Perspective

Recent developments in antibody therapeutics against prion disease

Karl Frontzek and Adriano Aguzzi

Institute of Neuropathology, University of Zurich, Zurich, Switzerland

Correspondence: Karl Frontzek (karl.frontzek@usz.ch) or Adriano Aguzzi (adriano.aguzzi@usz.ch)

Preclinical evidence indicates that prion diseases can respond favorably to passive immunotherapy. However, certain antibodies to the cellular prion protein PrP\text{C} can be toxic. Comprehensive studies of structure–function relationships have revealed that the flexible amino-terminal tail of PrP\text{C} is instrumental for mediating prion toxicity. In a first-in-human study, an anti-prion antibody has been recently administered to patients diagnosed with sporadic Creutzfeldt–Jakob’s disease, the most prevalent human prion disease. Moreover, large-scale serosurveys have mapped the prevalence of naturally occurring human anti-prion autoantibodies in health and disease. Here, we provide a perspective on the limitations and opportunities of therapeutic anti-prion antibodies.

Prions are self-propagating proteins leading to spongiform encephalopathies, progressive degenerative diseases of the central nervous system such as Creutzfeldt–Jakob’s disease (CJD) in humans and bovine spongiform encephalopathy (BSE) in cattle [1]. The pathogenic agent, the scrapie prion protein PrP\text{SC}, replicates by a repetitive cycle of growth and fragmentation [2]. PrP\text{SC} and its cellular counterpart PrP\text{C} share the identical amino acid sequence and are encoded by the prion protein gene \textit{Prnp}. \textit{Prnp} knock-out mice are resistant to prion toxicity [3], and genetic or pharmacological interventions leading to reduced levels of PrP\text{C} are sufficient to delay prion disease incubation times [4].

Prion disease research gained momentum in the mid-1990s after the BSE epizootic when predominantly young males were affected by new variant CJD which was suspected to be transmitted from BSE-infected livestock [1]. The prion-like spread of pathologically misfolded proteins was demonstrated in a variety of other neurodegenerative diseases such as Alzheimer’s and Parkinson’s disease [5] but no but therapy is available to date.

Recently, progress has been made in the research of therapeutic and diagnostic anti-prion antibodies, which we will discuss in this concise review. Polyclonal prion protein antiserum from rabbits raised against purified PrP\text{SC} reduced prion titers [6]. Transgenic expression of \textit{\mu} chains of anti-PrP\text{C} antibody 6H4 in prion-infected mice showed reduced prion attack rates and provided the first evidence of neuroprotection against prions in vivo [7]. This finding has been replicated by others [8] and a wide range of anti-PrP\text{C} antibodies and variants thereof were developed subsequently [9]. Antibody efficacy is epitope-dependent: antibodies targeting the flexible tail of PrP\text{C} (PrP\text{C}-FT) are neuroprotective while those targeting the globular domain (PrP\text{C}-GD) can induce neurodegeneration [10]. \textit{Bona fide} prion neurotoxicity is also contingent on the flexible tail of PrP\text{C} (PrP\text{C}-FT) since interstitial deletion mutants of PrP\text{C}-FT show a delayed and milder prion disease phenotype [11].

What is the possible mechanism of protective anti-prion antibodies? If PrP\text{SC} is the causal agent and PrP\text{C} is its receptor, then lowering levels of either PrP\text{C} or PrP\text{SC} by passive anti-prion immunotherapy should counteract prion neurotoxicity, whereas increased amounts of PrP\text{SC} will have dire consequences. Indeed, a subset of antibodies against PrP\text{C}-FT was reported to accelerate PrP\text{C} degradation which in turn led to reduced accumulation of PrP\text{SC} [12]. On the other hand, stabilization of ordered PrP\text{SC} aggregates is the proposed mechanism of action of polythiophenes, which were found to be potent anti-prion anionic compounds [13]. POM2, a neuroprotective anti-PrP\text{C}-FT antibody effective...
against toxic anti-PrPC-GD antibodies, toxic PrP\(^{\text{Sc}}\) mutants and prions, led to increased amounts of higher-order PrP\(^{\text{Sc}}\) aggregates [10,14]. Metabolic pathways deranged by prion infection, but restored by anti-prion immunotherapy, include reactive oxygen species and the unfolded protein response [9].

Antibody therapeutics have shown great potential in pre-clinical models of prion diseases [9] as well as in other neurodegenerative conditions such as Alzheimer’s disease [15] and Parkinson’s disease [16]. PRN100 is a humanized version of the anti-PrPC-GD antibody ICSM18 and was given as first-in-human, compassionate use therapy in subjects diagnosed with CJD. However, the results of this study are still undisclosed [17,18]. Two independent research groups have reported the PrP C-dependent toxicity of ICSM18 in vitro and in vivo [19,20]. Aducanumab is a fully human antibody against beta-amyloid which was derived from an anonymized library of B-cells from healthy elderly individuals and is currently a promising lead candidate against Alzheimer’s disease notwithstanding contradictory results from recent phase III clinical trials [21,22]. Low blood–brain barrier permeability is generally considered an unconquerable roadblock for the delivery of anti-bodies to the brain. In the case of Aducanumab, the successful crossing of the blood–brain barrier was established by dose- and time-dependent reduction in beta-amyloid [21]. Coupling of therapeutic antibodies with antibodies against endothelial surface receptors (‘brain shuttles’) elicits receptor-mediated transport of the therapeutic compound and enhanced brain uptake [23]. Overall, there is compelling evidence that human-derived antibodies show favorable pharmacological properties through reduced immunogenicity as induced by non-human motifs [24]. Naturally occurring human autoantibodies against misfolded proteins have been reported for beta-amyloid [25], SOD1 [26] and an artificial fragment of the prion protein [27] and others. Lacking a genuinely biological correlate, the physiological relevance of the mutated 21-mer PrPC-FT fragment from the latter study remains, however, unclear.

Mouse monoclonal anti-PrPC-GD antibodies such as POM1 cause severe neurodegeneration reminiscent of prions but do not propagate infectivity [28]. We have speculated that individuals carrying anti-PrPC-GD auto-antibodies may develop autoimmune encephalitis similar to other autoimmune encephalopathies where auto-antibodies against central nervous system surface proteins wreak havoc in the brain [29]. On the other hand, naturally occurring anti-PrP\(^{\text{Sc}}\)-FT autoantibodies could be exploited as anti-prion therapeutics. In an unselected, large hospital cohort of over 35 000 patients resulting in almost 50 000 blood samples, we did not find a

---

### Table 1. Summary of currently available treatment strategies against prions

| Compound group | Examples | Mode of action |
|----------------|----------|----------------|
| Anti-PrP\(^{\text{Sc}}\) antibodies | 6H4 [7], POM2 [10], PRN100/ICSM18 [18] | Lowering of PrP\(^{\text{Sc}}\) levels, inhibition of engagement of PrP\(^{\text{Sc}}\) flexible tail |
| Active immunization | Recombinant prion protein [33,34] | Generation of protective anti-PrP\(^{\text{Sc}}\) autoantibodies |
| Antioxidants | N-acetylcysteine [14], isoascorbate [14] | Scavenging of reactive oxygen species |
| Compounds targeting the unfolded protein response (UPR) | GSK2606414 [35] | Inhibition of protein kinase RNA-like endoplasmic reticulum kinase (ePERK) and prevention of UPR-induced translational repression |
| Inhibition of peripheral replication/neuroinvasion | Genetic ablation of B-cells [36], lymphotoxin \(\beta\)-receptor/immunoglobulin-hybrid [37] | Inhibition of prion replication in B-cells and follicular dendritic cells |
| Others/indirect effects | Doxycycline [38], flupirtine [39], quinacrine [40] | Lowering of PrP\(^{\text{Sc}}\) levels, anti-apoptotic, anti-inflammatory |
| Prion hyperstabilizers | Luminescent conjugated polythiophenes [13] | Stabilization of pathological prion aggregates |
| PrP\(^{\text{C}}\) stabilizers | dimethylsulfoxide (DMSO) [41], glycerol [41], trehalose [41] | Prevention of generation of de novo aggregates |
| Suppression of prion protein gene expression | Genetic knockout [3], antisense oligonucleotides [42] | Prion disease susceptibility correlates with PrP\(^{\text{C}}\) expression levels |
| Stimulators of autophagy | Lithium [43], tacrolimus [44], rapamycin [45] | Promotion of PrP\(^{\text{Sc}}\) degradation |
significant association between the presence of plasma anti-PrP<sup>C</sup> autoantibodies and specific pathologies [30]. Comparative sequence analyses of variable immunoglobulin fragments, however, showed an overlap between therapeutic monoclonal anti-PrP<sup>C</sup> antibodies and naturally occurring autoantibodies in publicly available immunological repertoires [30]. One might wonder whether the presence of protective naturally occurring autoantibodies is responsible for delayed clinical manifestations in individuals harboring germline mutations of the human prion protein gene PRNP. These individuals usually do not experience neurological symptoms until high age despite the presence of a pathogenic prion protein mutation [31]. A case-control study of PRNP mutation carriers and their PRNP wild-type family members, however, did not support this hypothesis but reinforced our previous results that anti-PrP<sup>C</sup> autoantibodies are not linked to specific conditions [32].

Besides antibodies, randomized clinical trials against human prion diseases were conducted using flupirtine, quinacrine and doxycycline but were unsuccessful [9]. We have summarized the most important therapeutic anti-prion compounds in Table 1. Other promising alternatives to antibodies involve rationally designed luminiscence conjugated polymers that act as hyperstabilizers of pathological prion protein aggregates [13]. Antisense oligonucleotides (ASOs) can reduce protein expression by targeted mRNA degradation and Prnp-binding ASOs given by intracerebroventricular injection extended the life span of prion-infected mice [42]. Due to its low prevalence and lack of prodromal markers, clinical phase III trials involving several hundred symptomatic individuals are not feasible in human prion disease. Current efforts are focused on pre-symptomatic trials in genetic prion disease individuals which might lead to adequately powered trials [46].

Despite intensive investigations, antibodies against PrP<sup>C</sup> have not yet yielded therapeutic success in human prions diseases. As more clinical trials against neurodegenerative diseases are underway, making the results accessible in the public domain will maximize their impact on transitional research. Identification of therapeutic, naturally occurring autoantibodies from large unselected patient cohorts and pooled human B-cell libraries may become transformative. Innovative immunoglobulin modifications such as bispecific [47] or intracellular antibodies [48] will yield antibodies with enhanced pharmacological properties.

Competing Interests
The authors declare no competing interests.

Author Contributions
K.F. and A.A. conceptualized, wrote, reviewed and edited the manuscript.

Abbreviations
ASOs, antisense oligonucleotides; BSE, bovine spongiform encephalopathy; CJD, Creutzfeldt–Jakob’s disease.

References
1. Aguzzi, A. and Falsig, J. (2012) Prion propagation, toxicity and degradation. Nat. Neurosci. 15, 936–939. https://doi.org/10.1038/nn.3120
2. Knowles, T.P., Waudby, C.A., Devlin, G.L., Cohen, S.I., Aguzzi, A., Vendruscolo, M. et al. (2009) An analytical solution to the kinetics of breakable filament assembly. Science 326, 1533–1537. https://doi.org/10.1126/science.1178250
3. Bueler, H., Aguzzi, A., Sailer, A., Greiner, R.A., Autenried, P., Aguet, M. et al. (1993) Mice devoid of PrP are resistant to scrapie. Cell 73, 1339–1347. https://doi.org/10.1016/0092-8674(93)90360-3
4. Aguzzi, A. and Frontzek, K. (2020) New paradigms of clinical trial design for genetic prion diseases. Lancet Neurol. 19, 284–285. https://doi.org/10.1016/S1474-4422(20)30029-6
5. Scheckel, C. and Aguzzi, A. (2018) Prions, prionoids and protein misfolding disorders. Nat. Rev. Genet. 19, 405–418. https://doi.org/10.1038/s41576-018-0011-4
6. Gabizon, R., McKinley, M.P., Groth, D. and Prusiner, S.B. (1988) Immunoaffinity purification and neutralization of scrapie prion infectivity. Proc. Natl. Acad. Sci. U.S.A. 85, 6617–6621. https://doi.org/10.1073/pnas.85.18.6617
7. Hoppen, F.L., Musahl, C., Arrogi, L., Klein, M.A., Rulicke, T., Oesch, B. et al. (2001) Prevention of scrapie pathogenesis by transgenic expression of anti-prion protein antibodies. Science 294, 178–182. https://doi.org/10.1126/science.1063093
8. White, A.R., Enever, P., Taeabi, M., Mushens, R., Linehan, J., Brandner, S. et al. (2003) Monoclonal antibodies inhibit prion replication and delay the development of prion disease. Nature 422, 80–83. https://doi.org/10.1038/nature01457
9. Aguzzi, A., Lakkananj, A.K.K. and Frontzek, K. (2018) Toward therapy of human prion diseases. Annu. Rev. Pharmacol. Toxicol. 58, 331–351. https://doi.org/10.1146/annurev-pharmtox-010617-052745
10. Sonati, T., Reimann, R.R., Falsig, J., Baral, P.K., O’Connor, T., Hornemann, S. et al. (2013) The toxicity of antiprion antibodies is mediated by the flexible tail of the prion protein. Nature 501, 102–106. https://doi.org/10.1038/nature12402
11. Flechsig, E., Shmerling, D., Hegyi, I., Faibler, A.J., Fischer, M., Cuzzio, A. et al. (2000) Prion protein devoid of the octapeptide repeat region restores susceptibility to scrapie in PrP knockout mice. Neuron 27, 399–408. https://doi.org/10.1016/S0896-6273(00)00046-5
Karapetyan, Y.E., Sferrazza, G.F., Zhou, M., Ottenberg, G., Spicer, T., Chase, P. et al. (2013) Unique drug screening approach for prion diseases identifies tacrolimus and astemizole as antiprion agents. Proc. Natl. Acad. Sci. U.S.A. 110, 7044–7049 https://doi.org/10.1073/pnas.1303510110

Cortes, C.J., Qin, K., Cook, J., Solanki, A. and Mastrianni, J.A. (2012) Rapamycin delays disease onset and prevents PrP plaque deposition in a mouse model of Gerstmann–Straussler–Scheinker disease. J. Neurosci. 32, 12396–12405 https://doi.org/10.1523/JNEUROSCI.6189-11.2012

Vallabh, S.M., Minikel, E.V., Schreiber, S.L. and Lander, E.S. (2020) Towards a treatment for genetic prion disease: trials and biomarkers. Lancet Neurol. 19, 361–368 https://doi.org/10.1016/S1474-4422(19)30403-X

Bardelli, M., Frontzek, K., Simonelli, L., Hornemann, S., Pedotti, M., Mazzola, F. et al. (2018) A bispecific immunotweezer prevents soluble PrP oligomers and abolishes prion toxicity. PLoS Pathog. 14, e1007335 https://doi.org/10.1371/journal.ppat.1007335

Messer, A. and Butler, D.C. (2020) Optimizing intracellular antibodies (intrabodies/nanobodies) to treat neurodegenerative disorders. Neurobiol. Dis. 134, 104619 https://doi.org/10.1016/j.nbd.2019.104619