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REVIEW ARTICLE

Recombinant Antibody Fragments for Immunotherapy of Parkinson's Disease

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Abstract

Parkinson's disease (PD) is the second most common age-related neurodegenerative disorder. Multiple genetic and environmental factors leading to progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SN) and consequent depletion of dopamine were described. Current clinical approaches, such as dopamine replacement or deep brain stimulation using surgically implanted probes, provide symptomatic relief but cannot modify disease progression. Therefore, disease-modifying therapeutic tools are urgently needed. Immunotherapy approaches, including passive transfer of protective antibodies and their fragments, have shown therapeutic efficacy in several animal models of neurodegenerative diseases, including PD. Recombinant antibody fragments are promising alternatives to conventional full-length antibodies. Modern computational approaches and molecular biology tools, directed evolution methodology, and the design of tissue-penetrating fusion peptides allowed for the development of recombinant antibody fragments with superior specificity and affinity, reduced immunogenicity, the capacity to target hidden epitopes and cross the blood-brain barrier (BBB), higher solubility and stability, the ability to refold after heat denaturation, and inexpensive large-scale production. In addition, antibody fragments do not induce microglia Fcγ receptor (FcγR)-mediated proinflammatory response and tissue damage in the central nervous system (CNS), because they lack the Fc portion of the immunoglobulin molecule. In the present review, we summarized data on recombinant antibody fragments evaluated as immunotherapeutics in preclinical models of PD and discussed their potential for developing therapeutic and preventive protocols for patients with PD.

Key points

Parkinson's disease (PD) is the second most common age-related multifactorial neurodegenerative disorder.

Current clinical approaches provide symptomatic relief but cannot modify disease progression.

Immunotherapy is a feasible therapeutic approach and has shown efficacy in several animal models of PD.

Recombinant antibody fragments, promising alternatives to full-length immunoglobulins, offer great opportunities for developing therapeutic and preventive protocols for patients with PD.

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1 Introduction

Parkinson's disease (PD) is the second most common age-related neurodegenerative disorder. Multiple genetic and environmental factors, including inflammation, mitochondrial dysfunction, oxidative stress, glymphatic system impairment, gut dysbiosis, and the accumulation of pathological aggregates of α-synuclein (α-syn) in the Lewy bodies and Lewy neurites with the subsequent progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SN) and consequent depletion of dopamine were described [1–5]. Current clinical approaches, such as dopamine replacement or deep brain stimulation using surgically implanted probes, provide symptomatic relief but cannot modify disease progression. Therefore, disease-modifying therapeutic tools are urgently needed.

In physiological conditions, α-syn, a 14 kDa cytosolic unfolded monomeric or soluble oligomeric (dimers and trimers) neuronal protein, participates in the regulation of synaptic vesicle trafficking, fusion, and neurotransmitter release [6]. However, various factors, including

phosphorylation and impaired proteasome function, can influence the folding and aggregation of α-syn following a prion-like mechanism, leading to protofibrils and fibrils [6]. Accumulation of intraneuronal aggregates of misfolded α-syn impairs mitochondrial function, calcium homeostasis, and autophagy. An increase in Ca^{2+} levels in neurons activates dopamine synthesis; still, this newly synthesized dopamine could not be properly incorporated into synaptic vesicles because of disrupted axonal transport caused by α-syn, and cytosolic dopamine concentration rises. Finally, these abnormalities lead to dopaminergic neuron degeneration [6]. Likewise, extracellular toxic forms of α-syn activate microglia and trigger oxidative stress and inflammatory response. Neuroinflammation and oxidative stress, in turn, lead to further α-syn modification, misfolding, and aggregation, creating a vicious cycle [6]. Therefore, the development of therapeutic molecules targeting toxic forms of α-syn without interfering with the physiological function of the protein is of considerable significance.

Immunotherapy approaches, including passive transfer of protective antibodies and their fragments, have shown therapeutic efficacy in several animal models of neurodegenerative diseases, such as Alzheimer's disease (AD), PD, frontotemporal dementia (FTD), Huntington´s disease (HD), and transmissible spongiform encephalopathies (TSEs) [7–16]. It has been demonstrated that these immunotherapeutics may target toxic extra and intracellular misfolded proteins involved in the pathogenesis of AD, PD, FTD, HD, or TSEs [10, 12, 17, 18]. However, results of clinical trials raised safety concerns because of inflammatory and autoimmunity-related adverse effects. Thus, anti-amyloid beta antibodies showed dose-related adverse effects, such as amyloid-related imaging abnormalities (ARIA), resulting in bleeding and brain swelling in participants, which may limit their use [16]. Also, the timing of immunotherapy and adequate monitoring of its effects are essential: early-stage patients should be included in clinical trials, and novel biomarkers and non-invasive diagnostic protocols must be developed. Currently, there are two anti-amyloid beta antibodies approved by the Food and Drug Administration (FDA); however, both antibodies carry a risk of ARIA. For this reason, baseline brain imaging is necessary to determine which patients may use these antibodies. Also, frequent periodic brain imaging during treatment is recommended to attenuate the risk of ARIA.

Recombinant antibody fragments are promising alternatives to full-length immunoglobulins and offer great opportunities for biomedicine. Modern computational approaches and molecular biology tools, directed evolution methodology, and the design of tissue-penetrating fusion peptides allowed the development of recombinant antibody fragments with superior specificity and affinity, reduced immunogenicity, the capacity to target hidden epitopes and cross the blood–brain barrier (BBB), higher solubility and stability, the ability to refold after heat denaturation, and inexpensive large-scale production [19–29]. In addition, antibody fragments do not induce microglia Fcγ receptor (FcγR)-mediated pro-inflammatory response and tissue damage in the central nervous system (CNS) because they lack the Fc portion of the immunoglobulin molecule [30, 31]. Finally, combining two or more fragments with different specificities and additional molecules, such as toxins or cytokines, allows the development of multifunctional constructs for simultaneously targeting multiple pathological pathways [21, 32, 33]. Thus, recombinant antibody fragments may be promising molecules for the prevention and treatment of several neurodegenerative diseases, such as AD, PD, FTD, HD, TSEs, tauopathies, and synucleinopathies.

There are different antibody formats currently being studied as therapeutic molecules in clinical trials and preclinical models of cancer, as well as infectious, neurological, and autoimmune diseases: antigen-binding fragments (Fab), single-chain fragment variable (scFv) consisting of the antigen-binding domains of Ig heavy (VH) and light (VL) chain regions, and single-domain antibody fragments (sdAbs), such as camelid heavy-chain variable domains (VHHs) and shark variable new antigen receptor (VNARs). A handful of recombinant antibody fragments have been approved by the FDA for therapeutic use in individuals with diabetic retinopathy, age-related macular degeneration, acquired thrombotic thrombocytopenic purpura, thrombosis, Crohn's disease, and rheumatoid arthritis [34].

In the present review, we summarized data on recombinant antibody fragments evaluated as immunotherapeutics in pre-clinical models of PD and discussed their potential for developing therapeutic and preventive protocols for patients with PD.

2 Clinical Trials Evaluating Traditional Immunotherapy Approaches: Active Immunization and Full-Length Antibody Administration

Several anti-α-syn immunotherapeutic strategies were evaluated in clinical trials but could not inhibit the progression of PD and failed to provide clinical benefit to the participants. Active immunization with different epitopes of α-syn and passive transfer of protective antibodies binding to different regions of α-syn were assessed. Here, we briefly describe all clinical trials that have concluded, have been withdrawn, are underway, or are currently recruiting participants.

2.1 Active immunization

2.1.1 AFFITOPE® PD01A and PD03A Active Immunization Studies in Healthy Participants and Patients with PD

Several active immunization Phase 1 studies assessing tolerability and safety and exploring the immunogenicity and therapeutic activity of AFFITOPE® PD01A and PD03A (NCT02758730, NCT01568099, NCT02618941, NCT02216188, and NCT02267434) have been completed (www.clinicaltrials.gov). One of these trials was withdrawn (NCT02758730), and four were completed without results posted. AFFITOPE® PD01A and PD03A (developed by AFFiRiS AG and owned by AC Immune SA) are short peptides affinity-selected from a phage display peptide library; they mimic an epitope in the C-terminal region of human α-syn and do not elicit an α-syn-specific T cell response [35]. The peptides were adsorbed to aluminum oxide and administered by subcutaneous injection. Specific anti-α-syn antibodies were detected in participants [36].

In 2023, AC Immune SA announced a new multicenter Phase 2 study to evaluate the safety, tolerability, immunogenicity, and pharmacodynamic effects of PD01A conjugated with keyhole limpet hemocyanin (KLH) in patients with early stages of PD (NCT06015841). This trial will be completed in 2028.

2.1.2 UB-312 Vaccine Candidate Studies in Healthy Participants and Patients with PD

UB-312 vaccine candidate (Vaxxinity, Inc) consists of the 10-residue C-terminal epitope of α-syn conjugated to a UBITh T helper peptide via a small peptide linker, which was shown to induce anti-oligomeric and fibrillar α-syn antibodies and prevent motor deficits in a mouse model of α-synucleinopathy [37, 38]. A first-in-human Phase 1 study to determine the safety, tolerability, and immunogenicity of UB-312 demonstrated that the vaccine can induce α-syn-specific antibodies; importantly, adverse events after three intramuscular injections were mild, transient, and self-resolving [39]. A new Phase 1b study (NCT05634876) to determine the safety, tolerability, and immunogenicity of UB-312 in individuals with PD is recruiting participants and is expected to be completed in 2025.

2.2 Passive Immunization

2.2.1 Passive Immunization with ABBV-0805

ABBV-0805 is a monoclonal antibody binding selectively to aggregated α-syn with very low affinity for monomers. In both prophylactic and therapeutic settings in mouse models of PD, ABBV-0805 decreased α-syn aggregates and prolonged survival in a dose-dependent manner [40]. On the basis of preclinical performance, a humanized version of this antibody was proposed to be evaluated in a Phase 1 clinical trial by AbbVie (NCT04127695), but was soon withdrawn.

2.2.2 Passive Immunization with MEDI1341

MEDI1341 is a high-affinity anti-α-syn antibody that binds to monomeric and aggregated forms of α-syn and blocks the uptake of aggregated α-syn into cells [41]. It has been shown that MEDI1341 enters the brain after intravenous infusion and reduces α-syn levels [41]. A multicenter, randomized, double-blind, placebo-controlled study in male and female subjects with Parkinson's disease conducted by AstraZeneca was completed in 2022, and no study results were posted (NCT04449484).

2.2.3 Passive Immunization with Cinpanemab (BIIB054)

Cinpanemab (BIIB054) is a high-affinity human IgG1 anti-α-syn antibody that binds to the N-terminal amino acid residues 1–10 of α-syn [42]. The authors demonstrated that BIIB054 inhibits α-syn spread and aggregation, reduces neuronal loss, and ameliorates motor impairments in a mouse model of PD [42].

Phase 1 clinical trials (NCT02459886 and NCT03716570) conducted by Biogen evaluated the safety, tolerability, immunogenicity, and serum pharmacokinetics (PK) profile of BIIB054 in healthy participants and patients with PD. Although the antibody was well tolerated and showed an acceptable safety profile, the study was completed without posting the results [43]. Another multicenter Phase 2 study (NCT03318523) did not provide evidence of efficacy and was closed; thus, Biogen discontinued the development of BIIB054 for PD.

2.2.4 Passive Immunization with Prasinezumab

PRX002/RG7935 (PRX002, prasinezumab) is a humanized IgG1 mAb binding to the C-terminus of α-syn and targeting aggregated forms of α-syn. This antibody has been shown to reduce intracellular α-syn accumulation and axonal pathology, prevent the loss of tyrosine hydroxylase (TH) in the striatum, and ameliorate behavioral deficits in a PD-like model [44, 45]. In a Phase 1 trial, PRX002 was safe and tolerable after three intravenous infusions every 4 weeks in patients with idiopathic PD [46]. Also, dose-dependent, rapid, and prolonged reduction in free serum α-syn and an increase of PRX002 concentrations in cerebrospinal fluid (CSF) were observed, suggesting that the antibody can cross the BBB and bind extracellular α-syn in the brain [46]. Subsequently,

in the PASADENA study (Hoffmann-La Roche), a Phase 2 multicenter, randomized, double-blind, and placebo-controlled trial (NCT03100149), individuals with early-stage PD across the USA and Europe received intravenous prasinezumab monthly for 52 weeks, and no significant effect on the disease progression compared with placebo was observed [47, 48]. However, eligible participants will continue receiving a low-dose or a high-dose treatment for an additional 52 months (Part 2) and, subsequently, will be invited to participate in Part 3 extension for an additional 260 weeks. Thus, this trial will be completed by September 2026.

Hoffmann-La Roche conducted another phase 2B multicenter study (NCT04777331) to evaluate prasinezumab in patients with early PD who are on stable symptomatic PD medication, and this trial is expected to be completed by the end of 2026.

3 Recombinant Antibody Fragments

3.1 Anti-α-syn Recombinant Antibody Fragments

An alternative immunotherapeutic strategy applies recombinant antibody fragments for destabilizing or inhibiting intracellular α-syn aggregation and accumulation of toxic fibrils (Table 1).

The first anti-α-syn high-affinity scFv antibody fragments were selected by Dr. Sierks's group from a large human scFv phage display library and shown to specifically bind the α-syn intracellularly and inhibit the formation of detergent-insoluble toxic synuclein aggregates [49–51]. Subsequently, the same group isolated a panel of scFvs binding to morphologically distinct oligomeric and/or fibrillary forms of α-syn and blocking cytotoxicity of aggregated α-syn [51–53]. Notably, some of these scFvs bound specifically to α-syn aggregates in the PD brain, suggesting their potential use as immunotherapeutics [53].

Fassler and collaborators isolated a novel scFv antibody designated sMB08 from a human antibody fragment library containing the repertoire from over 110 healthy individuals and demonstrated that the sMB08 antibody fragment binds with a high affinity to both human and mouse α-syn oligomers and preformed fibrils (PFF) [54]. Also, sMB08 scFv inhibited α-syn aggregation in vitro, entered differentiated human neuroblastoma SH-SY5Y cells, and protected them from α-syn oligomers/PFF- and PD brain extract-induced toxicity [54]. Notably, the sMB08 antibody detected α-syn in the striatum and cortex of patients with PD and dementia with Lewy bodies (DLB) [54]. Finally, the sMB08 scFv attenuated neuroinflammation, dopaminergic neuron loss, and motor dysfunction after intranasal injection in various pre-clinical models of PD [54]. Interestingly, while Fc containing full-length anti-α-syn antibodies increased the expression of proinflammatory cytokines TNF-α and IL-6 in the microglia after exposure to PFF, sMB08 scFv downregulated the expression of these cytokines in a dose-dependent manner, indicating a clear advantage of the latter over full-length immunoglobulin molecules [54].

Gupta and collaborators applied a different approach for the construction of anti-α-syn scFvs: they used the VH and VL sequences of a previously described conformation-specific anti-α-syn monoclonal antibody (Syn-F2) and combined them using a (Gly4Ser)3 linker [55, 56]. Two obtained scFvs, scFv-pF, and scFv-pC, specifically bound to α-syn fibrils and oligomers but not to monomers, and detected intracellular aggregates in the brain from individuals with Lewy body pathology [55, 56]. Furthermore, scFv-pF and scFv-pC could inhibit the seeding of α-syn aggregation and reduce α-syn toxicity in an SH-SY5Y cell model of PD [55, 56].

In an interesting study by Spencer et al., anti-oligomeric α-syn scFv was fused to the 38-amino-acid domain of apolipoprotein B (ApoB) binding to the low-density lipoprotein (LDL) receptor to enhance brain penetration [57]. Then, this modified scFv-ApoB was shown to reduce the accumulation of pathogenic α-syn in neurons and neuronal loss in the neocortex and hippocampus and ameliorate behavioral deficits in a mouse model of PD/DLB [58].

Two nanobodies, NbSyn2 and NbSyn87, binding to the highly exposed C-terminal region of α-syn and inhibiting α-syn aggregation, were selected from an immune single-domain camelid phage display antibody library [58]. These nanobodies were shown to reduce α-syn oligomer-induced cellular toxicity in vitro [59]. Subsequently, NbSyn87 was fused to a proteasome-targeting proline, aspartate or glutamate, serine, and threonine (PEST) motif, capable of modulating monomeric concentrations of target proteins, and evaluated in an animal model of PD. Unfortunately, this NbSyn87*PEST showed only a modest effect on motor function and induced an inflammatory response after gene therapy using an adeno-associated virus 5 (AAV5) vector [60]. In contrast, in the same study, the authors demonstrated that another intrabody, VH14 [61], binding to the critical determinant of the fibrillation process of α-syn, non-amyloid-β component (NAC), had a pronounced protective effect and minimal inflammatory response in the same experimental setting: fused to a PEST and delivered using AAV5 [60]. Previously, a scFv intrabody, NAC32, binding to the NAC fragment of α-syn, was isolated from a yeast-displayed non-immune human scFv library by sequential magnetic bead enrichment and flow cytometric sorting and shown to inhibit α-syn-induced cytotoxicity in vitro [62]. Subsequently, Chen and collaborators demonstrated that recombinant AAV5 expressing NAC32 scFv increases the survival of dopaminergic neurons and improves locomotor behavior after

Table 1 Anti-α-syn recombinant antibody fragments evaluated in preclinical models of Parkinson's disease (PD)

Recombinant antibody fragments	Target	Key points	Refs
A panel of scFvs	α-syn	Human scFvs were selected from a large scFv phage display library. These fragments: (a) Bound specifically to intracellular α-syn (b) Inhibited the formation of detergent-insoluble toxic α-syn aggregates (c) Bound specifically to morphologically distinct oligomeric and/or fibrillary forms of α-syn (d) Blocked cytotoxicity of aggregated α-syn (e) Bound specifically to α-syn aggregates in the PD brain	[49–53]
Human scFv sMB08	α-syn	This human scFv: (a) Bound with a high affinity to both human and mouse α-syn oligomers and pre-formed fibrils (PFF) (b) Inhibited α-syn aggregation in vitro (c) Entered into differentiated human neuroblastoma SH-SY5Y cells (d) Protected SH-SY5Y cells from α-syn oligomers/PFF- and PD brain extract-induced toxicity (e) Detected α-syn in the striatum and cortex of patients with PD and dementia with Lewy bodies (DLB) (f) Attenuated neuroinflammation, dopaminergic neuron loss, and motor dysfunction after intranasal injection in various preclinical models of PD	[54]
scFv-pF, scFv-pC	α-syn	These scFvs: (a) Bound specifically to α-syn fibrils and oligomers but not to monomers (b) Detected intracellular aggregates in the brain from individuals with Lewy body pathology (c) Inhibited the seeding of α-syn aggregation (d) Reduced α-syn toxicity in neuroblastoma cells	[55, 56]
Anti-oligomeric α-syn scFv fused to the 38-amino-acid domain ofApoB	α-syn	In a mouse model of PD/DLB, this fusion molecule: (a) Reduced the accumulation of pathogenic α-syn in neurons (b) Reduced neuronal loss in the neocortex and hippocampus (c) Ameliorated behavioral deficits	[57]
Single-domain VH nanobodies: NbSyn2 and NbSyn87	α-syn	These nanobodies: (a) Bound to the highly exposed C-terminal region of α-syn (b) Inhibited α-syn aggregation (c) Reduced α-syn oligomer-induced cellular toxicity in vitro NbSyn87, fused to a proteasome-targeting proline, aspartate or glutamate, serine, and threonine (PEST) motif, showed only a modest effect on motor function in an animal model of PD. NbSyn87*PEST fusion molecule induced an inflammatory response in mice.	[58–60]
VH14 single-domain nanobody	α-syn	This nanobody: (a) Bound to the critical determinant of the fibrillation process of α-syn, non-amyloid-β component (NAC) (b) Had a pronounced protective effect in an animal model of PD after fusion with PEST (c) Induced minimal inflammatory response	[60, 61]
scFv intrabody, NAC32	α-syn	This human scFv: (a) Bound to the NAC fragment of α-syn (b) Inhibited α-syn-induced cytotoxicity in vitro (c) Increased the survival of dopaminergic neurons (d) Improved locomotor behavior after intracranial administration to rats overexpressing α-syn	[62–64]
Single-domain camelid VH Nbα-syn01	α-syn	This single-domain nanobody: (a) Bound to the N-terminal region of α-syn (amino acids 43–56) known to participate in mediating the membrane fusion of α-syn and its aggregation (b) Inhibited α-syn aggregation (c) Inhibited toxicity in SH-SY5Y cells (d) Detected Lewy bodies in brain samples from individuals with PD and DLB	[65]
PFFNB2	α-syn	This nanobody: (a) Preferentially bound to α-syn fibrils but not α-syn monomers (b) Dissociated preformed PFFs (c) Inhibited α-syn toxicity in primary neurons in vitro (d) Prevented prion-like α-syn spreading in the mouse model of PD after intraventricular injection	[66]

scFv single-chain fragment variable, *α-syn* α-synuclein, *ApoB* apolipoprotein B

intracranial administration to rats overexpressing α-syn [63, 64].

Another single domain antibody, Nbα-syn01, was selected after screening an immune camelid VHH phage display library against monomeric α-syn and shown to bind to the N-terminal region of α-syn (amino acids 43–56) [65]. Notably, this region is known to participate in mediating the membrane fusion of α-syn and its aggregation in vitro and in vivo, suggesting that it can be an appropriate target for immunotherapy [65]. The authors demonstrated that Nbα-syn01 had a higher affinity toward α-syn fibrils compared with a monomeric form, inhibited α-syn aggregation and toxicity in SH-SY5Y cells, and detected Lewy bodies in brain samples from individuals with PD and DLB [65].

Butler and collaborators constructed a synthetic nanobody library and selected a panel of nanobodies that preferentially bind to α-syn fibrils but not α-syn monomers [66]. The most promising nanobody, PFFNB2, dissociated preformed PFFs, inhibited α-syn toxicity in primary neurons in vitro, and prevented prion-like α-syn spreading in the mouse model of PD after intraventricular injection [66].

Thus, there are currently many nanobodies binding to different regions of monomeric, oligomeric, and fibrillar α-syn. Some of them can prevent α-syn aggregation, and others can dissociate preformed PFFs. Importantly, they inhibited α-syn toxicity in primary neurons in vitro and ameliorated neuronal loss and behavioral deficits in preclinical models of the disease.

3.2 Anti-Leucine-Rich Repeat Kinase 2 (LRRK2) Recombinant Antibody Fragments

Some common mutations in the gene coding for leucine-rich repeat kinase 2 (LRRK2) have been linked to early-onset familial and late-onset sporadic PD, and the protein is considered an attractive target for immunotherapy of the disease [67–70]. This enzyme has two catalytic activities: GTPase activity mediated by the Roc domain and Ser/Thr protein kinase activity [71]. The Ser/Thr protein kinase activity is particularly interesting for drug development for PD, and numerous LRRK2 kinase inhibitors with improved specificity and pharmacokinetics and enhanced BBB crossing have been developed and tested in preclinical models [67–69]. As with anti-α-syn intrabodies, LRRK2-specific recombinant antibody fragments may have potential therapeutic value.

Notably, while ATP-competitive inhibitors are currently approved by the FDA, long-term inhibition of LRRK2 with these molecules was associated with toxic side effects, including kidney and lung abnormalities [69, 71]. However, in a recent study, Baptista and collaborators found that, despite morphological changes in the lungs of macaque monkeys caused by three different LRRK2 inhibitors, the respiratory function was not compromised [72]. Importantly, no morphological changes were detected in the kidney or brain, and those in the lungs disappeared when treatment stopped [72]. Certainly, clinical trials monitoring pulmonary function will need to define the dose and frequency of the inhibitor application for the optimum outcome.

That being said, designing molecules that bind outside the ATP-binding pocket may represent an interesting and promising approach. With this hypothesis in mind, Singh and collaborators isolated nanobodies binding to a different region of the LRRK2, capable of inhibiting kinase activity in human embryonic kidney 293 (HEK293) cells overexpressing LRRK2 [73].

3.3 Novel Approaches

The BBB may be an obstacle to antibody passage into the brain. Fortunately, recombinant antibody fragments can be easily designed and constructed by applying modern molecular biology tools, and efforts were made to produce tissue-penetrating fusion molecules [74]. Thus, anti-transferrin receptor (TfR) antibody fragments were used as a shuttle to transport antibodies of interest across the BBB by receptor-mediated transcytosis [75–79]. Recently, Clarke and collaborators reported the fusion of the TfR1-specific shark variable new antigen receptor (VNAR) recombinant antibody, TXB4, to a tropomyosin receptor kinase B (TrkB) receptor agonist antibody capable of enhancing neuronal survival and demonstrated that the TXB4-anti-TrkB multivalent antibody fragment crosses the BBB and accumulates in the brain of mice after peripheral administration [80]. Furthermore, the TXB4-anti-TrkB antibody prevented neuronal loss in a mouse model of PD [80].

Another promising and viable therapeutic approach may be based on the viral-vector-mediated gene delivery of target-specific antibody fragments. We have previously mentioned preclinical studies reporting the application of AAV5 expressing anti-α-syn antibody fragments [60, 63, 64]. Moreover, AAV vector-mediated delivery of anti-amyloid beta antibody fragments was successfully applied in preclinical models of AD [81]. Gene-mediated expression of antibody fragments in the brain allows for intracellular production of therapeutic antibodies targeting intracellular toxic protein aggregates. In addition, this approach makes unnecessary repeated injections of antibodies and prevents antibody loss by systemic elimination. Nowadays, many AAV vectors are approved by the FDA and are widely used for gene therapy in humans, suggesting the feasibility of their use in neurodegenerative diseases.

4 Concluding Remarks

Despite numerous clinical studies on passive immunotherapy in patients with PD using anti-α-syn full-length antibodies, α-syn-specific recombinant antibody fragments have

been evaluated only in pre-clinical models of the disease. However, a handful of recombinant antibody fragments have been approved by the FDA for therapeutic use in individuals with various pathologies, as mentioned above [34]. We think that many of the scFvs discussed in this review and shown to target α-syn and/or LRRK2 warrant further in vivo evaluation and may represent promising therapeutic approaches for PD. Importantly, patients with other synucleinopathies, such as multiple system atrophy (MSA) and DLB, can also benefit from treatment with anti-α-syn immunotherapeutics.

Antibody immunotherapy targets intra- and extracellular protein aggregates, blocking their propagation and toxic effects, and age-related neurodegenerative processes, such as inflammation and cell senescence [82]. The limited application of antibodies and their recombinant fragments may be overcome by discovering new potential targets and designing new antibodies binding to different epitopes or specific conformations of toxic protein aggregates. Likewise, the development of new platforms helping antibody fragments reach protective concentrations in the brain after systemic administration and applying an antibody cocktail targeting multiple pathologies involved in neurodegeneration may enhance the effectiveness of immunotherapy. Hopefully, recombinant antibody fragments will soon become available for the prevention and treatment of several neurodegenerative diseases.

Declarations

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