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Review

Opportunities for Conformation-Selective Antibodies in Amyloid-Related Diseases

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Abstract: Assembly of misfolded proteins into fibrillar deposits is a common feature of many neurodegenerative diseases. Developing effective therapies to these complex, and not yet fully understood diseases is currently one of the greatest medical challenges facing society. Slow and initially asymptomatic onset of neurodegenerative disorders requires profound understanding of the processes occurring at early stages of the disease including identification and structural characterisation of initial toxic species underlying neurodegeneration. In this review, we chart the latest progress made towards understanding the multifactorial process leading to amyloid formation and highlight efforts made in the development of therapeutic antibodies for the treatment of amyloid-based disorders. The specificity and selectivity of conformational antibodies make them attractive research probes to differentiate between transient states preceding formation of mature fibrils and enable strategies for potential therapeutic intervention to be considered.

Keywords: antibody; amyloids; conformation; prion; Alzheimer's; Parkinson's; fibrils, tau; Huntingtin; protein misfolding

1. Introduction

Correct protein folding is crucial for maintaining healthy biological functions. It is a complex process, dependent on optimum rates of transcription and translation, which can be negatively influenced in an undesirable environment, such as extreme pH, ionic strength, and oxidative stress. Incorrectly folded

proteins normally follow one of two pathways: they can be salvaged by chaperones, which facilitate a refolding process or they can be degraded by proteases. Some misfolded proteins manage however to escape this rigorous cellular quality control system, leading to unwanted intra- or extracellular aggregation and eventually insoluble fibril formation [1–3]. A large body of literature has shown that the formation and abnormal accumulation of fibrillar deposits of different proteins is a common characteristic associated with a number of diseases including systemic amyloidosis [4], and neurodegenerative disorders such as Alzheimer's [5], prion [6,7], Parkinson's [8,9] and Huntington's diseases [10].

Neurodegenerative diseases are multifactorial debilitating disorders afflicting millions of people worldwide. The financial burden to health organisations associated with diagnosis and treatment of these devastating disorders is billions of dollars each year, and is forecasted to increase even further with an expanding aging population [11]. Despite an enormous research effort, there are no effective disease-modifying treatments of amyloid-based disorders. The currently available treatment strategies for neurodegenerative diseases are limited and aim to compensate for neuronal loss by increasing the level of neurotransmitters. For example, acetylcholinesterase inhibitors are administered to elevate the acetylcholine level in the cortex of Alzheimer's disease (AD) patients [12]. Treatment with L-DOPA is regarded to be much more successful compared to acetylcholinesterase inhibitors for AD patients. It aims to increase the dopamine levels in the brains of PD patients with early onset of the disease and has proved to be of great help for patients in the first 5–7 years of disease [13,14]. Unfortunately, these therapies can only offer temporary, symptomatic relief rather than addressing the disease at its core.

Intensive research developments carried out in past years have however brought scientists a few steps closer to a better understanding of the complex, multifactorial mechanisms underlying neurodegenerative diseases. It is well established that a common structural feature of all amyloid structures is high content of a specific β-sheet conformation, in which β-strands are oriented perpendicular to the main fibril axis [3,15]. The ability of a wide range of proteins, with no evident similarity in primary sequences, to promote amyloid formation presents a challenge in successfully targeting amyloid-related diseases, but also implies similar underlying mechanisms.

It has been established that fibrils are formed during a multistep process initiated by misfolded, monomeric proteins, which undergo several conformational transitions before they reach a mature fibril state [5,16]. It is becoming increasingly apparent that amyloid-forming proteins exist in a complex dynamic equilibrium between soluble monomeric, oligomeric and various insoluble higher-order conformers (see Figure 1).

Understanding this aggregation process requires the identification of intermediate states which are formed during the transition from native proteins to ordered oligomers and fibrils [8,17–19]. The identification of species prone to aggregation, followed by the determination of intermediate conformers of the proteins prior to their transition to fibrils is paramount, not only to understanding the pathology of the disease, but also to inform the choice of the correct protein conformation to target with effective therapy.

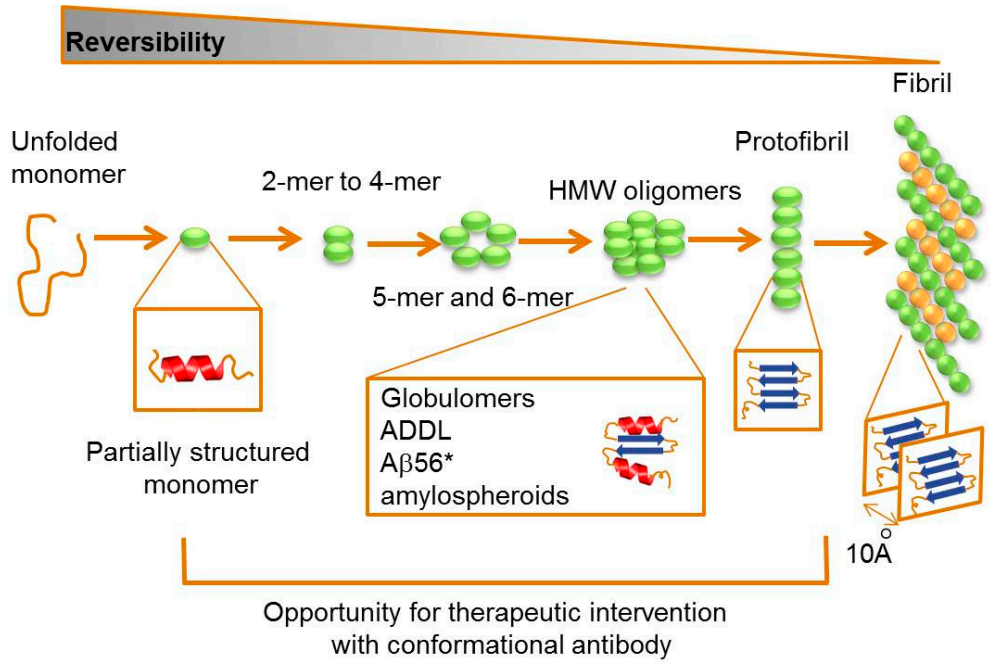

Figure 1. Schematic representation of the multistep aggregation process initiated by misfolded, monomeric proteins, which undergo several conformational transitions before they reach a mature fibril state. amyloid beta-derived diffusible ligands (ADDLs), Aβ*56, globulomers, amylospheroids, "tAβ" (toxic soluble Aβ) represents known intermediate High Molecular Weight (HMW) toxic species of Aβ.

Significant conformational differences observed between native, oligomeric and fibril forms raise the possibility to discriminate between these states with conformation-sensitive antibodies. Such antibodies are attractive because they are able to recognize 3-dimentional epitopes related to a specific aggregation conformer [20,21]. However, conformational antibodies able to recognise sequence-specific epitopes, in particular, linear epitopes within amyloidogenic proteins, offer additional structural insight into amyloid formation and stabilisation [22,23]. It appears that amyloid-specific antibodies act as stabilisers of the recognised fibrillar conformations [24]. On the other hand, sequence-specific antibodies [22,25], which are also able to recognise native conformation of a protein lead to fibril destabilisation. Such antibodies and antibody fragments can therefore find considerable applications as powerful tools in amyloid research, diagnostics and therapy, and are the focus of this review.

2. Alzheimer's Disease

Alzheimer's disease (AD) is the most common neurodegenerative disorder. Most AD cases are sporadic, although small percentage is familial Alzheimer's disease (FAD) associated with mutations in both the amyloid precursor protein (APP) and the presenilin (PSEN) genes [26]. The severe neurodegeneration in the hippocampus and neocortical regions of the brain which leads to the onset of AD is still not fully understood. Its pathology is thought to be linked to extracellular Aβ plaques which mainly comprise Aβ peptide [5,7] and intracellular neurofibrillary tangles consisting of hyperphosphorylated filaments of the microtubule-associated tau protein [27,28]. The amyloid cascade is thought to be initiated by gradual changes in Aβ protein metabolism, which leads to an increase in

total Aβ production and/or reduced clearance. Monomeric Aβ peptides with predominantly 38 to 43 residues are produced by a dysregulated sequential proteolysis of non-aggregating APP with β- and γ-secretases. Despite a significantly higher concentration of Aβ40 in the brain, it is Aβ42 that appears to be the main component of the plaques [5]. In some AD patients the changes in Aβ protein metabolism result in an increase of the Aβ42/Aβ40 ratio. This relative increase of Aβ42 is believed to initiate oligomerisation and formation of diffuse Aβ42 deposits, which causes the subtle first effects on synaptic functions. As the diffuse plaques undergo a transition into fibrillar deposits; this event is accompanied by inflammatory responses, increasingly severe and irreversible changes of synaptic functions, which eventually lead to widespread neuronal dysfunction and cell death [5]. In contrast, in patients with Down's syndrome and the Swedish APP mutation, the Aβ 42/40 ratio does not change. In both Down's syndrome and the Swedish mutation cases, development of AD appears to be related to the over production of all forms of Aβ [29,30].

2.1. Targeting Aβ Fibrils: Lessons Learnt

Initially, mature fibrils had been considered to be the main neurotoxic species responsible for the onset of AD, and thus were at the centre of early antibody development and both active and passive immunotherapies have been considered. Active immunotherapy (vaccine) involves injection of an antigen (in this case fibrillar Aβ) to stimulate the patient's immune system to produce antibodies with the aim to generate a long-term immune response with less drug administration. The first active immunotherapy trial (AN-1792) followed the encouraging results obtained from the immunization of AβPP transgenic mice with fibrillar Aβ. The AN-1792 trial was terminated in phase two, after 6% of the patients developed severe meningoencephalitis [31–33]. Although, the postmortem examination of brains from patients participating in the trial revealed a lower level of Aβ deposits suggesting that AN-1792 reached its target, passive immunotherapy using humanised anti-Aβ monoclonal antibodies was considered safer. A number of antibodies and antisera able to target specifically Aβ fibrils were generated in order to identify 'aggregation epitopes' to gain a better understanding and consequently prevention of the protein aggregation (see Table 1) [20,22,23]. Monoclonal antibodies specific for the C-terminus of Aβ indicated that diffusible plaques contain primarily Aβ42, while the dense core and neuritic plaques contain both Aβ40 and Aβ42. Aβ40 and Aβ42 sequences are identical with the exception of the two additional amino acids present at the C-terminal: Ile (residue 41) and Ala (residue 42). Interestingly, antibodies AMY-33 and 6F/3 raised against Aβ fragments (targeting 1–28 and 8–17 amino acids residues, respectively) were found to prevent self-association of Aβ peptide and were able to convert Aβ from a fibrillar to nonfibrillar conformation [25]. The inhibitory effect of these antibodies was thought to be through the recognition of the 'aggregating epitopes' believed to initiate the undesirable aggregation process. Bapineuzumab (Elan/Pfizer Inc./ Johnson & Johnson), a humanised monoclonal antibody, which was progressed to the clinic, was developed to target fibrillar Aβ and directed against Aβ1-5. Studies conducted by Miles *et al.* have revealed that bapineuzumab recognises the N-terminal end of Aβ in a helical conformation [34]. The preferential binding of this antibody for Aβ plaques implied that the helical conformation at N-terminus is either enriched or exists in an equilibrium with other conformational states in dense plaque deposits. Subsequent X-ray crystal structures of Aβ oligomers, protofibrils and fibrils have suggested that the N-terminal region of the

peptide is free to adopt a helical structure independent of the core cross- β structure [35,36]. Despite being effective in promoting clearance of Aβ plaque burden and lowering the total tau and phosphorylated-tau levels in cerebrospinal fluid (CSF), the antibody did not achieve the desired effect of stopping cognitive decline in a phase three clinical trial and the studies were stopped in August 2012. Treatment with bapineuzumab showed a high incidence of Aβ-related imaging abnormalities (ARIA), likely to be associated with impairment of the brain blood barrier (BBB). Due to safety reasons, a very low dose of the antibody was used in the final phase III study, which could explain why the desired clinical effects were not achieved [37].

Another antibody, which entered clinical trials, is gantenerumab (Roche, Basel, Switzerland), a human monoclonal IgG1 antibody that was selected and optimised by phage display technologies. Unlike other conformational antibodies, gantenerumab was found to bind to both central and N-terminal regions of Aβ (1–10 and 19–26) fibrils [38]. Further investigation with X-ray and NMR structures has revealed that the flexible N-terminal region provides initial binding contact points followed by interaction with the Aβ peptide central region, resulting in 70 pM avidity-driven binding. Interestingly this antibody did not alter plasma Aβ levels, suggesting unaffected systemic clearance of soluble Aβ and preferential interactions with aggregated Aβ in brain. Although gantenerumab has shown sensitive and dose-dependent binding to Aβ fibrils and plaques, large plaques (>400 μm^2) appeared to be relatively resistant to clearance as shown by the five month study, and this led to the termination of the phase three clinical trial in December 2014 [39]. Habitch *et al*. have described a conformational antibody fragment, called B10, which recognises both amyloid fibrils and protofibrils through a pattern-recognition mechanism [24]. It has shown strong binding to an anionic surface moiety of fibrils derived from Aβ (1–40) and Aβ (1–42) peptides as well as tissue-extracted AA (from serum amyloid A protein) and AL amyloid fibrils (derived from Ig light chains), but it does not recognise monomers. Although the studies have also shown that this antibody was able to bind to oligomeric conformations, it showed significantly lower affinity in comparison to the affinity for protofibrils and Aβ fibrils, indicating that this antibody fragment does not recognise all intermediate conformations. More importantly, the above studies imply that there are significant structural differences between Aβ fibrils and different oligomers which can be detected with antibodies.

Despite disappointing results from clinical studies targeting Aβ fibrils [37,40] crucial conclusions have been drawn suggesting that disease modifying drugs need to be administered much earlier, in the asymptomatic AD patients before the irreversible changes occur. Studies published by Bateman have indicated that Aβ42 levels in CFS begin to decline as early as 25 years before expected symptoms appear [41]. Additionally, passive Aβ immunotherapy AD Tg mice, in which APP is overexpressed has shown to reduce cerebral Aβ and improve cognition, especially if administered prophylactically [40]. Also, a number of second-generation active Aβ immunotherapies are currently being tested in clinical trials [42] More importantly, the observed lack of correlation between Aβ plaque density in the brain and the severity of dementia [43,44], together with a clear link between neuronal injury and soluble aggregated Aβ posit that the culprit behind AD may not necessary be fibril deposits, but soluble oligomeric, intermediate forms [45].

2.2. Targeting Intermediate Conformations

Despite a growing body of evidence pointing towards Aβ oligomers as the most toxic in AD disease, obtaining detailed molecular and structural characterisation and identifying the corresponding link to the activity of oligomers is still challenging. Different forms of soluble Aβ have often been ambiguous, with overlapping definitions based on the method of detection (e.g., biochemical or immunohistochemical analysis) [46]. Soluble Aβ oligomers often exhibit profound variability in secondary structure comprising a wide range of conformations ranging from amorphous aggregates, micelles, protofibrils, prefibrillar aggregates, amyloid beta-derived diffusible ligands (ADDLs), Aβ*56, globulomers, amylospheroids, "tAβ" (toxic soluble Aβ), "paranuclei," to annular protofibrils [18,19,45–48]. It is therefore critical to identify forms closely associated with the pathology of AD, and conformational antibodies and antibody fragments can serve as tools for investigating these Aβ states, and their dynamics as well as offering potential for immunotherapy [49–61] (see Figure 2 and Table1).

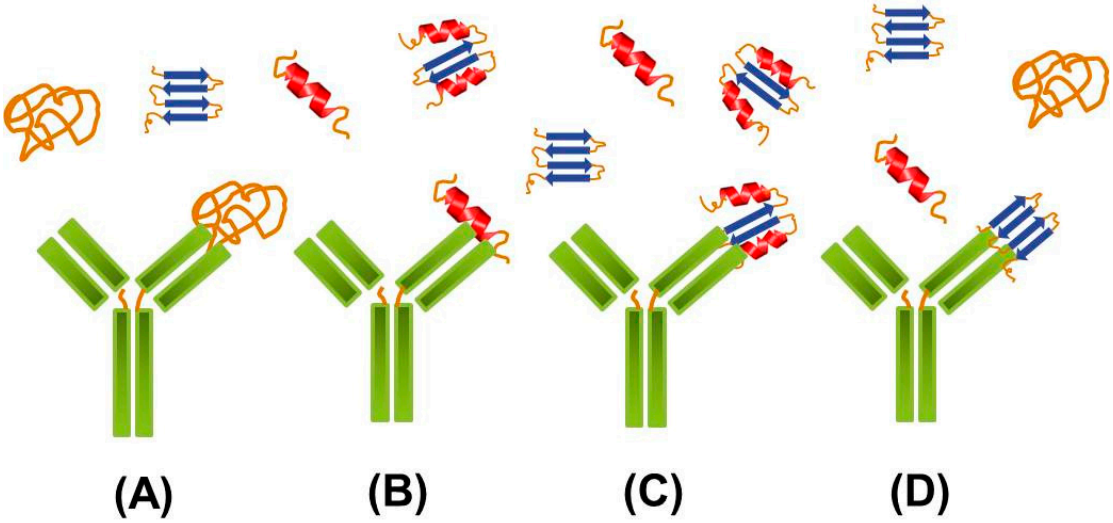

Figure 2. Concept of conformational antibody targeting four different conformations of Aβ. Examples of antibodies binding: (**A**) a conformational epitope of unfolded protein, (**B**) a partially structured Aβ monomer, (**C**) an oligomeric form of Aβ, (**D**) a fibrillary form.

For example, structural information about amylogenic residues 18–41 within the Aβ peptide was obtained with the aid of the CDR3 loop of a shark Ig new antigen receptor (IgNAR) serving as a scaffold in crystallography [49]. The crystal structure has revealed that the predominant oligomeric species is a tightly associated Aβ dimer, with paired dimers forming a tetramer confined within four IgNAR domains, thus preventing uncontrolled Aβ formation.

A number of single domain camelid antibody fragments have demonstrated different functionalities ranging from the prevention of mature fibril formation, inhibition of aggregation and extracellular toxicity [50], and the stabilisation of small non-toxic Aβ species [21,51,52]. These antibody fragments do not recognise monomeric or fibrillar forms of Aβ, but define previously unknown, but critical, conformations, which hold promise for therapeutic targeting. For example, a KW1 antibody fragment was able to discriminate between oligomers and fibrils and differentiate between different types of Aβ oligomers (Aβ (1–40) and Aβ (1–42)). KW1 recognises oligomers through a hydrophobic and aromatic

surface motif of Aβ residues 18–20 (see Table1). Studies appear to suggest that this antibody fragment modulates rather than blocks Aβ assembly and achieves this by transforming oligomers into nonfibrillar aggregates [21].

Antibody A11 has also been reported to recognise specifically a generic epitope common to prefibrillar oligomers but not monomers or fibrils [53]. Interestingly, the A11 antibody was able to recognise small focal or punctuate deposits in AD, but not diffuse plaques or other plaque types, indicating that plaques may not represent an accumulation of prefibrillar oligomers.

Table 1. Passive antibodies generated to target different forms of Aβ.

Antibody	Selective for	Recognition mechanism and mode of action	Reference
Amy-33	Fibrils	Aβ 1–28 aa residues, preventing self-association and disintegrating fibrils into a nonfibrilar conformation	[25]
6F/3	Fibrils	8–17 aa residues, preventing self-association and disintegrating fibrils into a nonfibrilar conformation	[25]
Bapineuzumab	Fibrils	1–5 aa of N-terminal end of Aβ in helical conformation	[34–36]
Gantenerumab	Fibrils	Targeting 1–10 aa and 19–26 aa	[38]
B10	Fibrils, Protofibrils	Pattern-recognition mechanism, binding to an anionic surface moiety	[24]
KW1	Oligomers	Hydrophobic and aromatic surface motif of Aβ residues 18–20	[21]
A11	Prefibrillar oligomers	A generic epitope common to prefibrillar oligomers	[51,53]
Crenezumab	Protofibrils, oligomers	Recognition of multiple conformational epitopes (aa13–14 relevant) and promoting disintegration of Aβ	[54]
SAR228810	Protofibrils and LMW Aβ	Binds to congophilic amyloid plaques (present only in AD patients)	[55]
A-887755	Aβ20-43 globulomers, condensed and hydrophobic oligomers	Recognition of the relevant structural motif located in the C-terminal part of the Aβ sequence between amino acids 20 and 42 Recognises N-terminally truncated Aβ oligomers	[56]
Solanezumab	Intermediate conformations of soluble, monomeric Aβ	Targeting mid region of Aβ	[57]
BAN2401	soluble Aβ protofibrils	It is thought to either enhance clearance of Aβ protofibrils and/or to neutralize toxic effects on neurons in the brain	[57]
Grafted AMyloid-Motif AntiBODIES, 'Gammabodies'	Aβ, α-synuclein and islet amyloid polypeptide	Prevents amyloid formation and further aggregation by stabilising benign intermediates	[60]
Aducanumab (BIIB037)	Soluble and insoluble Aβ	Preferentially binds parenchymal over vascular Aβ	[61]

Another antibody, crenezumab was designed to target multiple conformational protofibrillar epitopes of Aβ, including oligomeric forms while inhibiting aggregation and promoting disaggregation of Aβ at the same time [54]. In contrast to other antibodies, crenezumab was produced as an IgG4 to reduce the risk of ARIA. Interestingly, this indicated an importance of amino acids 13–14 in the fibrillation process.

In contrast, a humanised antibody, SAR228810, has been designed to recognise and target protofibrils and low molecular weight Aβ [55]. In addition, antibody A-887755, generated against Aβ20–42 globulomers, condensed and hydrophobic oligomers, showed promising high affinity/avidity for immobilised globulomers and was able to detect endogenous Aβ species but not in non-demented control patients or in vascular Aβ deposits [56].

A number of antibodies targeting intermediate conformations have now entered clinical trials. One of these is solanezumab (Eli Lilly and Company) that has been developed to target the mid-region of soluble, monomeric Aβ. A dose-dependent increase of the Aβ42 in CSF was demonstrated in a Phase 2 clinical trial, but with no effect on tau, amyloid PET levels. Although the antibody showed a small but significant cognitive improvement in the number of patients suffering from a mild form of AD, unfortunately it failed to achieve the success criteria in a phase three clinical trial [57].

BAN2401 (Eisai, Tokyo, Japan/BioArctic Neuroscience, Stockholm, Sweden), a humanised version of mAb158 antibody is believed to be the first antibody designed to target selectively soluble Aβ protofibrils and is currently in a phase 2b study, having shown a favourable safety profile [57].

Meli *et al*. have generated a panel of intrabodies selectively recognising Aβ oligomers and targeting different subcellular compartments [58,59]. The antibody fragment called scFvA13 was expressed in endoplasmic reticulum (ER) with the aim of capturing oligomers at subcellular sites during their formation and modulating their assembly and activity. This approach provides selective control over intra- and extracellular fractions of biologically-active Aβ oligomers in living cells without interfering with the maturation and processing of APP protein. More importantly this work has pointed out that intracellular Aβ can transform into pathological oligomer conformers at the ER, and that these are involved in the deregulation of two independent pathways of cellular homeostasis and synaptic signalling. In an interesting approach to targeting AD, small amyloidogenic peptides (6–10 residues) from Aβ42 have been grafted into the CDR3 loop of a VH domain. The resulting 'gammabodies' prevented Aβ formation and further aggregation by stabilising benign intermediates [60]. Exploiting homotypic interactions to recognise Aβ conformers enabled the identification of important structural differences between oligomers and fibrils. It has been shown that the central region Aβ (18–21) of soluble oligomers undergoes a conformational transition to form β-sheet within fibrils. In addition, gammabodies, displaying the hydrophobic C-terminal sequence of Aβ peptide, exhibited similar immunogenicity with both oligomers and fibrils, indicating structural similarity in both Aβ oligomer and fibril conformers. Interestingly, it appears that the vast majority of antibodies successfully inhibiting protein aggregation described in the literature appear to target residues 18–20 of Aβ, which can be ascribed with great confidence as key residues in the Aβ formation.

Owing to the dynamic equilibrium between different conformations of Aβ it still remains debatable whether it is sufficient to concentrate on one of the 'most toxic' species or to target a number of distinct conformers in parallel. However, recently announced promising results from a pre-specified interim analysis of PRIME, the Phase 1b study of aducanumab (BIIB037) (Biogen Idec.) appear to indicate that the key to successful treatment of AD might be targeting soluble and insoluble species simultaneously [61]. Aducanumab (BIIB037) is a human recombinant monoclonal antibody, which has been selected from a population of elderly, healthy donors and cognitively stable patients. The antibody has been shown to target both Aβ soluble oligomers and insoluble fibrils deposited into the Aβ plaque in the brain of AD patients. Reported data from a Phase 1b clinical trial revealed a statistically significant dose- and time-

dependent reduction of Aβ plaque in the brain. In addition, treatment with aducanumab demonstrated a statistically significant slowing of clinical impairment in patients with prodromal or mild AD [61]. Instances of ARIA were also reported with other anti Aβ antibodies, and in the case of aducanumab (BIIB037 were dependent on dose- and apolipoprotein E4-(ApoE4) status. Despite the side effects, the majority of patients with ARIA continued treatment at lower dose. Promising results from a Phase 1b clinical trial strongly support the feasibility of antibodies as therapeutic candidates for AD treatments, and show sufficient ability of the antibody to penetrate the BBB in order to generate a desired effect. Based on these encouraging data, aducanumab is going to be advanced to a Phase 3 clinical program (readout planned for February 2022) which will reveal whether antibody therapy will have the desired medical impact in the treatment of AD.

The knowledge acquired from unsuccessful clinical studies has led to three secondary preventive trials for AD now being conducted: the Anti-Amyloid Treatment in Alzheimer's Disease Prevention Trial (A4), Alzheimer's Prevention Initiative (API) and the Dominantly Inherited Alzheimer's Network Trial (DIAN). The API and DIAN trials involve presymptomatic patients carrying APP or presenilin mutations, whereas the A4 trials have been designed for asymptomatic elderly subjects at risk of developing AD [40,57,62]. The goal of these trials is to validate the preclinical phase of AD and identify the starting point of the AD treatment, as well as the validation of potential biomarkers and disease-modifying drug candidates. Currently, a number of antibodies are being re-evaluated. Genentech, the Banner Institute and the National Institutes of Health have partnered to test the crenezumab antibody (API trial) in 300 subjects selected from a large Columbian family who carry the mutant gene (PS1 E280A) and are 30 years of age or older. PS1 E280A) is known to lead to AD onset at a young age, followed by 10–15 year period of progressive decline in cognition and clinical function [63]. It is a 5-year trial with readout planned for 2018. Gantenerumab and solanezumab will be tested in 210 individuals of 18–80 years of age as part of the DIAN trial. These include individuals who are known to have AD-causing mutation or have a 50% chance of having an autosomal dominant AD mutation (e.g., family member with a known AD-causing mutation) and individuals who are within −15 to +15 years of their parental age of AD symptom onset [readout: 2019]. Additionally, as part of the A4 trial, solanezumab is now being tested in 1000 subjects of 70 years of age or older, who were selected based on the following criteria: no genetic predisposition, lack of clinical AD symptoms but positive PET scans for brain Aβ Readout is expected at the end of 2016.

2.3. Natural Anti-Aβ Antibodies

Interestingly, it has been found that the level of anti-Aβ antibodies measured in AD patients is significantly lower compared to healthy volunteers [64–66]. There is thus an increasing interest in naturally occurring polyclonal anti-Aβ antibodies and an IVIG approach to AD treatment [18,57,64–69]. Prophylactic, regular IVIG treatment with autoantibodies against-Aβ has shown to reduce by more than 40% the risk of developing AD [66]. It is thought that autoantibodies have an ability to inhibit Aβ aggregation through preferentially binding to dimers and trimers and interfering with oligomeric forms of Aβ. As the epitope mapping demonstrated, natural anti-Aβ antibodies are predominantly directed towards a mid-/C-terminal epitope of Aβ starting at 28aa, and it is believed that these antibodies recognize a common conformational epitope rather a distinct peptide sequence [67–69]. Aβ peptide in

plaques is deposited in such a manner that the N-terminal part of the peptide is pointing out towards the surface of the plaque. This explains why autoantibodies are not capable of clearing senile plaques, although a reduction of fleecy-like plaques was observed. This suggests that autoantibodies could be beneficial for AD patients but only when administered at the very early stage of the disease. Different clinical pilot studies have demonstrated that IVIG decreased the level of Aβ in CSF, improved cognition, and led to stabilisation of AD symptoms [18,57,64–69]. Although there is evidence from these clinical pilot studies suggesting the benefit of administering autoantibodies, it is often difficult to obtain statistically relevant results. Therefore, an increasing emphasis should be given to careful classification and selection of AD patients for clinical trials, and the development of highly sensitive biomarker diagnostics for use with presyptomatic patients.

2.4. Targeting Tau

AD drug development has long been dominated by Aβ-based therapeutic approaches. However, AD pathology is also associated with intracellular neurofibrillary tangles formed by hyperphosphorylated filaments of tau protein. In its native state tau is a soluble and unfolded cytoplasmic protein, essential for assembly of microtubules. It binds to tubulin and acts as a stabilising agent during polymerisation into microtubules in neurones. However, as a result of post-translational modifications, including hyperphosphorylation, glycosylation, ubiquitination, glycation, polyamination, nitrosylation, and truncation, the tau protein can undergo a transformation from an unfolded into a higher order structure prone to aggregation and subsequent fibrilisation [70–73]. Normal tau contains 2–3 mol phosphate/mol of the protein [70]. In AD, tau becomes abnormally hyperphosphorylated and undergoes additional electrostatic modification that enables it to form a side chain-side chain interaction, leading to a tau-tau dimer. Once these dimers are formed and adopt a stable structure, they can begin a process of nucleation, forming oligomers, which transforms into filaments, termed paired helical filaments (PHFs) and eventually intraneuronal tangles (iNFTs) [27,28]. Upon cell death iNFTs are released and become extraneuronal tangles (eNFTs).

The mechanism of action of tau antibodies remains controversial. For a number of years tau was considered to be purely intracellular. Sigurdsson *et al*. reported antibodies inside neurons and proposed that antibody-mediated clearance of intracellular tau aggregates follows the lysosomal pathway [74,75]. Further studies have revealed that tau antibody uptake into neurons correlates well with tau levels. It has been suggested that this occurs primarily via clathrin-dependent Fcγ receptor endocytosis, and is required for acute tau clearance [76]. In addition, passive immunisation of JNPL3 mice with a PHF1 antibody that detects the pSer396/404 tau epitope present on both normal adult brain tau and PHF-tau showed reduced tau pathology and improved motor function compared to controls [77]. Morgan *et al*. have reported that intracerebral injection of tau-5, a monoclonal antibody against a non-phosphorylated epitope in the middle region of tau, also effectively and acutely reduced intracellular tau pathology [78]. A study carried out by D'Abramo demonstrated that P301L mice treated with MC1, a conformational monoclonal antibody specific for PHF-tau, resulted in a reduced rate of tau pathology progression [79]. In contrast, administration of DA31, a high affinity tau sequence antibody, did not show the anticipated effects. While DA31 antibody was designed to recognise the amino acid region 150–190 of tau; MC1 detects a very specific early pathological tau conformation produced by the intramolecular association

between the extreme N-terminus and the third microtubule repeat domain of tau. It appears that in tau mutant P301L mice, specificity of an antibody, rather than its affinity, plays a crucial role in promoting tau pathology clearance. Interestingly, despite promising results the survival rate of the P301L treated mice did not improve upon administration of MC1.

Unlike Aβ, the tau protein has a crucial physiological function. Thus design of antibodies targeting specific motifs associated with the toxic species is paramount in order to avoid disastrous consequences, such as destabilization of the microtubes and subsequent interference with axonal transport and cytoskeletal integrity [27–28,71–73]. One of the approaches is to design an antibody to target the phosphorylation sites that are characteristic for toxic species. For example, phosphorylation at thr231 occurs at an early stage of the neurofibrillary tangles NFTs formation, is shown to inhibit the binding of tau to microtubules, and appears in all types of NFTs: pretangle phospho-tau aggregates (pre-NFTs), intraneuronal NFTs (iNFTs), and extraneuronal NFTs (eNFTs). This specific phosphorylation appears to precede tau oligomerisation. In contrast, phosphorylation at ser202 and thr205 has been related to late phospho-tau changes. An alternative is to target conformations that are only associated with the aggregated forms of tau [71–73]. Additionally, as tau aggregates are formed, antibodies can take an advantage from the avidity effect and increase the apparent selectivity for toxic species over tau monomer.

However, a growing body of work has indicated that tau aggregates produced in one cell can escape or can be released into the extracellular environment and initiate aggregation in neighbouring or connected cells [80–82]. Development of therapeutic antibodies to prevent tau aggregates from spreading between cells by targeting disease-relevant species in the extracellular space offers an attractive approach for AD intervention, previously thought to be intractable for intracellular protein aggregates. Promising outcomes of immunotherapy against tau were demonstrated in tau-overexpressing non-neuronal cell models and in a transgenic mouse model for tauopathy [83,84]. A study led by Yanamandra has revealed the ability of the antibody to reduce 'seeding' activity of tau fibrils in P301S tau transgenic mice [83]. The antibodies showed a significant decrease in hyperphosphorylated, aggregated and insoluble tau, leading to reduced microglial activation, and improved cognitive deficits.

Furthermore, a number of antibodies have also been generated to gain a better understanding of the effect of oligomeric species on pathology. T22 antibody revealed elevated levels of the oligomeric form of tau in AD brain samples, which appears to contribute to neurofibrillary tangle (NFTs) formation [72]. Administration of the T22 antibody has reduced the level of pathogenic tau oligomers and led to the rescue of tauopathy-related motor and cognitive functions in mice. Subsequent studies conducted by Kayed *et al*. demonstrated that tau oligomer specific antibodies administered intraperitoneally in the JNPL3 mouse model resulted in a reduced tau oligomer level and improved motor test performance [85].

Although studies described above imply that passive immunotherapy in mutant tau models may be efficacious in reducing the development of tau pathology, further work is required to select specific tau epitopes to target.

3. Lewy Body Diseases: Targeting α-synuclein

Misfolding of a small 14kDa protein, α-synuclein, into fibrillar aggregates, a main component of Lewy bodies and Lewy neuritis, has been associated with Parkinson's disease (PD), PD with dementia (PDD), and dementia with Lewy bodies (DLB) [8,9,86]. Braak *et al*. have proposed that progression of

PD follows a stereotypical and topographical pattern between different parts of the brain, which depends partly on the vulnerability of specific neuron types [87,88]. α-Synuclein itself appears to be a key factor in mediating transmission of disease pathology from one brain area to another [89–94]. In its native state, monomeric α-synuclein is a soluble and primarily unfolded protein expressed in the brain and predominantly localised at the presynaptic terminals of neurons. The protein is comprised of three distinct domains: a positively charged N-terminal region (1–60 residues) involved in binding to membranes, upon which the α-synuclein protein becomes more ordered by adopting an α-helical structure; a middle region (61–95 residues), called a non-Aβ compartment (NAC), which undergoes significant structural changes during the self-association process resulting in the well-recognised β-sheet rich conformation, and a third section, which is highly negatively charged and unstructured (96–140 residues). Although it is thought that N and C-terminal domains are not directly involved in the α-synuclein fibrillation process, there is evidence suggesting they are likely to play important roles in the stabilisation of protofibrils and fibrils [95].

For a number of years, like the tau protein, α-synuclein has been considered to be primarily intracellular. Thus, the discovery that disease-relevant α-synuclein conformers can accumulate in the membrane, be secreted into an extracellular environment and spread to neighboring cells and/or anatomically connected brain regions (Braak's hypothesis) provides strong rationale to develop antibodies targeting disease-relevant species in the extracellular space inhibiting cell to cell spreading [87–94]. Monoclonal antibodies, Syn211 and Syn303, specific for a misfolded form of α-synuclein are thought to block the uptake of α-synuclein preformed fibrils in primary neurons and subsequent propagation of α-synuclein pathology to other neurons [96]. Intraperitoneal administration of these antibodies in a mouse model of sporadic PD led to a significant reduction of Lewy pathology and dopaminergic cell loss in the brain, and improved motor coordination and grip strength. Whereas Syn211 recognises human 121–125 residues (C-terminal region), species-independent Syn303 targets the first five residues of the N-terminus of α-synuclein and appears to recognise only misfolded α-synuclein. Although Syn211 exhibits higher efficacy in preventing α-synuclein pathology in comparison to Syn303, nevertheless it seems that both conformation and affinity are critical factors in determining successful immunotherapy. In addition, passive administration with the 9E4 antibody, which recognizes 118–126 residues showed a reduced level of accumulation of calpain-cleaved α-synuclein aggregates in the neocortex and hippocampus [97].

In 2001, Uversky *et al.* described α-synuclein fibril formation as a conformational shift from the unfolded native state towards the partially folded and prone-to-aggregation species that first initiates dimerization, followed by the formation of soluble oligomer and protofibrils of increasing size, eventually resulting in the insoluble fibrils and development of Lewy bodies' [98]. The level of accumulated α-synuclein in PD appears to be strongly linked to the balance between the rates of α-synuclein synthesis, aggregation and clearance. Factors such as oxidative stress, post-translational modifications, proteolysis, and concentration of fatty acids, phospholipids and metal ions have been shown to shift this equilibrium towards fibril formation [8,9].

To help characterise early events in amyloid formation and gain a better understanding of the mechanism leading to PD pathology, antibodies and antibody fragments have been used as molecular probes to define morphologically distinct conformations of α-synuclein, which could be amenable to therapeutic intervention [23]. Monoclonal antibodies: mAb38F and mAb38E2 have shown high affinity

and selectivity for large α-synuclein oligomers [99]. They did not bind to Aβ or tau but were able to recognise pathological α-synuclein present in the brains of patients with Lewy body disorders. Additionally, these antibodies were shown to detect the pathology earlier in α-synuclein transgenic mice in comparison to antibodies targeting linear epitopes. For example, a single chain antibody fragment (syn-10H scFv) from a phage display library was selected for its ability to bind to larger, oligomeric conformers of α-synuclein in PD tissue and fluid samples [100]. The oligomer-sensitive antibodies can thus serve as relevant tools to gain a further understanding of the pathogenic mechanisms behind Lewy body-based disorders and contribute to the development of potential candidates for both immunotherapy and biomarkers. Correlation of increased levels of α-synuclein protofibrils present in the spinal cord of (Thy-1)-h[A30P] α-synuclein transgenic mice with motor deficits also implies toxicity of the protofibrils. Protofibril-specific mAb47 antibody reduces levels of protofibrils without affecting monomeric α-synuclein in the spinal cord, but did not exert a desirable effect in the brain [101]. It is important to note that the transgenic mouse model used in this study express α-synuclein at much higher levels in the spinal cord than in the whole brain, which could explain the significant reduction of α-synuclein protofibrils in the spinal cord.

As the nitration of pathological inclusions has also been identified in α-synucleopahies, the 5G4 antibody, which binds to high molecular weight nitrated β-sheet rich α-synuclein oligomers and fibrils, could be used as a tool for morphological localization of disease-specific α-synuclein in the human brain [102].

A large proportion of high affinity antibodies reported in the literature show specificity for the C-terminal domain of α-synuclein, suggesting that upon binding to membrane, the C-terminal portion of α-synuclein will likely penetrate the membrane and become exposed to the extracellular environment, where an antibody could recognise it [97,103]. Additionally, antibodies able to recognise different epitopes within the C-terminal domain revealed structural variation within that region, suggesting that the C-terminal domain plays a role in stabilization or rearrangement of protofilaments during fibril assembly. Single-domain camelid antibodies NbSyn2 and NbSyn87 were found to bind to aggregated as well as monomeric forms of α-synuclein by recognising 118–131 and 137–140 residues of the C-terminal region, respectively. A time-dependent decrease in apparent affinity of these antibodies observed during fibril formation and maturation implies a change in α-synuclein conformation or limited exposure of the C-terminal domain within the fibril structure [103].

In vitro studies suggest that binding of α-synuclein to membranes may affect the kinetics and pathways of α-synuclein aggregation [104,105]. Contradictory studies report that binding of α-synuclein to the membrane can lead to the inhibition, or promote aggregation and formation of β-sheet-rich and α-helical aggregates, which indicates that the mechanism underlying the pathology of PD is complex and not yet fully understood. Further study revealing that the heterogeneity of the aggregates formed upon binding to phospholipid membranes is strongly dependent on the experimental conditions adds an additional level of complexity. The proposal that the increased level of monomeric α-synuclein observed in PD may act as a reservoir for the protein aggregation led to the development of antibodies such as intrabodies D5 and D10 targeting monomeric α-synuclein, and VH14 and NAC32, raised against a different region of NAC section of α-synuclein [106,107]. These antibodies showed promising effects in inhibiting α-synuclein aggregation and rescuing α-synuclein toxicity.

4. Huntington's Disease: Targeting Huntingtin

Abnormal expansion of the N-terminal polyglutamine (polyQ) stretch in the first exon of the Huntingtin protein (Htt) leads to protein misfolding, aggregation and neurodegeneration known as Huntington's disease (HD) [10,108]. In the wild-type Htt the first exon contains on average 16–20 glutamine residues. In HD, the number of glutamine repeats present within the first exon increases significantly to reach between 35–250 repeats with the onset of the disease being inversely correlated to the length of polyQ tract. An increase in the polyQ length also promotes caspase and calpain activation leading to greater production of toxic species prone to aggregate into inclusion bodies in the brain of HD patients. Thus, targeting the first exon of mutated Htt protein (mHttexon1) appears to be an attractive strategy to treat HD patients. The ability of anti-mHttexon1 antibodies and antibody fragments to reduce the aggregation and associated neurotoxicity has proven to be strongly dependent on the targeted epitope [109]. Specifically targeting the Htt sequence between 1 and 17, which has been shown to play a key role in aggregation and HD pathology, seems to be an obvious choice. Thus far two intrabodies: VL12.3 [110,111] and scFv(C4) [112,113] raised against this region showed binding to the N-terminal of exon 1 of mHtt. Both antibodies appeared to diminish aggregation and associated neurotoxicity. The minimal binding epitope of VL12.3 was identified as EKLMKAFESLKSFQ, which comprised the N-terminal residues 5–18 of Htt and the first residue of the polyQ stretch. Unfortunately, VL12.3 has also appeared to enhance the level of the antigen-antibody complex in the nucleus, which could increase toxicity through disrupting cytoplasmic and nuclear trafficking of htt scFv(C4) preferentially binds to soluble mutated Htt (mHTT) N-terminal fragments, and has a weak affinity for endogenous full length Htt, which is likely to be the result of epitope inaccessibility on full-length Htt. scFv(C4) appears to neutralize the toxic effects of mHtt exon 1 by stabilizing N-terminal mHTT exon 1 fragments in a non-toxic conformation. Fusion of the PEST region of mouse ornithine decarboxylase (mODC) to the C-terminus of the antibody, to create a bifunctional entity with enhanced clearance, led to a ~80–90% reduction of Htt exon 1 protein fragments with 72 glutamine repeats (httex1-72Q) in ST14A cells compared to scFv-C4 alone [114].

In contrast, Khoshnan *et al*. generated a number of antibodies: MW1 to MW6 that preferentially bind to the polyQ domain of mHtt compared to Htt, and MW7-8 which recognises the poly-proline section and the eight residues in the C-terminal domain of mHttexon1 [115]. Interestingly, further analysis revealed that antibodies specific for polyQ region of Htt induced a pronounced cell death and Htt aggregation, whereas antibodies recognising the proline-rich domain reduced Htt aggregation and subsequent cytotoxicity.

Promoting the clearance of mHtt has also been considered as an alternative approach to HD treatment. mHtt undergoes a complex proteolysis involving protease cleavage, proteasomal degradation and lysosomal/autophagic degradation [116]. A better understanding of this complex mechanism is crucial in the development of successful therapy. A characteristic property of mHtt is the number of caspase and calpain cleavage sites that produce N-terminal fragments more toxic than the full-length mHtt protein. Use of antibody fragments such as Happ1 Happ3 intrabodies raised against the C-terminal, proline-rich region of mHtt exon1 have demonstrated the ability to increase clearance of mHtt but not wild type Htt [94,97]. Interestingly, it turned out that Happ1 required calpain cleavage at position AA15

to induce and increase degradation of Huntingtin exon 1 fragments suggesting that the C-terminal proline-rich region is essential to maintain the stability of the protein [117].

5. Transmissible Spongiform Encephalopathy: Targeting Prion

In contrast to other neurodegenerative disorders, prion-related diseases also known as transmissible spongiform encephalopathies (TSEs) are infectious [6,7]. In the native state human prion protein PrP^{C} is composed of an unstructured N-terminal domain (residues 1–124) and a well-defined C-terminal globular region (residues 125–231). Disease is caused by the conformational transformation of ubiquitously expressed cellular prion protein (PrP^{C}) into pathogenic, highly insoluble prion protein conformer (PrP^{Sc}). Once PrP^{Sc} is generated, a misfolding process known as templated conformational change is initiated leading to protein aggregation and onset of disease. Despite having the same amino acid sequence, PrP^{C} and PrP^{Sc} are structurally very different. Upon association with pathogenic PrP^{SC}, PrP^{C} initially composed of 45% helices and few β-sheets undergoes significant conformational transition resulting in a structure characterised by ~30% helices and ~45% β-sheets. Whereas PrP^{C} is monomeric, soluble in non-ionic detergent and entirely degraded in the proteinase K digestion process, PrP^{SC} is insoluble, partially resistant to PK degradation and prone to aggregation. It is this partial PK degradation that produces infectious fragments referred to as the PK-resistant core of PrP^{SC} [6,7].

Understanding prion diseases requires detailed structural insights into PrP^{C} and PrP^{Sc}. This can be achieved with the use of a range of antibodies directed against many different epitopes of PrP^{C}, (N-terminal octarepeat region, central unstructured region, globular C-proximal domain) providing valuable tools for prion research and diagnostics [118]. With the aid of antibodies, Sonati *et al*. reported interesting observations regarding toxicity of the prion protein [119]. Deletion of the octapeptide repeats within the N-terminal domain led to a lack of neurotoxicity. Encouraging evidence also suggests that in prion diseases, such as Creutzfeldt-Jakob disease (CJD), the intrinsically flexible N terminal region (AGAAAAGA) of prion protein plays a critical role in the conversion of the ubiquitous cellular PrP^{C} into a misfolded oligomeric conformation, PrP^{SC}. The solved crystal structure of full length PrP in complex with a nanobody (Nb484), which inhibited prion propagation [120], revealed β-enrichment in the palindromic motif as an early event in the conversion of PrP^{C} to PrP^{SC}. These studies have shown that the palindromic motif adopts a stable and fully extended configuration to form a three-stranded antiparallel β-sheet with the β1 and β2 strands. This demonstrates that PrP^{C} can adopt a more intricate β0-β1-α1-β2-α2-α3 structural organization than the established β1-α1-β2-α2-α3 prion-like fold. The noteworthy structural feature of the PrP:Nb484 complex is the substantial stabilisation of the β2-α2 loop region, being structurally flexible in all known human PrP structures. This observed loop rigidity induced by Nb484 is thought to have relevant biological implications. Significant stabilisation of PrP^{C} in complex with an antibody opens the possibility to carry out soaking experiments to study the interactions of potential small molecules with the flexible part of PrP, linking a nanobody-stabilised conformation with discovery of new chemical entities [120]. Wei *et al*. identified and isolated anti-prion specific antibodies, which bound strongly to both PrP^{C} and PrP^{SC}, from sera and CSF of healthy individuals, and showed significant inhibition and ability to disrupt preformed PrP fibrils [121]. Determination of this unique epitope revealed that the antibody targets only five amino acids located at the N-terminus of PrP106-110 indicating the importance of this discrete region in fibril formation.

There is a body of evidence substantiating prion immunotherapy as effective in curing an infected cell as outlined in the review by Rovis and Legname [122]. Anti-PrP^C antibodies have shown ability to reduce the level of PrP^C that could undergo conformational transformation into pathogenic PrP^{SC}. Although the mechanism by which antibodies prevent this transition from PrP^C to PrP^{SC} is still not fully understood, a number of strategies have been proposed. It is thought that anti-prion antibodies function by lowering expression of PrP^C and prompting redistribution of PrP^C from the sites critical for prion conversion or by preventing PrP^C:PrP^{SC} complex formation. Alternatively, antibodies could stabilise non-toxic PrP^C conformation or mature fibrils. Other groups of antibodies have been designed to target PrP^{SC} species aimed at interfering with PrP^{SC} trafficking and promote degradation or inhibit PrP^{SC} from interacting with PrP^C molecules. Interestingly, a significant number of promising antibodies do not target pathogenic PrP^{SC}, but PrP^C, which is thought to serve as a reservoir for prion transformation. Although initial concerns regarding the feasibility of immunotherapy were raised when a number of anti-PrP antibodies triggered a pro-apoptotic signalling cascade in neurons that is reminiscent of PrP, these were alleviated after a study showing that co-expression of anti-PrP antibodies with PrP^C expressed at physiological levels showed no autoimmune responses and no effect on various immune cell populations [123].

Alternative antibody formats such as recombinant Fab fragments, camelid antibody fragments and scFvs have also been successful at clearing pathogenic prion from infected cells [122] (and references within). The advantage of VHs and scFvs is the single polypeptide sequence used for the gene transfer-based passive immunisation. Studies conducted by Moda *et al*. [124] and Wuertzer *et al*. [125] investigated vectors for antibody gene delivery to both periphery and brain areas and highlighted two: AAV2 and AAV9 showing a promising delay in the onset of clinical signs of the disease, prolonged survival time, milder neuropathological changes, reduced PrP^{SC} in brain and more importantly, no inflammation and neurotoxicity. An additional benefit of intrabodies is the ability to design them to target a specific cellular compartment. As an example, anti-PrP scFv successfully retained PrP^C in the ER compartment and prevented PrP^{SC} formation, while a secretory version of the same intrabody re-directed PrP^C to proteasome and impaired PrP^C association to exosomes [126]. By fusing anti-PrP^C scFv with cell-penetrating peptide (CPP) penetratin [127], the antibody fragment was able to cross BBB. Unfortunately, it was also found in nuclei and thus further alteration would be required if this fused anti-PrP^C scFv were to be considered as a potential therapeutic.

6. Concluding Remarks

For a number of years, passive immunotherapy was considered to be an unsuitable approach to treat neurodegenerative diseases, mainly due to the large size of antibodies, which may limit their ability to cross the BBB. It is reported that only 0.1%–0.2% of circulating antibodies cross BBB and enter the brain or CSF [128]. Limited BBB uptake and subsequent brain exposure requires that antibodies have an extremely high affinity for the target or are administered at large doses to achieve the desired therapeutic effect. To improve their potency, antibodies may take advantage of the avidity effect, as long as multiple binding sites are present in close proximity within the aggregated targets. Other routes to increase BBB uptake and enhancing the therapeutic effect of antibodies may include incorporation of sequences that have naturally occurring BBB permeability, engineering bispecific antibodies or fusing antibody fragments to proteins, such as transferrin, insulin or leptin to facilitate receptor-mediated transcytosis [128].

Furthermore, with the very slow, and initially asymptomatic, progression of the neurodegenerative disorders, it is extremely important to understand the early stages of the disease development and structural characteristics of toxic species underlying neurodegeneration. Determination of the binding epitopes of conformational antibodies, which selectively bind with high affinity to these toxic species can contribute to a better understanding of fibril formation and offers an exciting opportunity to develop immunotherapy and biomarkers for neurodegeneration diseases. In addition, the fact that epitopes of conformational antibodies are widely distributed, yet distinct and non-overlapping between oligomeric and various insoluble conformers, suggests fundamental differences in the structural organization of the polypeptide backbone between these amyloid structures. Owing to dynamic equilibrium between transient, yet relevant conformations, the structural characterization of neurotoxic species still remains challenging. Use of antibodies as research tools to stabilize intermediate conformations ranging from monomers, oligomers, protofibril to fibril can facilitate necessary target validation for therapeutic intervention. In addition, tool conformational antibodies generated to stabilise specific conformations could potentially enable small molecule screening campaigns aimed at the discovery of new chemical matter able to re-direct the aggregation pathway towards non-toxic species [129]. The existence of differentiated and transient conformers also raises a fundamental question related to all protein aggregation-driven disorders: which is the most relevant species behind the pathology? Is it a number of distinct conformations that are equally important?

Antibody-mediated studies concerning α-synuclein, Aβ, prion and tau protein aggregation also seem to indicate that they share key biophysical and biophysical characteristics, implying that the protein misfolding and plaque accumulation could occur through the same or similar fundamental biophysical mechanisms. In addition, emerging studies indicating the important role of prion protein in other neurodegenerative diseases highlights the complexity and interconnection between different neurodegenerative disorders.

It is clear that significant progress has been made towards understanding the multifactorial mechanisms behind amyloid-based diseases, and animal models developed to mimic amyloid-related diseases have played a pivotal role. A number of reviews have been published describing animal models mimicking different neurodegenerative diseases in detail, including associated benefits and disadvantages [130–133]. Animal models provide opportunity to follow the disease development and progression and have been widely used to study factors that promote the Aβ plaque formation. They have also contributed considerably to the current knowledge on the toxic species and became an essential tool in the development and testing of new immunotherapies, prior to advancing a potential drug candidate into clinical trials. For example, APP transgenic mice model provided strong evidence for the toxicity of Aβ oligomers, as many of the pathological and functional changes in mice occur prior to plaque development. Also, findings generated in PS1 FAD mutant mice showing elevated level of Aβ42 and no plaques, have supported the toxic role of soluble Aβ. Tg2576 and 3xTg-AD mice models identified a 56kDa oligomeric toxic form of Aβ, which results in memory impairment in these mice, and when injected into rats disrupts cognitive functioning [130–132]. Thus, animal models can serve as complementary tools to validate conformational antibodies targeting specific oligomeric toxic forms of proteins and will continue to play a central role in improving existing knowledge of amyloid-based diseases and in preclinical testing of the current and novel therapeutic interventions.

Despite a number of advantages, animal models raise a few issues. For an animal model to be useful, the transgenic organism must exhibit the essential pathological, physiological or behavioural features of the human disease. Despite taking advantage of mutations, such as identified in FAD, none of the animal models fully reproduce all pathological conditions of the human disease. This raises an important question as to which determinants: behaviour, synapse, dendritic responses, reduction in toxic species, inflammation, astroglial and microglial responses become the best measure and guide for drug development? It is important to remember that regardless of the impressive morphological resemblance to neurodegenerative disorders, extrapolating Tg mouse results to humans is not straight forward, as the triggering events of complex aging processes, and hence the exact pathway to the disease, are not necessarily the same in each individual [130–132].

With encouraging results from animal models and more antibodies entering clinical trials, the hope is that passive immunotherapy will soon start to impact neurodegenerative disease to the benefit of patients.

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Author Contributions

Both authors contributed to the text, format and figures of this review.

Conflicts of Interest

A. Lawson holds shares and share options in UCB.

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