibits the capacity of cells to degrade $PrP^{Sc}$ (84). Hence, promoters of autophagy and lysosomal degradation could be therapeutic against prions (85; 86). Lithium has been shown to enhance the clearance of $PrP^{Sc}$ in cultured cell lines by inducing autophagy (87), and also slightly reduced cellular $PrP^{C}$ levels. Rapamycin and tacrolimus, which also promote autophagy, showed similar results (88; 89).

Trehalose is an alpha linked disaccharide synthesized by fungi and plants to protect them against environmental stress conditions by preventing protein denaturation. In cell culture, trehalose induces autophagy and may improve the clearance of misfolded proteins (90). $PrP^{Sc}$ from prion infected cell cultures was rapidly cleared by treatment with trehalose (91). Similarly, imatinib, another autophagy promoting compound, abolished $PrP^{Res}$ levels in cell cultures (92).

### Chaperone therapy

Molecular chaperones interact with other proteins and assist them in attaining a stable conformation. They represent an important quality control system that prevents misfolding and aggregation. In yeast the Hsp104 disaggregase can solubilize cytosolic aggregates of Sup35, the yeast prion Ψ (93). A triad of Hsp110/70/40 was identified as a mammalian minimal disaggregase (94). Upregulation of Hsp70 alone afforded neuroprotection in model systems (95). However, it is unclear whether this triad could be exploited therapeutically against prion diseases.

Chemical chaperones are small molecules that bind to proteins and restore their function by refolding them and letting them attain a stable structure. In spite of their non-specific mode of action and low affinity, their ability to eliminate protein aggregates makes them attractive as therapeutics. Methyl amines and glycerol have been effective in blocking the conversion of $PrP^{C}$ to $PrP^{Sc}$ in cell culture models (96). Also anthracyclines, porphyrins and diazo dyes were also effective in blocking prion replication in the *in vitro* assays (97), yet in vivo results were discouraging.

## Active immunotherapy against prion disease

Immunization strategies have shown promise in various protein misfolding disorders (98). Active immunization against prions is hindered by the widespread expression of the cellular prion protein $PrP^{C}$ in the body, leading to self-tolerance. Immunization with small prion fragments designed to fit into known grooves of major histocompatibility complex II (MHC-II) binding pockets elicited anti-$PrP^{C}$ immunity and antibodies derived thereof reduced Proteinase K-resistant $PrP^{Sc}$ levels in a prion-infected tumor transplant (99). Active immunization of mice with recombinant prion protein in mice delayed prion disease when the immunogen was administered prophylactically and, to a lesser extent, when animals were already infected (100). Clinical disease induced through orally administered prions was attenuated after vaccination of mice (101; 102) and deer (103). However, another report failed to show differences in disease susceptibility through prophylactic prion vaccination in deer suffering from chronic wasting disease (CWD), a prion disease of deer and elk (104). A modest disease delay was achieved after immunization with recombinant prion protein fragments and intraperitoneal prion inoculation (105; 106). Attempts to break self-tolerance using a combined DNA and protein vaccination regime yielded mixed results (107; 108).

Because the immune system is tolerant to self-antigens, antibodies derived from immunizations often lack the affinity needed for effective therapy. Addressing the molecular whereabouts of $PrP^{C}$ self-tolerance, one study found that even small amounts of extraneuronal $PrP^{C}$ abolished an efficient immune response (109). A delay in disease onset was achieved by Freund's adjuvant, suggesting a benefit through an unspecific activation of the immune system (110). Another study suggested a strongly neuroprotective effect through *post-hoc* immunostimulation against prions using repetitive administration of CpG oligodeoxynucleotides (CpG-ODN) that are suggested to stimulate innate immunity (111). A chronic CpG-ODN treatment, however, was shown to induce profound immunosuppression with lymphoid follicle destruction, hepatotoxicity and hemorrhagic ascites (112). Moreover, repetitive immunization of mice and increases their susceptibility to peripherally induced prion disease through reduced prion clearance and/or size of follicular dendritic cells (FDCs) networks suggesting that individual immune states, e.g. hyperactivated or depressed, may predispose to prion disease vulnerability (113).

## Passive immunotherapy against the prion protein

The first proof-of-concept for prion immunotherapy demonstrated a reduction in prion infectivity through exposure of cell-free, purified prions with PrP-specific antisera (114). Passive prion immunotherapy through diminishing $PrP^{Sc}$ levels *in vitro* was exhibited when

the monoclonal anti-PrP antibodies 6H4, SAF32, SAF61 or Fab-fragments of the PrP-specific antibodies D13, D18, R1 and R2 were given to chronically prion infected N2A neuroblastoma cells (115-118). When D13 was given as a bivalent antibody (D13-IgG), widespread neuronal apoptosis was observed suggesting neuronal death through cross-linking of $PrP^{C}$, a finding that was not seen with the holo-IgG molecule of D18 (119). When a single-chain fragment (scFv) of D18 was engineered into the adeno-associated virus 9 (AAV9) vector and transduced into RML-infected mice brains, prolonged survival of inoculated mice was observed (120). The toxic effects of D13 were reproduced in a second study (41).

Transgenic overexpression of an $IgM^{a}$ µ chain of the anti-PrP antibody 6H4 reduced prion infectivity and levels of $PrP^{Sc}$ in prion-infected mice (121), and peripheral injections with the monoclonal anti-PrP antibodies 8B4, 8H4 and 8F9 led to a decrease in clinical disease onset (122), as were injections with the PrP-α1 helix targeting antibody 31C6 (123). 31C6 was reported to be protective against prion disease when given as late as clinical signs had already manifested, albeit through intraventricular application (124).

The safety profile of the anti-PrP antibodies ICSM18 and ICSM35 is highly controversial (125). One report stated no drug-related toxicity in mice of both compounds after stereotaxic injections of 2 µg of antibody (126). However, in a dose-escalation study with ICSM18, the allegedly safe dosage of 2 µg of antibody already showed drug-attributable neurotoxic effects raising concerns about the suitability of ICSM18 to clinical trials (127). Of note, POM1 is a monoclonal antibody directed against a similar epitope as ICSM18 shows severe neurotoxicity *ex vivo* and *in vivo* (39; 41; 128; 129).

Human autoantibodies recognizing the mutant prion fragment $PrP^{A117V}_{106\text{-}126}$ from commercially available, pooled immunoglobulins were proposed to be protective against $PrP^{A117V}_{106\text{-}126}$-induced neuronal death *in vitro* through microglial uptake of the mutated fragment (130; 131). However $PrP^{A117V}_{106\text{-}126}$ does not exist in nature, and therefore such speculations are implausible.

When the octapeptide repeat domain of the prion protein (OR) at its N-terminus was targeted in prion-infected mice through intraventricular delivery of the anti-$PrP^{C}$-OR antibody 4H11, no disease amelioration was observed (132). Instead, 4H11-injected animals showed behavioral deficits and heightened neuronal loss and astrogliosis (132). Because the N-terminus of PrP is crucial for neurotoxicity, (39; 41), it will be important to investigate the differences between 4H11 and -which was shown to be safe when given intracerebrally in doses up to 6 µg (41).

## Targeting the peripheral replication and neuroinvasion of prions

Genetic blockade of B-cell maturation ablated the onset of prion disease after peripheral prion inoculation (133). In the light of these findings one might speculate that pharmacological ablation of B-cells, e.g. through the anti-CD20 antibody rituximab, could

afford post-exposure prophylaxis. Initial prion accumulation occurs in secondary lymphoid organs prior to neuroinvasion (134) whereas other prion strains, so-called “neurotropic” prions, can primarily invade the central nervous system without the need of peripheral replication (135). Early studies have argued for the requirement of mature $PrP^{C}$-expressing follicular dendritic cells (FDCs) for prion neuroinvasion: ablation of differentiated B-cells prevented peripheral scrapie pathogenesis due to the lack of FDC maturation signals secreted by B-cells (133) and mice lacking either expression of $PrP^{C}$ on mature FDCs or lacking mature FDCs did not succumb to peripherally initiated prion disease (136). As FDCs depend on lymphotoxins and tumor necrosis factor from B-cells for development and maintenance, they provide an opportunity to target prion replication (137). Administration of a hybrid protein consisting of lymphotoxin $\beta$ receptor and human immunoglobulin (LT$\beta$R-Ig) dedifferentiated FDCs through inhibition of the lymphotoxin $\alpha/\beta$ pathway and led to a delay of prion disease upon peripheral inoculation (138), even when LT$\beta$R-Ig was given late during the disease course – but not upon intracerebral inoculation (139). Dedifferentiation of FDCs through a single injection of soluble human TNF receptor linked to the Fc portion of human immunoglobulin IgG1 (huTNFR:Fc) also showed a decreased disease susceptibility to peripherally adminstered prions (140).

FDCs trap immune complexes through binding to Fc$\gamma$ receptors. They also bind opsonized antigens via the complement receptors CD21/CD35. Pharmacological and genetic ablation of the complement factor C3 or its receptor CD21/CD35 prolonged incubation times in peripherally prion-inoculated mice (141; 142). Hence, complement activation through $PrP^{Sc}$ may lead to more FDC-bound $PrP^{Sc}$ and hence favor prion replication. Neither membrane-bound nor secreted immunoglobulins did alter prion neuroinvasion (142). Circulating immune complexes bound to $PrP^{Sc}$ may not play a role in prion pathology since deletion mutants of a variety of Fc$\gamma$ receptors had no effect on prion incubation times (142).

The sympathetic nervous system (SNS) innervates secondary lymphoid organs and experimental evidence pointed towards an involvement of the SNS in prion pathogenesis as splanchnic nerves are an early replication site after peripheral prion inoculation (143) and accumulation of prions was found in sympathetic and sensory ganglia as well (144). A transient pharmacological ablation of the SNS through injection of 6-hydroxydopamine (6-OHDA) or anti-nerve-growth factor antibodies (anti-NGFAb) led to delayed scrapie onset after peripheral inoculation (145).

In a study addressing the cells responsible for conveying prions to the gut-associated lymphoid tissue (GALT) after oral exposure, microfold cells (M-cells) (146), specialized epithelial cells, were depleted through application of a monoclonal antibody against receptor activator of NF-$\kappa$B ligand (RANKL) (147). M-cell depletion led to a reduced prion uptake into

FDCs without modifying FDC status and prevented disease onset after oral prion exposure (147).

### Therapies against prion disease in humans

To date, no clinical trial against prion diseases has succeeded. One inherent limitation of this rare disease concerning double-blind, randomized controlled multicenter trials on large patient groups is the low prevalence of prion diseases. Rare diseases are less likely to be funded through industry, and indeed a systemic review found only one out of seven trials in CJD to have an industrial sponsor in contrast to an overall average of three out of four industry-sponsored studies (148; 149).

Due to the lack of a prion disease-specific disease rating scale, initial clinical studies were performed using cognitive test batteries not specifically designed to address prion disease phenotypes (27) or using survival as outcome measure (150). Limited sample sizes and heterogeneous endpoints lead to therapeutic interventions being published as case reports. Yet case reports are intrinsically flawed by publication bias: an exceptional treatment success is more likely to be published than a treatment failure. Extension of the endpoint-based primary outcome, i.e. survival, in prion disease trials towards neuropsychological, psychiatric and other functional ranking systems may improve power calculations for future trials (151).

### Palliative care in prionopathies

With currently no effective therapy available against prion diseases, all medical care is essentially supportive and palliative. Primarily, nursing efforts are laid out to keep the patients safe, i.e. by providing walking assistance through walkers and wheelchairs and – during the terminal stage – a hospital bed with regular skin and mouth care and assistance during food intake (152). Specifically, pyrexia, i.e. broad variations in body temperature was suggested to be a common symptom which, if left untreated, may lead enhanced agitation that could be alleviated through the use of fans and tepid sponge baths (153). Further distressing symptoms that need to be addressed carefully are myoclonic jerking, heightened sensory sensitivity, shortness of breath, incontinence and constipation (153).

Strict preventive measures in agriculture and in human medicine have reduced the incidence of variant Creutzfeldt-Jakob's disease to a near complete disease extinction (154). Although the current WHO Tables on Tissue Infectivity Distribution in Transmissible Spongiform Encephalopathies (155) does not formally deem blood or urine from non-vCJD diseased patients as infectious, some reports suggest detectable infectivity of urine from sCJD patients and transmissibility of blood from human gPrD patients to primates (156; 157). Hence,

preventive actions have to be followed by all personnel working with non-vCJD prionopathies as well, including but not limited to wearing appropriate protective gear and gaining knowledge about the relative infectivity of different human tissues (158). On another note, as CJD patients need both palliative and mental health care, it was suggested that the development of multidisciplinary guidelines can improve patient care through the development of sophisticated treatment schemes (159).

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**Figure Legends**

**Figure 1:** Upon encountering a prion propagon, the cellular $PrP^{C}$ is converted and incorporated into $PrP^{Sc}$. The conversion of $PrP^{C}$ to $PrP^{Sc}$ probably begins on the plasma membrane and continues throughout the endocytic pathway. PrP aggregates were also observed in the cytoplasm and may originate through ERAD and/or leakage from defective endosomes and lysosomes. Potential therapeutic points of intervention include prion clearance (antibody therapy) and prion replication, including hyperstabilization of aggregates. Intracellular targets include enhancers of autophagy and of lysosomal function, as well as modulators of the UPS and chemical chaperones.

**Figure 2:** Immunotherapy is quickly evolving as an attractive therapeutic strategy against prion disease. The monoclonal antibody POM2 binds to a degenerate epitope in the octoapeptide repeat region of $PrP^{C}$ and protects against prion-induced neurodegeneration. The red dots indicate the N terminus of prion protein, also called the flexible tail (FT), which is intrinsically disordered. The ordered globular domain (GD) of $PrP^{C}$ is represented in magenta. The interaction between a $F(ab)_1$ fragment of the POM2 antibody (cyan and grey structures) and its cognate epitope on $PrP^{C}$ (depicted in cyan) is visualized in the blow-up. Solid lines (blue) indicate the interactions.