Recent developments in antibody therapeutics against prion disease

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Preclinical evidence indicates that prion diseases can respond favorably to passive immunotherapy. However, certain antibodies to the cellular prion protein PrPC can be toxic. Comprehensive studies of structure–function relationships have revealed that the flexible amino-terminal tail of PrPC 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 PrPSc, replicates by a repetitive cycle of growth and fragmentation [2]. PrPSc and its cellular counterpart PrP0 share the identical amino acid sequence and are encoded by the prion protein gene Prnp. Prnp knock-out mice are resistant to prion toxicity [3], and genetic or pharmacological interventions leading to reduced levels of PrP0 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 PrPSc reduced prion titers [6]. Transgenic expression of μ chains of anti-PrPC antibody 6H14 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 C antibodies and variants thereof were developed subsequently [9]. Antibody efficacy is epitope-dependent: antibodies targeting the flexible tail of PrP C (PrP C-FT) are neuroprotective while those targeting the globular domain (PrP C-GD) can induce neurodegeneration [10]. Bona fide prion neurotoxicity is also contingent on the flexible tail of PrP C (PrP C-FT) since interstitial deletion mutants of PrP C-FT show a delayed and milder prion disease phenotype [11].

What is the possible mechanism of protective anti-prion antibodies? If PrPSc is the causal agent and PrP C is its receptor, then lowering levels of either PrP0 or PrPSc by passive anti-prion immunotherapy should counteract prion neurotoxicity, whereas increased amounts of PrPSc will have dire consequences. Indeed, a subset of antibodies against PrP C-FT was reported to accelerate PrP C degradation which in turn led to reduced accumulation of PrPSc [12]. On the other hand, stabilization of ordered PrPSc 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 C-FT antibody effective...
against toxic anti-PrPC-GD antibodies, toxic PrPC mutants and prions, led to increased amounts of higher-order PrPSc 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 autoantibodies may develop autoimmune encephalitis similar to other autoimmune encephalopathies where autoantibodies against central nervous system surface proteins wreak havoc in the brain [29]. On the other hand, naturally occurring anti-PrPSc-FI 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

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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 luminescent 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 intra-cellular 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