Published in final edited form as:

Pharmacol Ther. 2013 June ; 138(3): 311–322. doi:10.1016/j.pharmthera.2013.01.013.

Immunotherapy for neurodegenerative diseases: focus on α -synucleinopathies

Elvira Valera and Eliezer Masliah*

Department of Neurosciences, University of California, San Diego, La Jolla, California 92093

Abstract

Immunotherapy is currently being intensively explored as much-needed disease-modifying treatment for neurodegenerative diseases. While Alzheimer's disease (AD) has been the focus of numerous immunotherapeutic studies, less attention has been paid to Parkinson's disease (PD) and other neurodegenerative disorders. The reason for this difference is that the amyloid beta ($A\beta$) protein in AD is a secreted molecule that circulates in blood and is readily recognized by antibodies. In contrast, α -synuclein (α -syn), tau, huntingtin and other proteins involved in neurodegenerative diseases have been considered to be exclusively of intracellular nature. However, the recent discovery that toxic oligomeric versions of α -syn and tau accumulate in the membrane and can be excreted to the extracellular environment has provided a rationale for the development of immunotherapeutic approaches for PD, dementia with Lewy bodies, frontotemporal dementia, and other neurodegenerative disorders characterized by the abnormal accumulation of these proteins. Active immunization, passive immunization, and T cell-mediated cellular immunotherapeutic approaches have been developed targeting $A\beta$, α -syn and tau. Most advanced studies, including results from phase III clinical trials for passive immunization in AD, have been recently reported. Results suggest that immunotherapy might be a promising therapeutic approach for neurodegenerative diseases that progress with the accumulation and propagation of toxic protein aggregates. In this manuscript we provide an overview on immunotherapeutic advances for neurodegenerative disorders, with special emphasis on α -synucleinopathies.

Keywords

Immunotherapy; neurodegenerative disease; α -synucleinopathies; α -synuclein; active immunization; passive immunization; vaccination; Parkinson's disease; Multiple system atrophy; dementia with Lewy bodies; α -synuclein propagation

1. Introduction

The lack of disease-modifying treatments for neurodegenerative diseases explains the need for developing new therapies that target the molecular origins of the pathology. Interestingly, many of these neurodegenerative diseases are accompanied with an abnormal

© 2012 Elsevier Inc. All rights reserved.

*To whom correspondence may be addressed at: Department of Neurosciences, University of California, San Diego, La Jolla, CA 92093-0624, USA. emasliah@ucsd.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conflict of interest:

None

accumulation of soluble proteins in insoluble intracellular or extracellular aggregates, and it is yet to be determined whether the toxicity resides in the insoluble aggregates and/or their soluble oligomeric precursors. In the last decade there has been an increased interest in the development of immunotherapies focused on the clearance of these aggregates and oligomers, as they are believed to be critical components in the neurodegeneration pathways.

Initially, immunotherapeutic approaches for neurodegenerative disorders were focused on targeting and clearing extracellular protein aggregates, the most relevant example being amyloid beta (A β) peptide accumulation in Alzheimer's disease (AD). However, intracellular accumulation of toxic proteins, a hallmark of numerous neurodegenerative diseases, is currently in the spotlight for the development of new immunotherapies after the discovery that these aggregates may accumulate in the plasma membrane and can be secreted to the extracellular environment. Such is the case of α -synuclein (α -syn), tau, prion protein (PrP) and huntingtin, for which immunotherapeutic approaches are being developed for the clearance of aggregates and reversal of cognitive deficits associated with their accumulation. Accordingly, immunotherapy might represent a plausible strategy for the management of several neurodegenerative disorders, specifically targeting membrane-bound or extracellular forms of aggregation-prone proteins.

2. Immunotherapy for Alzheimer's disease, Frontotemporal Dementia, and other neurodegenerative diseases

The state of progress of immunotherapeutic studies for neurodegenerative disorders depends on the specific disease and the nature of the toxic protein accumulation. First and foremost, AD has been the target of extensive work for the development of immunotherapies, and currently a number of ongoing clinical trials focus on the clearance of A β aggregates via immunotherapy (Table 1). Initial pre-clinical studies showed that immunization against the A β peptide could provide protection and reversal of the AD pathology in animal models (Schenk et al., 1999). Since then, several types of A β immunotherapy for AD have been under investigation, involving either direct immunization with intact A β , with synthetic fragments of A β conjugated to a carrier protein, or passive immunization with monoclonal antibodies directed against the A β peptide (Delrieu et al., 2012; Morgan, 2006) (Table 1). However, several adverse events have been described, such as meningoencephalitis with the synthetic peptide AN1792, and vasogenic edema and microhemorrhages with the monoclonal antibody Bapineuzumab (Delrieu et al., 2012; Orgogozo et al., 2003; Uro-Coste et al., 2010). Although A β immunotherapy has resulted in the clearance of amyloid plaques in AD patients, this clearance has not always resulted in significant reduction of progressive neurodegeneration (Holmes et al., 2008). An initial Phase II clinical trial of the monoclonal antibody Solanezumab showed a good safety profile with encouraging indications from biomarkers in cerebrospinal fluid and plasma (Farlow et al., 2012). However, very recently, two separate Phase III clinical trials of Solanezumab missed the goal of significantly slowing the progression of AD, but the pooled results of the two trials found 34% to 42% less cognitive decline in mild AD patients compared to those on placebo at 18 months. Earlier in 2012, a Phase III clinical trial of the intravenous form of the monoclonal antibody Bapineuzumab was halted since it did not improve cognition or daily functioning of patients compared to placebo. Importantly, in a previous Phase II study of Bapineuzumab, patients with one or two alleles of APOE ϵ 4 showed no treatment effect and were more likely to suffer vasogenic edema. No significant differences were found in the primary efficacy analysis either (Salloway et al., 2009). Phase III studies with the intravenous immunoglobulin Gammagard have not shown improvement in most of the symptoms of AD, but nor did they show any further decline on measures of cognition, memory, daily functioning or mood over the three-year long trial. In an attempt to overcome the difficulties

encountered to date and reduce potential adverse effects, exciting new experimental approaches are currently being investigated. These new approaches include antibodies recognizing specific conformational epitopes, single chain variable fragment (scFv) antibodies, or intrabodies (Pul et al., 2011). For more detailed information on the topic of AD immunotherapies, the reader is referred to numerous reviews published to date (Citron, 2010; Corbett et al., 2012; Fernández et al., 2012; Fu et al., 2010; Golde et al., 2009; Lemere & Masliah, 2010; Menéndez-González et al., 2011; Morgan, 2011; Panza et al., 2012; Town, 2009; Wang et al., 2010).

Other potential immunotherapeutic targets for the treatment of AD are secretases and tau. A study by Atwal and colleagues (Atwal et al., 2011) showed that treatment with an anti-beta-secretase 1 (BACE1) antibody reduced endogenous BACE1 activity and A β production in human cell lines expressing the amyloid precursor protein and in cultured primary neurons. The anti-BACE1 antibody also reduced A β concentrations in the CNS of mice and monkeys, consistent with the uptake of the antibody across the brain blood barrier. Passive immunotherapy targeting BACE1 might provide a potential approach for treating AD, although therapeutic success with anti-BACE1 would depend on improving antibody uptake into the brain (Yu et al., 2011).

More recently, some groups have also investigated the possibility of immunotherapy against tau for the treatment not only of AD, but also of other tauopathies (Panza et al., 2012; Ubhi & Masliah, 2011). Abundant tau-positive neurofibrillary lesions constitute a defining neuropathological characteristic of AD (Grundke-Iqbal et al., 1986; Lee et al., 1988; Mandelkow & Mandelkow, 1993; Wolozin et al., 1986), but filamentous tau pathology is also central to a number of other disorders, such as progressive supranuclear palsy, corticobasal degeneration, and familial frontotemporal dementia (FTD) and parkinsonism linked to chromosome 17 (Buée & Delacourte, 1999; Robert & Mathuranath, 2007; Spillantini & Goedert, 1998). FTD is the clinical syndrome caused by degeneration of the frontal lobe of the brain, degeneration that may also extend to the temporal lobe (Froelich-Fabre et al., 2004; Neary et al., 2005; Snowden et al., 2007). Approximately 30–50% of FTD cases present with tau pathology at post-mortem (Froelich-Fabre et al., 2004; Seelaar et al., 2011; Shi et al., 2005; Taniguchi et al., 2004). In AD, FTD, and other tauopathies, hyperphosphorylated tau accumulates within neurons forming intracellular neurofibrillary tangles (Grundke-Iqbal et al., 1986; Hampel & Teipel, 2004; Iwatsubo et al., 1994; Lee et al., 1988; Mandelkow & Mandelkow, 1993; Wolozin et al., 1986). Interestingly, it has been observed that cognitive impairment in AD patients correlates well with the degree of tau pathology (Arriagada et al., 1992; Bierer et al., 1995), therefore removing tau aggregates has become a promising therapeutic approach for the treatment of tauopathies (Götz et al., 2012; Lasagna-Reeves et al., 2011). Several studies have shown that both active and passive immunization targeting the tau epitopes that are involved in hyperphosphorylation and pathological accumulation of the protein successfully reduce tau aggregates *in vivo* and slow or prevent behavioral deficits in mouse models of tauopathy (Asuni et al., 2007; Boutajangout et al., 2011; Boutajangout et al., 2010; Chai et al., 2011; Gu & Sigurdsson, 2011; Lasagna-Reeves et al., 2011; Troquier et al., 2012; Wisniewski & Boutajangout, 2010).

The development of immunotherapies for other neurodegenerative diseases, such as Huntington's disease (HD), is much less advanced. An *in vitro* study showed that aggregation of expanded polyglutamine repeat sequences expressed in COS-7 cells was dramatically reduced by co-expression of scFv antibodies to the huntingtin protein (Lecerf et al., 2001). In 2003, Miller and colleagues reported the first study examining the use of a DNA vaccine against mutant huntingtin in a HD mouse model (Miller et al., 2003). Finally, intrabody-mediated suppression of HD neuropathology *in vivo* was later achieved using a

Drosophila model (Wolfgang et al., 2005). Another neurodegenerative disease that has been the subject of preliminary immunotherapy studies is amyotrophic lateral sclerosis (ALS). Toxicity of mutant superoxide dismutase-1 (SOD1) in ALS is linked to its propensity to misfold and aggregate, and both active and passive immunotargeting of differently folded states of SOD1 have provided therapeutic benefit in mutant SOD1 transgenic mice (Gros-Louis et al., 2010; Takeuchi et al., 2010; Urushitani et al., 2007).

More advanced is the work on transmissible spongiform encephalopathies, such as Creutzfeldt-Jacob disease (CJD) in humans and scrapie in other species, for the immunotherapeutic removal of PrP aggregates (Alexandrenne et al., 2009; Sigurdsson et al., 2002). Native PrP is expressed throughout life and creates immune tolerance, which initially proved to be a challenge for the development of effective vaccinations in experimental animals (Bade & Frey, 2007). Despite the initial difficulties, there has been substantial progress in preclinical models, but none of the experimental approaches has yet demonstrated alleviation of symptomatic disease. However, reports have confirmed the *in vivo* efficacy of passive immunization in preventing prion diseases after intraperitoneal injection of anti-PrP antibodies (White et al., 2003).

Finally, immunotherapy has also been explored as a potential treatment for Parkinson's disease (PD) and related neurodegenerative diseases (Masliah et al., 2005; Masliah et al., 2011; Rohn, 2012), but despite promising preclinical results only one immunotherapeutic treatment is currently undergoing clinical studies for PD (Table 1). Many reviews have focused on immunotherapy for AD and less attention has been paid to PD and related neurodegenerative disorders. In this review, we present an overview of the recent advances in immunotherapy for α -synucleinopathies, discussing the results reported to date, future prospects and the rationale for further development of immunotherapies for PD and related neurodegenerative disorders.

3. Parkinson's disease, Dementia with Lewy bodies and Multiple System Atrophy: the spectrum of neurodegeneration associated with α -synuclein aggregation

Lewy body diseases (LBD) are one of the most common pathologic types of dementia in the elderly, following AD and vascular dementia. LBD are a heterogeneous group of diseases characterized by motor and cognitive impairments (McKeith et al., 2000), and the presence of intraneuronal eosinophilic cytoplasmic inclusions called Lewy bodies (LBs). The primary structural component of LBs is the protein α -synuclein (α -syn) (Spillantini et al., 1997; Takeda et al., 1998; Wakabayashi et al., 1997). LBD include dementia with Lewy bodies (DLB), Parkinson's disease dementia (PDD) and idiopathic PD, all of which are characterized by specific patterns of neurodegeneration associated with the accumulation of α -syn. PD results from the death of dopaminergic neurons in the substantia nigra, while in DLB and PDD additional degeneration of cholinergic neurons in the basal forebrain is also observed (Schulz-Schaeffer, 2010; Spillantini & Goedert, 2000). PD involves grey matter loss in frontal areas, while in PDD and DLB this loss extends to temporal, occipital and subcortical areas, accompanied by significant occipital atrophy when compared to PD (Burton et al., 2004). In addition to LBD, other neurodegenerative disorders are also associated with intracellular accumulation of α -syn, such as Multiple system atrophy (MSA) (Dickson et al., 1999; Papp & Lantos, 1994; Spillantini, 1999; Wakabayashi et al., 1998), AD (Hamilton, 2000; Iwai, 2000), and Gaucher disease (Mazzulli et al., 2011). The occurrence of α -syn aggregates unveils a potential continuum between these conditions, which could be lumped together under the term of α -synucleinopathies (Farrer et al., 1999).

α -syn is a synaptic protein predominantly expressed in neurons of the neocortex, hippocampus, substantia nigra, thalamus, and cerebellum (Iwai et al., 1995). It was first cloned from the neuromuscular junction of the electric eel *Torpedo californica* (Maroteaux et al., 1988), and five years later identified in human brains as the precursor protein of the non-A β component of amyloid (NACP) plaques in AD (Uéda et al., 1993). Under physiological conditions, α -syn is located in neuronal synaptic terminals and is specifically upregulated in a discrete population of presynaptic terminals during acquisition-related synaptic rearrangement (Fortin et al., 2005; George et al., 1995). Under pathological conditions, α -syn aggregation is central to the neuropathological changes observed in α -synucleinopathies (Lashuel et al., 2002; Tsigelny et al., 2007), and blocking this process would be a key mechanism for preventing α -syn toxicity.

Although the presence of fibrillar aggregates of α -syn is the main characteristic of α -synucleinopathies, it is now widely accepted that the oligomeric form of α -syn, rather than the fibrillar form, is the toxic species (Danzer et al., 2007; Lashuel et al., 2002; Winner et al., 2011). α -syn oligomers can also be released to the extracellular environment and be assimilated by neighboring cells in a mechanism that we will henceforth call “propagation” (Angot & Brundin, 2009; Desplats et al., 2009; Lee et al., 2010a). Aggregation of α -syn is nucleation-dependent, and it has been suggested that α -syn can propagate its misfolding through a prion-like spreading (Lee et al., 2010b). Therefore, the mechanisms of α -syn-induced neurodegeneration can be related either to the oligomer toxicity or to the propagation and prion-like behavior of the small aggregates. Until recently, most of the immunotherapeutic approaches had been focused on targeting extracellular protein aggregates, for example A β in AD, and there has been a reluctance to target intracellular proteins such as α -syn and tau. However, as mentioned above, the discovery that α -syn and tau oligomers can penetrate and accumulate in the plasma membrane and that they can be secreted and propagate extracellularly (Angot & Brundin, 2009; Danzer et al., 2007; Desplats et al., 2009; Jones et al., 2012; Kfoury et al., 2012; Lee et al., 2005; Meraz-Ríos et al., 2010) provided a clear rationale for immunotherapy.

To date there are no disease-modifying treatments for α -synucleinopathies. Interestingly, recent preclinical studies have been successful in clearing intraneuronal α -syn aggregates by immunotherapy, focusing on stimulating or restoring the ability of the immune system to fight the disease (Masliah et al., 2005; Masliah et al., 2011; Näsström et al., 2011; Reynolds et al., 2010; Vekrellis & Stefanis, 2012). Humoral immunization against α -syn can occur in one of two forms, active or passive immunity. Active immunization involves stimulating the immune system to produce antibodies against α -syn aggregates, while passive immunization involves administering anti- α -syn antibodies to the patient, which confers temporary protection against the disease. It is also worth mentioning a third type of immunotherapy based in cellular-mediated immunity. Cell-mediated immunity is the immune response that involves the activation of phagocytes, natural killer cells, antigen-specific cytotoxic T lymphocytes, and the release of various cytokines in response to an antigen. Cell-based immunotherapies are proven to be effective for the treatment of some types of cancer (Restifo et al., 2012), and they have also been explored for the potential treatment of α -synucleinopathies (Reynolds et al., 2007; Reynolds et al., 2010).

4. α -synuclein as immunotherapeutic target

Under specific circumstances, α -syn has the tendency to aggregate and accumulate, resulting in the cellular toxicity and cell death observed in α -synucleinopathies. It has been shown that the ability of α -syn to form oligomers is accompanied by increased *in vivo* toxicity (Winner et al., 2011). If the mechanisms that clear α -syn oligomers are compromised, or the rate of aggregation is increased due to polymorphisms or genomic multiplications of its gene

(SNCA), enhanced α -syn accumulation activates pathways that lead to cell death (Lücking & Brice, 2000). Additionally, there is strong evidence for cell-to-cell propagation of α -syn and thus the spreading of the pathology (Danzer et al., 2012; Desplats et al., 2009). It has been shown that α -syn and its aggregates are released from neuronal cells via exocytosis, without compromising membrane integrity. Upon exposure, neurons and glial cells have the ability to take up extracellular α -syn oligomers through endocytosis and form inclusion bodies (Desplats et al., 2009; Lee et al., 2010a). Moreover, astrocytes exposed to α -syn undergo changes in their gene expression profile reflecting an inflammatory response (Lee et al., 2010a), and while neuronal deposition of α -syn serves as a pathological hallmark of PD and DLB, α -syn-positive protein aggregates are also detected in astrocytes and oligodendrocytes (Wakabayashi et al., 2000). Accumulation of α -syn in oligodendrocytes is the hallmark of MSA, and it impacts the neurotrophic support provided by these cells to neurons and contributes to neurodegeneration (Ubhi et al., 2010). Finally, circulating α -syn or activated astrocytes can activate microglia, which leads to increased generation of reactive oxygen species, nitric oxide and cytokine production, and further neurodegeneration (Lee et al., 2010a).

The use of immunotherapeutic approaches for α -synucleinopathies presents numerous advantages. Active or passive immunization against α -syn increases the clearance of toxic aggregates by autophagy or by macrophage activation. It also might reduce extracellular α -syn propagation and thus promote neuroprotection. Immunization must target specific species or configurations to prevent adverse side effects and autoimmune inflammatory responses. But there are also other important considerations, as immunization might promote inflammation, target native endogenous proteins and induce autoimmunity. Immunotherapies must be designed to minimize these potential adverse effects for developing the safest treatments.

5. Active immunization against α -synuclein

The effect of active immunization against α -syn has been studied in a mouse model of LBD (Masliah et al., 2005). When transgenic mice expressing human α -syn under the control of the platelet-derived growth factor- β (PDGF β) promoter were immunized with recombinant human α -syn, it was observed that the mice that produced higher affinity antibodies also showed decreased accumulation of α -syn in neuronal cell bodies and synapses. Furthermore, this reduction of α -syn was associated with reduced neurodegeneration. Western blot analysis showed that oligomeric human α -syn was more abundant in the membrane fraction, which is consistent with the fact that abnormal accumulation of human α -syn is associated with the translocation of this protein from the cytosol to the membrane (Tsigelny et al., 2008). It was also observed that internalized anti-human α -syn antibodies reacted with human α -syn within lysosomes, which suggests that circulating antibodies might recognize abnormally aggregated human α -syn associated with the neuronal membrane and lead to its clearance via lysosomal activation. Increased cathepsin-D immunoreactivity and colocalization with human α -syn was also observed using exogenously administered monoclonal antibodies against human α -syn, further supporting this hypothesis. These results are consistent with studies showing that vaccination is effective experimentally in other mouse models of neurodegenerative diseases, reducing the accumulation of A β (Bach et al., 2009), tau (Troquier et al., 2012), PrP (Sigurdsson et al., 2002) and huntingtin (Luthi-Carter, 2003; Miller et al., 2003). Moreover, a similar mechanism of clearance of membrane-bound proteins by circulating antibodies has been described for viral infections (Garzón et al., 1999; Ubol et al., 1995).

Importantly, active immunization against α -syn did not trigger a neuroinflammatory response in immunized mice compared to adjuvant only-treated mice, and no differences

were observed in microglial (Iba-1) or astroglial (GFAP) markers between groups (Masliah et al., 2005). Inflammation is frequently reported as a side effect in active immunization paradigms, and these results confirm there is no significant inflammatory response when transgenic mice are vaccinated with a human α -syn antigen. However, it is important to consider that active immunization has been associated in some cases with vasculitis and autoimmune responses (Menéndez-González et al., 2011; Wilcock & Colton, 2008). For this reason, the current line of research is focused on passive immunization using antibodies that recognize specific regions of α -syn. In this sense, the antibodies produced by immunized mice that showed higher affinity, were directed against the C-terminal (CT) region of α -syn (Masliah et al., 2005). Epitope mapping analysis showed that in vaccinated mice the antibodies recognized epitopes within the CT region of human α -syn, including amino acids 85–99, 109–123, 112–126, and 126–138 (Masliah et al., 2005). Affinity of these antibodies was measured using the serum of vaccinated mice as primary detection solution in Western blots or immunohistochemical staining of non-immunized transgenic mice brains.

Preventive immunization approaches may be more efficacious and associated with fewer side effects than therapeutic immunization. It has been recently reported that preventive A β immunization is a safe therapeutic approach, lacking adverse CNS immune system activation or other serious side effects, in both aged and juvenile non-human primates (Kofler et al., 2012). Immunotherapy might be most effective in preventing or slowing the progression of the disease when patients are immunized before or in the earliest stages of the disease onset (Lemere & Masliah, 2010). If immunization is administered before the buildup of intraneuronal α -syn aggregates that might act as seeds for more protein deposition, it might be protective by limiting aggregate formation at an early stage of the disease. Preventive vaccination might lead to more robust and long-lasting humoral immune responses, higher antibody titers and, therefore, more effective treatments.

Recently, AFFiRiS AG, an Austria-based biotech company, started Phase I clinical studies of the PD vaccine AFFITOPE PD01. AFFITOPE PD01 consists of a peptide-carrier conjugate and is formulated with aluminum hydroxide as immunological adjuvant (Schneeberger et al., 2012). The peptides used in the vaccine are designed to be too small to induce an α -syn-specific T cell response, thus avoiding T cell autoimmunity. Immunization with AFFITOPE PD01 reduced the level of cerebral α -syn in two independent mouse models of α -synucleinopathies and ameliorated α -syn-triggered neuropathological alterations such as neuronal cell loss and dendritic density loss. This represents the first active immunization study to be tested clinically in PD, and opens the door to further immunotherapeutic advances focused on α -syn.

6. Passive immunization against α -synuclein

Passive immunization allows for targeting and inducing the clearance of toxic α -syn aggregates in multiple neuronal populations and at different stages of propagation simultaneously, avoiding the potential side effects of active immunization. Therefore, after the promising results obtained with active immunization, the effect of passive immunization was analyzed in the transgenic mice expressing human α -syn under the regulatory control of the PDGF β promoter. Exogenous administration of antibodies against human α -syn reduced behavioral and neuropathological deficits in this mouse model (Masliah et al., 2011). A group of antibodies designed to recognize and bind different regions of α -syn (including N-terminal and C-terminal epitopes) was tested (Figure 1), and the most specific for human α -syn was found to be 9E4, which recognizes an epitope in the CT region of the protein. Immunization with antibodies against other regions of α -syn (amino acids 1–4, 40–54, 91–99, 109–112, 125–140) was performed side-to-side for comparison purposes (unpublished data). Passive immunization with 9E4 ameliorated motor behavior and learning deficits

measured by the Morris water maze, and improved synaptic pathology in the transgenic mice as confirmed by electron microscopy, PSD95 and synapsin I immunoreactivity. Passive immunization with 9E4 also reduced the accumulation of calpain-cleaved α -syn aggregates in neocortex and hippocampus (Figure 1). Additionally, 9E4 did not perturb the microvasculature as confirmed by Zo-1 immunostaining (Masliah et al., 2011). FITC-labeled 9E4 trafficked into the CNS and localized to lysosomes, proving that the antibodies could cross the brain blood barrier and bind α -syn. It is possible that the antigen-antibody complexes are endocytosed and transferred to the lysosomal compartment for degradation via autophagy (Figure 2). Perera and colleagues have reported a similar mechanism for the monoclonal antibody against the epidermal growth factor receptor (EGFR), mAb 806 (Perera et al., 2007). Following binding to EGFR, mAb 806 is internalized through dynamin-dependent, clathrin-mediated endocytosis. Internalized mAb 806 localizes to early endosomes and subsequently traffics and accumulates in lysosomal compartments, suggesting that clearance of antibody-targeted membrane proteins might use endocytosis and lysosomal degradation as a common clearance mechanism. The internalization of α -syn after passive immunization has also been studied using biomolecular fluorescence complementation, which demonstrated the internalization of monoclonal antibodies against the CT region of α -syn in living cells, together with a reduction in the formation of dimers and oligomers (Näsström et al., 2011). The results of this study also suggest that treatment with α -syn antibodies may promote the turnover of α -syn leading to a decrease in its secreted forms. This is of relevance considering the recent hypothesis on α -syn prion-like propagation properties (Dunning et al., 2012; Lema Tomé et al., 2012; Mougenot et al., 2012).

Several studies have shown that, similar to α -syn, immunotherapy can reduce the accumulation of other membrane-bound and intracellular proteins such as tau (Sigurdsson, 2008), PrP (Pankiewicz et al., 2006) and huntingtin (Wolfgang et al., 2005). In the SH-SY5Y human dopaminergic cell line, α -syn exists physiologically as a lipid-bound oligomer and a soluble monomer (Leng et al., 2001). As antibodies against α -syn CT show higher affinity for α -syn, a possible explanation is that the CT portion of the protein penetrates the membrane and is exposed to the extracellular medium, where antibodies could recognize it. However, other options have been explored for targeting intracellular α -syn aggregates. Intrabodies, or intracellular antibodies, are gene-engineered antibodies that are expressed intracellularly to modulate the function of their targets. Usually, cell transfections or infections with scFv cDNA-containing plasmids or viruses are the means to express scFv antibodies intracellularly (Chen et al., 1994). Two anti- α -syn scFv antibodies, D10 and NAC32, have shown promising effects as intrabodies in inhibiting α -syn aggregation and rescuing α -syn toxicity (Lynch et al., 2008; Zhou et al., 2004). Unfortunately, the application of recombinant DNA technology faces technical and safety challenges. First, the conformation and structural stability of the intrabodies within the cell is affected by the reducing conditions of the intracellular environment. Also, there are safety concerns surrounding the application of transfected DNA in human clinical therapy, especially in the case of viral-based vectors. An alternative approach around these problems would be fusing protein transduction domains to scFv antibodies to create cell-permeable antibodies or transbodies (Heng & Cao, 2005), but to date there is no study on α -syn aggregate clearance that supports this approach.

In conclusion, pre-clinical results suggest that passive immunization can be of therapeutic value for the treatment of α -synucleinopathies, as it has the potential to activate the clearance of toxic α -syn aggregates via autophagy and microglia clearance, and it might also prevent propagation and thus the spreading of the disease. At least three studies to date suggest that immunotherapy with antibodies against α -syn might represent a potentially useful approach to treat PD, DLB, and related neurodegenerative diseases, and that targeting

the CT region of the protein in particular might be of therapeutic value. However, more detailed studies using antibodies that recognize other regions or post-translational modifications of α -syn are needed.

7. α -synuclein propagation as immunotherapeutic target: reactive gliosis, MSA, and neural grafts

The antibodies against α -syn used for passive immunotherapy can potentially recognize the oligomerized protein at different points of the neurotoxic cascade (Figure 3). The first possible scenario would be antibodies targeting intracellular α -syn aggregates within neurons or glial cells. As mentioned above, it is possible that circulating antibodies may recognize abnormal α -syn accumulating in the neuronal plasma membrane (Lee et al., 2002), and stimulate α -syn degradation via autophagy (Figure 2). Evidence of this mechanism is provided by the observation that the 9E4 antibody colocalizes with α -syn in autophagosomes in immunized α -syn transgenic mice. Also the inhibitor of autophagy, 3MA, blocks the effects of the 9E4 antibody in neuronal cells expressing α -syn (Masliah et al., 2011).

The alternative to cell surface recognition would be antibodies targeting extracellular α -syn aggregates released after cell death or while propagating to other cells (Figure 3). α -syn is a neuronal synaptic protein that has been shown to also accumulate within astrocytes in LBD and within oligodendrocytes in MSA, through a process of neuron-to-glia propagation (Desplats et al., 2009; Kisos et al., 2012; Lee et al., 2010a). Blocking α -syn propagation from neurons to other neurons and to glial cells would be of tremendous relevance, specifically in MSA where propagation seems to be a crucial mechanism in the development of the disease (Marques & Outeiro, 2012). Furthermore, during extracellular propagation, α -syn aggregates would be easily accessible to circulating antibodies, thus potentially increasing the efficacy of the immunotherapy. Extracellular α -syn aggregates can be recognized and removed by microglia (Lee et al., 2008), the first and main form of active immune defense of the central nervous system. Activated phagocytic microglia is able to engulf foreign or aberrant materials and display the resulting molecules for T cell activation (Gehrmann et al., 1995). Results suggest that microglia may be the major scavenger cells for extracellular α -syn aggregates in the brain parenchyma, and that the clearance of these aggregates may be regulated by the activation state of microglia (Lee et al., 2008). It has been observed that microglial cells are also involved in the clearance of extracellular A β by taking up soluble forms through macropinocytosis and receptor-mediated pathways. Fibrillar forms of A β interact with the innate immune receptor complex at the cell surface, initiating intracellular signaling cascades that stimulate phagocytosis (Lee & Landreth, 2010). Evidence suggests that microglial cells are important players in acute and chronic neurodegenerative diseases of the CNS, which appear to be associated with a limited or dysfunctional microglial phagocytosis (Napoli & Neumann, 2009).

The transmission of α -syn to astroglia leads to astrogliosis and inflammatory responses, release of inflammatory cytokines and neuronal death. Astrocytes express no or low levels of α -syn, but they are capable of taking up α -syn aggregates from the culture media (Lee et al., 2010a). After internalization, α -syn maintains its aberrant conformation and moves through the endosomal pathway destined to be degraded in the lysosome. The uptake of neuron-derived α -syn by astrocytes strongly correlates with the production of cytokines, such as IL-1 α , IL-1 β and IL-6 (Lee et al., 2010a). These results suggest that the accumulation of neuron-derived α -syn leads to inflammatory responses, but a possible extracellular action of α -syn in inducing astroglial inflammatory reactions cannot be ruled out. Recent studies have also shown clearance of the A β protein by astrocytes, which imply that they might play a part in the inactivation of certain toxic extracellular protein aggregates

by removing them from the cell environment (Thal, 2012). Moreover, although transmission of α -syn can occur without apparent cell death, degenerating neurons would provide large amounts of α -syn and thereby may accelerate the propagation. Microglia are also responsible for elevated pro-inflammatory cytokines in brain pathological conditions (Streit et al., 2004), and extracellular aggregated α -syn has been shown to cause a variety of inflammatory responses in microglial cells (Thomas et al., 2007). In addition to the direct effect of α -syn on microglia, astrocytes may also mediate microglial activation (Min et al., 2006).

Removal of extracellular aberrant aggregates of α -syn through immunotherapy would be a potentially beneficial treatment to prevent astroglial and microglial activation and the neuroinflammatory responses derived from this activation. Antibodies against α -syn specifically target and aid in the clearance of extracellular α -syn aggregates by microglia, thereby preventing their actions on neighboring cells (Figure 3). Antibody-mediated clearance of extracellular α -syn appears to be a process selective for microglia and only applicable to aggregates, not monomers (Bae et al., 2012). When complexed with the antibody, α -syn aggregates are internalized through Fc γ receptors and undergo different intracellular trafficking pathways than α -syn aggregates alone, being delivered to lysosomes more efficiently, which may account for faster degradation. Stereotaxic administration of antibodies against α -syn into the brains of transgenic mice expressing human α -syn under the control of the PDGF β promoter prevents neuron-to-astroglia transmission of α -syn and leads to increased localization of α -syn and the antibody in microglia (Bae et al., 2012). Moreover, passive immunization is also accompanied by a reduction of functional deficits and amelioration of neurodegeneration, which suggests that it might exert therapeutic and preventive effects through promotion of microglia-mediated clearance of extracellular α -syn (Bae et al., 2012).

Blocking the propagation of α -syn oligomers from neurons to glial cells would also represent a key immunotherapeutic approach for MSA. MSA is a neurodegenerative disorder characterized by autonomic failure and motor impairment, parkinsonism, cerebellar ataxia and pyramidal signs (Gilman et al., 2008), commonly non-responsive to dopaminergic therapy. Parkinsonian features predominate in 80% of patients (MSA-P subtype), reflecting striato-nigral neurodegeneration, while cerebellar ataxia is the major motor feature in 20% of patients (MSA-C subtype), which is a consequence of olivopontocerebellar atrophy (Gilman et al., 2008). MSA is characterized by neuronal loss in the striatum, cerebellum, brainstem and cortex, and this neurodegeneration is also accompanied by astrogliosis and microgliosis (Wakabayashi & Takahashi, 2006; Yoshida, 2007). The pathological hallmark of MSA is the presence of glial cytoplasmic inclusions (GCIs) within oligodendrocytes, which induce degeneration and consequent myelin loss (Papp et al., 1989; Tsuboi et al., 2005). The principal component of GCIs is fibrillar α -syn, but also ubiquitin, tubulin, MAP2, p25 and other proteins can be found (Cairns et al., 1997; Chiba et al., 2011; Gai et al., 1999; Nakamura et al., 1998; Wakabayashi et al., 1998). As it occurs with astrocytes, oligodendrocytes express low levels of α -syn of their own (Kisos et al., 2012; Richter-Landsberg et al., 2000). An emerging hypothesis about the early stages of the disease states that oligodendrocytes might endocytose α -syn of neuronal origin and accumulate protein aggregates with toxic consequences. The transmission of aggregation-prone proteins has also been reported with A β , polyglutamine proteins and tau (Brundin et al., 2010; Frost & Diamond, 2010; Lee et al., 2010b). Treatments focused on halting α -syn extracellular prion-like transmission would therefore be of extreme relevance for MSA.

Immunotherapeutic approaches designed to target the extracellular propagation of α -syn aggregates would also be of interest for preventing the spreading of the disease to grafted neuronal stem cells (Figure 3). Clinical studies on neural grafting started in the late 1980s,

when dopaminergic neurons derived from the human embryonic brain were transplanted into the striatum of patients with PD (Lindvall et al., 1989; Madrazo et al., 1988). Recently, a new body of evidence has emerged showing host-to-graft propagation of LB and α -syn pathology in long term transplants (Hansen et al., 2011; Li et al., 2008), due to the transmission of aberrant α -syn aggregates from the surrounding tissues (Desplats et al., 2009). In toxin-induced rodent models of MSA, striatal grafts survive and exert functional effects, but oligodendrocytes expressing host-specific α -syn migrate into the graft tissue after 3 months of survival (Stefanova et al., 2009). Available data suggest that the majority of grafted cells in humans are functionally impaired after a decade due to the progression of Lewy-like inclusions from host to grafted neurons, but recipients can still experience long-term symptomatic relief, and immunotherapy might be used as a preventive measure to block the spreading of the pathology to the grafted cells.

8. Other potential targets for immunotherapy in α -synucleinopathies

Since its discovery, α -syn has been traditionally in the spotlight for treatments for LBD and related α -synucleinopathies, but other potential targets must also be considered. First, the possibility of cell-based immunotherapy with T cells has been investigated (Laurie et al., 2007; Reynolds et al., 2007; Reynolds et al., 2010). Cellular immunization has been shown to reduce trafficking of cytotoxic T cells, microglial activation and neuroinflammatory responses. When activated regulatory T cells are transferred to MPTP-treated mice, they induce a decrease of immunoreactive microglia and protect dopaminergic neuronal bodies in the substantia nigra (Reynolds et al., 2010). Regulatory T cells mediate neuroprotection through suppression of microglial responses to aggregated, nitrated α -syn, thereby slowing down the neurodegenerative events associated with microglial neuroinflammatory responses.

Another potential target for immunotherapy in α -synucleinopathies is tau. Since tau may coaggregate with α -syn in LBs and GCIs (Cairns et al., 1997; Galloway et al., 1989), immunotherapy against tau would be potentially effective in the clearance of those aggregates but not in the removal of toxic soluble oligomers. However, the effect of tau immunotherapy in α -synucleinopathies remains to be tested.

Finally, several genes other than *SNCA* have been associated with familial or sporadic PD. Seven validated genes have been identified so far: parkin (*PARK2*), PTEN-induced kinase 1 (*PINK1*), DJ-1 (*PARK7*), *ATP13A2*, Leucine-rich repeat kinase 2 (*LRRK2*), as well as two recently identified possibly causative genes, vacuolar protein sorting 35 (*VPS35*) and eukaryotic translation initiation factor 4G1 (*EIF4G1*) (Chartier-Harlin et al., 2011; Lesage & Brice, 2012; Matsumine et al., 1998; Ramirez et al., 2006; Valente et al., 2012; van Duijn et al., 2001; Zimprich et al., 2011; Zimprich et al., 2004). Furthermore, alterations in certain genes, including *GBA* and *UCHL1*, do not cause PD but appear to modify the risk of developing the condition in some families (Lincoln et al., 1999; Lwin et al., 2004; Sutherland et al., 2009). The identification of the genes for recessive parkinsonism *PINK1*, *DJ-1*, and parkin provided evidence of a causal relationship between mitochondrial function and PD. Each of these regulates responses to cellular stresses, including oxidative stress and depolarization of the mitochondrial membrane. *PINK1* and parkin also promote autophagic removal of depolarized mitochondria, thus modulating mitochondrial dynamics. Mutations in all genes linked to PD lead to enhanced sensitivity to mitochondrial toxins and oxidative stress (McCoy & Cookson, 2012). Despite the interesting pharmacological targets these genes provide, they don't seem to be suitable for immunotherapeutic approaches, as many of the mutations or polymorphisms that affect them result in loss of function rather than in accumulation of toxic forms. However, new advances in the identification of genes that

provide susceptibility to α -synucleinopathies might unveil potential targets that could help prevent or cure these diseases in the future.

9. Conclusions and final considerations

The results obtained to date on immunotherapy for the treatment of neurodegenerative diseases support the view that this approach might have therapeutic potential for disease modification, although many questions remain unanswered including how exactly immunotherapy works. Promising results have been obtained for both active and passive immunization in preclinical studies, but caution must be exercised when advancing from mouse models to clinical trials in order to maximize efficacy and minimize autoimmune and vascular adverse effects. One example comes from passive immunotherapy against A β , where effector functions appear not to be critical for the clearance of A β aggregates by passive immunization in mice (Tamura et al., 2005), and are suspected to be responsible for Fc-mediated responses that result in vasogenic edema in humans (Golde et al., 2009). Another question that needs to be addressed is whether immunotherapy can only be useful as preventive treatment or it could also be effective once the disease becomes symptomatic (Lemere & Masliah, 2010). In the latter situation, clearance of abnormal protein aggregates might not improve cognitive or motor deficits, but it might prevent or delay further deterioration. Despite these considerations, immunotherapy presents many advantages, as it has the potential to target abnormal protein aggregates simultaneously at different points of the neurodegeneration cascade and blocks the spread of the disease in a prion-like manner. Propagation of small aggregates is suspected to be a primary pathological event in several neurodegenerative diseases (Frost & Diamond, 2010; Goedert et al., 2010; Lee et al., 2010b; Luk et al., 2012) and elicits inflammatory reactions in astroglia and microglia (Lee et al., 2010a). However, an in-depth study on the contribution of propagation at different stages of the disease will clarify how immunotherapy might be effective in each case. Additionally, targeting propagation would also be of great relevance as a preventive measure for increasing the functional life of neural grafts (Brundin et al., 2010; Li et al., 2008). Mechanistic insights learned from AD immunotherapy will help in the development of successful immunotherapies for other neurodegenerative diseases and improve future clinical trial design (Citron, 2010; Golde et al., 2009; Tarawneh & Holtzman, 2009). Epitope selection and mode of administration of the therapy (intravenous vs. central) are fundamental considerations for therapeutic efficacy (Town, 2009). In the case of immunotherapy against α -syn and tau, specific considerations regarding the primarily intracellular nature of the aggregates should also be taken into account. Targeting specific conformational epitopes in the oligomeric or aggregated species might prevent potential complications from interfering with the physiological function of endogenous proteins (Lambert et al., 2009). Improving and/or preventing further deterioration of neurological function and minimizing vascular adverse effects should be a priority in the development of new immunotherapeutic approaches, and undoubtedly future immunotherapy studies for neurodegenerative diseases will benefit from the lessons learned in AD (Schenk et al., 2005; Wiessner et al., 2012). In conclusion, numerous preclinical studies and current Phase III clinical trials suggest that immunotherapy might constitute a potentially effective disease-modifying treatment for several neurodegenerative disorders, as it targets the molecular origin of the disease.

Acknowledgments

Supported by NIH grants AG18440, AG022074, NS044233 and by Neotope Biosciences.

Abbreviations

Aβ	amyloid beta
AD	Alzheimer's disease
ALS	Amyotrophic lateral sclerosis
BACE1	beta-site amyloid precursor protein cleaving enzyme 1
CJD	Creutzfeld-Jacob disease
CNS	Central nervous system
CT	C-terminal
DLB	Dementia with Lewy bodies
EGFR	epidermal growth factor receptor
FTD	Frontotemporal dementia
GCI s	glial cytoplasmic inclusions
HD	Huntington's disease
LB s	Lewy bodies
LBD	Lewy body diseases
MSA	Multiple system atrophy
NAC	Non-A β component of the AD amyloid
NACP	NAC precursor
PD	Parkinson's disease
PDD	Parkinson's disease dementia
PDGFβ	Platelet-derived growth factor- β
PrP	prion protein
scFv	Single-chain variable fragment
SOD1	Superoxide dismutase-1
α-syn	α -synuclein

References

- Adolfsson O, Pihlgren M, Toni N, Varisco Y, Buccarello AL, Antonello K, et al. An effector-reduced anti- β -amyloid (A β) antibody with unique β binding properties promotes neuroprotection and glial engulfment of A β . *J Neurosci*. 2012; 32:9677–9689. [PubMed: 22787053]
- Alexandrenne C, Hanoux V, Dkhissi F, Boquet D, Couraud JY, Wijkhuisen A. Curative properties of antibodies against prion protein: a comparative in vitro study of monovalent fragments and divalent antibodies. *J Neuroimmunol*. 2009; 209:50–56. [PubMed: 19232746]
- Angot E, Brundin P. Dissecting the potential molecular mechanisms underlying alpha-synuclein cell-to-cell transfer in Parkinson's disease. *Parkinsonism Relat Disord*. 2009; 15(Suppl 3):S143–S147. [PubMed: 20082977]
- Arriagada PV, Growdon JH, Hedley-Whyte ET, Hyman BT. Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. *Neurology*. 1992; 42:631–639. [PubMed: 1549228]

- Asuni AA, Boutajangout A, Quartermain D, Sigurdsson EM. Immunotherapy targeting pathological tau conformers in a tangle mouse model reduces brain pathology with associated functional improvements. *J Neurosci*. 2007; 27:9115–9129. [PubMed: 17715348]
- Atwal JK, Chen Y, Chiu C, Mortensen DL, Meilandt WJ, Liu Y, et al. A therapeutic antibody targeting BACE1 inhibits amyloid- β production in vivo. *Sci Transl Med*. 2011; 3 84ra43.
- Bach P, Tschäpe JA, Kopietz F, Braun G, Baade JK, Wiederhold KH, et al. Vaccination with Abeta-displaying virus-like particles reduces soluble and insoluble cerebral Abeta and lowers plaque burden in APP transgenic mice. *J Immunol*. 2009; 182:7613–7624. [PubMed: 19494285]
- Bade S, Frey A. Potential of active and passive immunizations for the prevention and therapy of transmissible spongiform encephalopathies. *Expert Rev Vaccines*. 2007; 6:153–168. [PubMed: 17408366]
- Bae EJ, Lee HJ, Rockenstein E, Ho DH, Park EB, Yang NY, et al. Antibody-Aided Clearance of Extracellular α -Synuclein Prevents Cell-to-Cell Aggregate Transmission. *J Neurosci*. 2012; 32:13454–13469. [PubMed: 23015436]
- Bierer LM, Hof PR, Purohit DP, Carlin L, Schmeidler J, Davis KL, et al. Neocortical neurofibrillary tangles correlate with dementia severity in Alzheimer's disease. *Arch Neurol*. 1995; 52:81–88. [PubMed: 7826280]
- Blennow K, Zetterberg H, Rinne JO, Salloway S, Wei J, Black R, et al. Effect of immunotherapy with bapineuzumab on cerebrospinal fluid biomarker levels in patients with mild to moderate Alzheimer disease. *Arch Neurol*. 2012; 69:1002–1010. [PubMed: 22473769]
- Bohrmann B, Baumann K, Benz J, Gerber F, Huber W, Knoflach F, et al. Gantenerumab: a novel human anti-A β antibody demonstrates sustained cerebral amyloid- β binding and elicits cell-mediated removal of human amyloid- β . *J Alzheimers Dis*. 2012; 28:49–69. [PubMed: 21955818]
- Boutajangout A, Ingadottir J, Davies P, Sigurdsson EM. Passive immunization targeting pathological phospho-tau protein in a mouse model reduces functional decline and clears tau aggregates from the brain. *J Neurochem*. 2011; 118:658–667. [PubMed: 21644996]
- Boutajangout A, Quartermain D, Sigurdsson EM. Immunotherapy targeting pathological tau prevents cognitive decline in a new tangle mouse model. *J Neurosci*. 2010; 30:16559–16566. [PubMed: 21147995]
- Brundin P, Melki R, Kopito R. Prion-like transmission of protein aggregates in neurodegenerative diseases. *Nat Rev Mol Cell Biol*. 2010; 11:301–307. [PubMed: 20308987]
- Buée L, Delacourte A. Comparative biochemistry of tau in progressive supranuclear palsy, corticobasal degeneration, FTDP-17 and Pick's disease. *Brain Pathol*. 1999; 9:681–693. [PubMed: 10517507]
- Burton EJ, McKeith IG, Burn DJ, Williams ED, O'Brien JT. Cerebral atrophy in Parkinson's disease with and without dementia: a comparison with Alzheimer's disease, dementia with Lewy bodies and controls. *Brain*. 2004; 127:791–800. [PubMed: 14749292]
- Cairns NJ, Atkinson PF, Hanger DP, Anderton BH, Daniel SE, Lantos PL. Tau protein in the glial cytoplasmic inclusions of multiple system atrophy can be distinguished from abnormal tau in Alzheimer's disease. *Neurosci Lett*. 1997; 230:49–52. [PubMed: 9259461]
- Chai X, Wu S, Murray TK, Kinley R, Cella CV, Sims H, et al. Passive immunization with anti-Tau antibodies in two transgenic models: reduction of Tau pathology and delay of disease progression. *J Biol Chem*. 2011; 286:34457–34467. [PubMed: 21841002]
- Chartier-Harlin MC, Dachsel JC, Vilarinho-Güell C, Lincoln SJ, Leprêtre F, Hulihan MM, et al. Translation initiator EIF4G1 mutations in familial Parkinson disease. *Am J Hum Genet*. 2011; 89:398–406. [PubMed: 21907011]
- Chen SY, Bagley J, Marasco WA. Intracellular antibodies as a new class of therapeutic molecules for gene therapy. *Hum Gene Ther*. 1994; 5:595–601. [PubMed: 7914435]
- Chiba Y, Takei S, Kawamura N, Kawaguchi Y, Sasaki K, Hasegawa-Ishii S, et al. Immunohistochemical localization of aggresomal proteins in glial cytoplasmic inclusions in multiple system atrophy. *Neuropathol Appl Neurobiol*. 2011
- Citron M. Alzheimer's disease: strategies for disease modification. *Nat Rev Drug Discov*. 2010; 9:387–398. [PubMed: 20431570]

- Corbett A, Smith J, Ballard C. New and emerging treatments for Alzheimer's disease. *Expert Rev Neurother.* 2012; 12:535–543. [PubMed: 22550982]
- Danzer KM, Haasen D, Karow AR, Moussaud S, Habeck M, Giese A, et al. Different species of alpha-synuclein oligomers induce calcium influx and seeding. *J Neurosci.* 2007; 27:9220–9232. [PubMed: 17715357]
- Danzer KM, Kranich LR, Ruf WP, Cagsal-Getkin O, Winslow AR, Zhu L, et al. Exosomal cell-to-cell transmission of alpha synuclein oligomers. *Mol Neurodegener.* 2012; 7:42. [PubMed: 22920859]
- Delrieu J, Ousset PJ, Caillaud C, Vellas B. 'Clinical trials in Alzheimer's disease': immunotherapy approaches. *J Neurochem.* 2012; 120(Suppl 1):186–193. [PubMed: 21883222]
- Desplats P, Lee HJ, Bae EJ, Patrick C, Rockenstein E, Crews L, et al. Inclusion formation and neuronal cell death through neuron-to-neuron transmission of alpha-synuclein. *Proc Natl Acad Sci USA.* 2009; 106:13010–13015. [PubMed: 19651612]
- Dickson DW, Liu W, Hardy J, Farrer M, Mehta N, Uitti R, et al. Widespread alterations of alpha-synuclein in multiple system atrophy. *Am J Pathol.* 1999; 155:1241–1251. [PubMed: 10514406]
- Dunning CJ, Reyes JF, Steiner JA, Brundin P. Can Parkinson's disease pathology be propagated from one neuron to another? *Prog Neurobiol.* 2012; 97:205–219. [PubMed: 22115849]
- Farlow M, Arnold SE, van Dyck CH, Aisen PS, Snider BJ, Porsteinsson AP, et al. Safety and biomarker effects of solanezumab in patients with Alzheimer's disease. *Alzheimers Dement.* 2012; 8:261–271. [PubMed: 22672770]
- Farrer M, Gwinn-Hardy K, Hutton M, Hardy J. The genetics of disorders with synuclein pathology and parkinsonism. *Hum Mol Genet.* 1999; 8:1901–1905. [PubMed: 10469843]
- Fernández PL, Britton GB, Rao KS. Potential Immunotargets for Alzheimer's Disease Treatment Strategies. *J Alzheimers Dis.* 2012
- Fortin DL, Nemani VM, Voglmaier SM, Anthony MD, Ryan TA, Edwards RH. Neural activity controls the synaptic accumulation of alpha-synuclein. *J Neurosci.* 2005; 25:10913–10921. [PubMed: 16306404]
- Froelich-Fabre S, Skoglund L, Ostojic J, Kilander L, Lindau M, Glaser A, et al. Clinical and molecular aspects of frontotemporal dementia. *Neurodegener Dis.* 2004; 1:218–224. [PubMed: 16908993]
- Frost B, Diamond MI. Prion-like mechanisms in neurodegenerative diseases. *Nat Rev Neurosci.* 2010; 11:155–159. [PubMed: 20029438]
- Fu HJ, Liu B, Frost JL, Lemere CA. Amyloid-beta immunotherapy for Alzheimer's disease. *CNS Neurol Disord Drug Targets.* 2010; 9:197–206. [PubMed: 20205640]
- Gai WP, Power JH, Blumbergs PC, Culvenor JG, Jensen PH. Alpha-synuclein immunolocalization of glial inclusions from multiple system atrophy brain tissue reveals multiprotein components. *J Neurochem.* 1999; 73:2093–2100. [PubMed: 10537069]
- Galloway PG, Bergeron C, Perry G. The presence of tau distinguishes Lewy bodies of diffuse Lewy body disease from those of idiopathic Parkinson disease. *Neurosci Lett.* 1989; 100:6–10. [PubMed: 2548130]
- Garzón J, DeFelipe J, Rodríguez JR, DeAntonio I, García-España A, Sánchez-Blázquez P. Transport of CSF antibodies to Galpha subunits across neural membranes requires binding to the target protein and protein kinase C activity. *Brain Res Mol Brain Res.* 1999; 65:151–166. [PubMed: 10064886]
- Gehrmann J, Matsumoto Y, Kreutzberg GW. Microglia: intrinsic immune effector cell of the brain. *Brain Res Brain Res Rev.* 1995; 20:269–287. [PubMed: 7550361]
- George JM, Jin H, Woods WS, Clayton DF. Characterization of a novel protein regulated during the critical period for song learning in the zebra finch. *Neuron.* 1995; 15:361–372. [PubMed: 7646890]
- Gilman S, Wenning GK, Low PA, Brooks DJ, Mathias CJ, Trojanowski JQ, et al. Second consensus statement on the diagnosis of multiple system atrophy. *Neurology.* 2008; 71:670–676. [PubMed: 18725592]
- Goedert M, Clavaguera F, Tolnay M. The propagation of prion-like protein inclusions in neurodegenerative diseases. *Trends Neurosci.* 2010; 33:317–325. [PubMed: 20493564]
- Golde TE, Das P, Levites Y. Quantitative and mechanistic studies of Abeta immunotherapy. *CNS Neurol Disord Drug Targets.* 2009; 8:31–49. [PubMed: 19275635]

- Götz J, Ittner A, Ittner LM. Tau-targeted treatment strategies in Alzheimer's disease. *Br J Pharmacol*. 2012; 165:1246–1259. [PubMed: 22044248]
- Gros-Louis F, Soucy G, Larivière R, Julien JP. Intracerebroventricular infusion of monoclonal antibody or its derived Fab fragment against misfolded forms of SOD1 mutant delays mortality in a mouse model of ALS. *J Neurochem*. 2010; 113:1188–1199. [PubMed: 20345765]
- Grundke-Iqbal I, Iqbal K, Quinlan M, Tung YC, Zaidi MS, Wisniewski HM. Microtubule-associated protein tau. A component of Alzheimer paired helical filaments. *J Biol Chem*. 1986; 261:6084–6089. [PubMed: 3084478]
- Gu J, Sigurdsson EM. Immunotherapy for tauopathies. *J Mol Neurosci*. 2011; 45:690–695. [PubMed: 21739165]
- Hamilton RL. Lewy bodies in Alzheimer's disease: a neuropathological review of 145 cases using alpha-synuclein immunohistochemistry. *Brain Pathol*. 2000; 10:378–384. [PubMed: 10885656]
- Hampel H, Teipel SJ. Total and phosphorylated tau proteins: evaluation as core biomarker candidates in frontotemporal dementia. *Dement Geriatr Cogn Disord*. 2004; 17:350–354. [PubMed: 15178952]
- Hansen C, Angot E, Bergström AL, Steiner JA, Pieri L, Paul G, et al. α -Synuclein propagates from mouse brain to grafted dopaminergic neurons and seeds aggregation in cultured human cells. *J Clin Invest*. 2011; 121:715–725. [PubMed: 21245577]
- Heng BC, Cao T. Making cell-permeable antibodies (Transbody) through fusion of protein transduction domains (PTD) with single chain variable fragment (scFv) antibodies: potential advantages over antibodies expressed within the intracellular environment (Intrabody). *Med Hypotheses*. 2005; 64:1105–1108. [PubMed: 15823695]
- Holmes C, Boche D, Wilkinson D, Yadegarfar G, Hopkins V, Bayer A, et al. Long-term effects of Abeta42 immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled phase I trial. *Lancet*. 2008; 372:216–223. [PubMed: 18640458]
- Iwai A. Properties of NACP/alpha-synuclein and its role in Alzheimer's disease. *Biochim Biophys Acta*. 2000; 1502:95–109. [PubMed: 10899435]
- Iwai A, Masliah E, Yoshimoto M, Ge N, Flanagan L, de Silva HA, et al. The precursor protein of non-A beta component of Alzheimer's disease amyloid is a presynaptic protein of the central nervous system. *Neuron*. 1995; 14:467–475. [PubMed: 7857654]
- Iwatsubo T, Hasegawa M, Ihara Y. Neuronal and glial tau-positive inclusions in diverse neurologic diseases share common phosphorylation characteristics. *Acta Neuropathol*. 1994; 88:129–136. [PubMed: 7985493]
- Jones EM, Dubey M, Camp PJ, Vernon BC, Biernat J, Mandelkow E, et al. Interaction of tau protein with model lipid membranes induces tau structural compaction and membrane disruption. *Biochemistry*. 2012; 51:2539–2550. [PubMed: 22401494]
- Kfoury N, Holmes BB, Jiang H, Holtzman DM, Diamond MI. Trans-cellular propagation of Tau aggregation by fibrillar species. *J Biol Chem*. 2012; 287:19440–19451. [PubMed: 22461630]
- Kisoh H, Pukaß K, Ben-Hur T, Richter-Landsberg C, Sharon R. Increased Neuronal α -Synuclein Pathology Associates with Its Accumulation in Oligodendrocytes in Mice Modeling α -Synucleinopathies. *PLoS ONE*. 2012; 7:e46817. [PubMed: 23077527]
- Kofler J, Lopresti B, Janssen C, Trichel AM, Masliah E, Finn OJ, et al. Preventive immunization of aged and juvenile non-human primates to beta-amyloid. *J Neuroinflammation*. 2012; 9:84. [PubMed: 22554253]
- La Porte SL, Bollini SS, Lanz TA, Abdiche YN, Rusnak AS, Ho WH, et al. Structural basis of C-terminal β -amyloid peptide binding by the antibody ponzumab for the treatment of Alzheimer's disease. *J Mol Biol*. 2012; 421:525–536. [PubMed: 22197375]
- Lambert MP, Velasco PT, Viola KL, Klein WL. Targeting generation of antibodies specific to conformational epitopes of amyloid beta-derived neurotoxins. *CNS Neurol Disord Drug Targets*. 2009; 8:65–81. [PubMed: 19275637]
- Lasagna-Reeves CA, Castillo-Carranza DL, Jackson GR, Kaye R. Tau oligomers as potential targets for immunotherapy for Alzheimer's disease and tauopathies. *Curr Alzheimer Res*. 2011; 8:659–665. [PubMed: 21605039]

- Lashuel HA, Hartley D, Petre BM, Walz T, Lansbury PT. Neurodegenerative disease: amyloid pores from pathogenic mutations. *Nature*. 2002; 418:291. [PubMed: 12124613]
- Laurie C, Reynolds A, Coskun O, Bowman E, Gendelman HE, Mosley RL. CD4+ T cells from Copolymer-1 immunized mice protect dopaminergic neurons in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model of Parkinson's disease. *J Neuroimmunol*. 2007; 183:60–68. [PubMed: 17196666]
- Lecerf JM, Shirley TL, Zhu Q, Kazantsev A, Amersdorfer P, Housman DE, et al. Human single-chain Fv intrabodies counteract in situ huntingtin aggregation in cellular models of Huntington's disease. *Proc Natl Acad Sci USA*. 2001; 98:4764–4769. [PubMed: 11296304]
- Lee CY, Landreth GE. The role of microglia in amyloid clearance from the AD brain. *J Neural Transm*. 2010; 117:949–960. [PubMed: 20552234]
- Lee HJ, Choi C, Lee SJ. Membrane-bound alpha-synuclein has a high aggregation propensity and the ability to seed the aggregation of the cytosolic form. *J Biol Chem*. 2002; 277:671–678. [PubMed: 11679584]
- Lee HJ, Patel S, Lee SJ. Intravesicular localization and exocytosis of alpha-synuclein and its aggregates. *J Neurosci*. 2005; 25:6016–6024. [PubMed: 15976091]
- Lee HJ, Suk JE, Bae EJ, Lee SJ. Clearance and deposition of extracellular alpha-synuclein aggregates in microglia. *Biochem Biophys Res Commun*. 2008; 372:423–428. [PubMed: 18492487]
- Lee HJ, Suk JE, Patrick C, Bae EJ, Cho JH, Rho S, et al. Direct transfer of alpha-synuclein from neuron to astroglia causes inflammatory responses in synucleinopathies. *J Biol Chem*. 2010a; 285:9262–9272. [PubMed: 20071342]
- Lee SJ, Desplats P, Sigurdson C, Tsigelny I, Masliah E. Cell-to-cell transmission of non-prion protein aggregates. *Nat Rev Neurol*. 2010b; 6:702–706. [PubMed: 21045796]
- Lee VM, Otvos L, Schmidt ML, Trojanowski JQ. Alzheimer disease tangles share immunological similarities with multiphosphorylation repeats in the two large neurofilament proteins. *Proc Natl Acad Sci USA*. 1988; 85:7384–7388. [PubMed: 2459703]
- Lema Tomé CM, Tyson T, Rey NL, Grathwohl S, Britschgi M, Brundin P. Inflammation and α -Synuclein's Prion-like Behavior in Parkinson's Disease-Is There a Link? *Molecular neurobiology*. 2012
- Lemere CA, Masliah E. Can Alzheimer disease be prevented by amyloid-beta immunotherapy? *Nat Rev Neurol*. 2010; 6:108–119. [PubMed: 20140000]
- Leng Y, Chase TN, Bennett MC. Muscarinic receptor stimulation induces translocation of an alpha-synuclein oligomer from plasma membrane to a light vesicle fraction in cytoplasm. *J Biol Chem*. 2001; 276:28212–28218. [PubMed: 11337491]
- Lesage S, Brice A. Role of mendelian genes in "sporadic" Parkinson's disease. *Parkinsonism Relat Disord*. 2012; 18(Suppl 1):S66–S70. [PubMed: 22166458]
- Li JY, Englund E, Holton JL, Soulet D, Hagell P, Lees AJ, et al. Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation. *Nat Med*. 2008; 14:501–503. [PubMed: 18391963]
- Lincoln S, Vaughan J, Wood N, Baker M, Adamson J, Gwinn-Hardy K, et al. Low frequency of pathogenic mutations in the ubiquitin carboxy-terminal hydrolase gene in familial Parkinson's disease. *Neuroreport*. 1999; 10:427–429. [PubMed: 10203348]
- Lindvall O, Rehnström S, Brundin P, Gustavii B, Aastedt B, Widner H, et al. Human fetal dopamine neurons grafted into the striatum in two patients with severe Parkinson's disease. A detailed account of methodology and a 6-month follow-up. *Arch Neurol*. 1989; 46:615–631. [PubMed: 2786405]
- Lücking CB, Brice A. Alpha-synuclein and Parkinson's disease. *Cell Mol Life Sci*. 2000; 57:1894–1908. [PubMed: 11215516]
- Luk KC, Kehm V, Carroll J, Zhang B, O'Brien P, Trojanowski JQ, et al. Pathological α -synuclein transmission initiates Parkinson-like neurodegeneration in nontransgenic mice. *Science*. 2012; 338:949–953. [PubMed: 23161999]
- Luthi-Carter R. Progress towards a vaccine for Huntington's disease. *Mol Ther*. 2003; 7:569–570. [PubMed: 12718897]

- Lwin A, Orvisky E, Goker-Alpan O, LaMarca ME, Sidransky E. Glucocerebrosidase mutations in subjects with parkinsonism. *Mol Genet Metab*. 2004; 81:70–73. [PubMed: 14728994]
- Lynch SM, Zhou C, Messer A. An scFv intrabody against the nonamyloid component of alpha-synuclein reduces intracellular aggregation and toxicity. *J Mol Biol*. 2008; 377:136–147. [PubMed: 18237741]
- Madrazo I, León V, Torres C, Aguilera MC, Varela G, Alvarez F, et al. Transplantation of fetal substantia nigra and adrenal medulla to the caudate nucleus in two patients with Parkinson's disease. *N Engl J Med*. 1988; 318:51. [PubMed: 3336384]
- Magga J, Puli L, Pihlaja R, Kanninen K, Neulamaa S, Malm T, et al. Human intravenous immunoglobulin provides protection against A β toxicity by multiple mechanisms in a mouse model of Alzheimer's disease. *J Neuroinflammation*. 2010; 7:90. [PubMed: 21138577]
- Mandelkow EM, Mandelkow E. Tau as a marker for Alzheimer's disease. *Trends Biochem Sci*. 1993; 18:480–483. [PubMed: 8108862]
- Marques O, Outeiro TF. Alpha-synuclein: from secretion to dysfunction and death. *Cell Death Dis*. 2012; 3:e350. [PubMed: 22825468]
- Masliah E, Rockenstein E, Adame A, Alford M, Crews L, Hashimoto M, Seubert P, Lee M, Goldstein J, Chilcote T, Games D, Schenk D. Effects of alpha-synuclein immunization in a mouse model of Parkinson's disease. *Neuron*. 2005; 46:857–868. [PubMed: 15953415]
- Masliah E, Rockenstein E, Mante M, Crews L, Spencer B, Adame A, et al. Passive immunization reduces behavioral and neuropathological deficits in an alpha-synuclein transgenic model of Lewy body disease. *PLoS ONE*. 2011; 6:e19338. [PubMed: 21559417]
- Matsumine H, Yamamura Y, Hattori N, Kobayashi T, Kitada T, Yoritaka A, et al. A microdeletion of D6S305 in a family of autosomal recessive juvenile parkinsonism (PARK2). *Genomics*. 1998; 49:143–146. [PubMed: 9570960]
- Mazzulli JR, Xu YH, Sun Y, Knight AL, McLean PJ, Caldwell GA, et al. Gaucher disease glucocerebrosidase and α -synuclein form a bidirectional pathogenic loop in synucleinopathies. *Cell*. 2011; 146:37–52. [PubMed: 21700325]
- McCoy MK, Cookson MR. Mitochondrial quality control and dynamics in Parkinson's disease. *Antioxid Redox Signal*. 2012; 16:869–882. [PubMed: 21568830]
- McKeith IG, Ballard CG, Perry RH, Ince PG, O'Brien JT, Neill D, et al. Prospective validation of consensus criteria for the diagnosis of dementia with Lewy bodies. *Neurology*. 2000; 54:1050–1058. [PubMed: 10720273]
- Menéndez-González M, Pérez-Piñera P, Martínez-Rivera M, Muñiz AL, Vega JA. Immunotherapy for Alzheimer's disease: rational basis in ongoing clinical trials. *Curr Pharm Des*. 2011; 17:508–520. [PubMed: 21375481]
- Meraz-Ríos MA, Lira-De León KI, Campos-Peña V, De Anda-Hernández MA, Mena-López R. Tau oligomers and aggregation in Alzheimer's disease. *J Neurochem*. 2010; 112:1353–1367. [PubMed: 19943854]
- Miller TW, Shirley TL, Wolfgang WJ, Kang X, Messer A. DNA vaccination against mutant huntingtin ameliorates the HDR6/2 diabetic phenotype. *Mol Ther*. 2003; 7:572–579. [PubMed: 12718899]
- Min KJ, Yang MS, Kim SU, Jou I, Joe EH. Astrocytes induce hemeoxygenase-1 expression in microglia: a feasible mechanism for preventing excessive brain inflammation. *J Neurosci*. 2006; 26:1880–1887. [PubMed: 16467537]
- Morgan D. Immunotherapy for Alzheimer's disease. *J Alzheimers Dis*. 2006; 9:425–432. [PubMed: 16914881]
- Morgan D. Immunotherapy for Alzheimer's disease. *J Intern Med*. 2011; 269:54–63. [PubMed: 21158978]
- Mougenot AL, Nicot S, Bencsik A, Morignat E, Verchère J, Lakhdar L, et al. Prion-like acceleration of a synucleinopathy in a transgenic mouse model. *Neurobiol Aging*. 2012; 33:2225–2228. [PubMed: 21813214]
- Nakamura S, Kawamoto Y, Nakano S, Akiguchi I, Kimura J. Cyclin-dependent kinase 5 and mitogen-activated protein kinase in glial cytoplasmic inclusions in multiple system atrophy. *J Neuropathol Exp Neurol*. 1998; 57:690–698. [PubMed: 9690673]

- Napoli I, Neumann H. Microglial clearance function in health and disease. *Neuroscience*. 2009; 158:1030–1038. [PubMed: 18644426]
- Näsström T, Gonçalves S, Sahlin C, Nordström E, Screpanti Sundquist V, Lannfelt L, et al. Antibodies against alpha-synuclein reduce oligomerization in living cells. *PLoS ONE*. 2011; 6:e27230. [PubMed: 22073131]
- Neary D, Snowden J, Mann D. Frontotemporal dementia. *Lancet Neurol*. 2005; 4:771–780. [PubMed: 16239184]
- Orgogozo JM, Gilman S, Dartigues JF, Laurent B, Puel M, Kirby LC, et al. Subacute meningoencephalitis in a subset of patients with AD after Abeta42 immunization. *Neurology*. 2003; 61:46–54. [PubMed: 12847155]
- Ostrowitzki S, Deptula D, Thurfjell L, Barkhof F, Bohrmann B, Brooks DJ, et al. Mechanism of amyloid removal in patients with Alzheimer disease treated with gantenerumab. *Arch Neurol*. 2012; 69:198–207. [PubMed: 21987394]
- Pankiewicz J, Prelli F, Sy MS, Kascsak RJ, Kascsak RB, Spinner DS, et al. Clearance and prevention of prion infection in cell culture by anti-PrP antibodies. *Eur J Neurosci*. 2006; 23:2635–2647. [PubMed: 16817866]
- Panza F, Frisardi V, Solfrizzi V, Imbimbo BP, Logroscino G, Santamato A, et al. Immunotherapy for Alzheimer's disease: from anti- β -amyloid to tau-based immunization strategies. *Immunotherapy*. 2012; 4:213–238. [PubMed: 22339463]
- Papp MI, Kahn JE, Lantos PL. Glial cytoplasmic inclusions in the CNS of patients with multiple system atrophy (striatonigral degeneration, olivopontocerebellar atrophy and Shy-Drager syndrome). *J Neurol Sci*. 1989; 94:79–100. [PubMed: 2559165]
- Papp MI, Lantos PL. The distribution of oligodendroglial inclusions in multiple system atrophy and its relevance to clinical symptomatology. *Brain*. 1994; 117(Pt 2):235–243. [PubMed: 8186951]
- Perera RM, Zoncu R, Johns TG, Pypaert M, Lee FT, Mellman I, et al. 111In-Labeled chimeric monoclonal antibody, ch806, targeting the epidermal growth factor receptor deletion variant de2–7 (EGFRvIII). *Neoplasia*. 2007; 9:1099–1110. [PubMed: 18084617]
- Pul R, Dodel R, Stangel M. Antibody-based therapy in Alzheimer's disease. *Expert Opin Biol Ther*. 2011; 11:343–357. [PubMed: 21261567]
- Ramirez A, Heimbach A, Gründemann J, Stiller B, Hampshire D, Cid LP, et al. Hereditary parkinsonism with dementia is caused by mutations in ATP13A2, encoding a lysosomal type 5 P-type ATPase. *Nat Genet*. 2006; 38:1184–1191. [PubMed: 16964263]
- Restifo NP, Dudley ME, Rosenberg SA. Adoptive immunotherapy for cancer: harnessing the T cell response. *Nat Rev Immunol*. 2012; 12:269–281. [PubMed: 22437939]
- Reynolds AD, Banerjee R, Liu J, Gendelman HE, Mosley RL. Neuroprotective activities of CD4+CD25+ regulatory T cells in an animal model of Parkinson's disease. *J Leukoc Biol*. 2007; 82:1083–1094. [PubMed: 17675560]
- Reynolds AD, Stone DK, Hutter JA, Benner EJ, Mosley RL, Gendelman HE. Regulatory T cells attenuate Th17 cell-mediated nigrostriatal dopaminergic neurodegeneration in a model of Parkinson's disease. *J Immunol*. 2010; 184:2261–2271. [PubMed: 20118279]
- Richter-Landsberg C, Gorath M, Trojanowski JQ, Lee VM. alpha-synuclein is developmentally expressed in cultured rat brain oligodendrocytes. *J Neurosci Res*. 2000; 62:9–14. [PubMed: 11002283]
- Rinne JO, Brooks DJ, Rossor MN, Fox NC, Bullock R, Klunk WE, et al. 11C-PiB PET assessment of change in fibrillar amyloid-beta load in patients with Alzheimer's disease treated with bapineuzumab: a phase 2, double-blind, placebo-controlled, ascending-dose study. *Lancet Neurol*. 2010; 9:363–372. [PubMed: 20189881]
- Robert M, Mathuranath PS. Tau and tauopathies. *Neurol India*. 2007; 55:11–16. [PubMed: 17272893]
- Rohn TT. Targeting alpha-synuclein for the treatment of Parkinson's disease. *CNS Neurol Disord Drug Targets*. 2012; 11:174–179. [PubMed: 22483285]
- Salloway S, Sperling R, Gilman S, Fox NC, Blennow K, Raskind M, et al. A phase 2 multiple ascending dose trial of bapineuzumab in mild to moderate Alzheimer disease. *Neurology*. 2009; 73:2061–2070. [PubMed: 19923550]

- Schenk D, Barbour R, Dunn W, Gordon G, Grajeda H, Guido T, et al. Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature*. 1999; 400:173–177. [PubMed: 10408445]
- Schenk DB, Seubert P, Grundman M, Black R. A beta immunotherapy: Lessons learned for potential treatment of Alzheimer's disease. *Neurodegener Dis*. 2005; 2:255–260. [PubMed: 16909006]
- Schneeberger A, Mandler M, Mattner F, Schmidt W. AFFITOME® technology in neurodegenerative diseases: the doubling advantage. *Hum Vaccin*. 2010; 6:948–952. [PubMed: 20980801]
- Schneeberger A, Mandler M, Mattner F, Schmidt W. Vaccination for Parkinson's disease. *Parkinsonism Relat Disord*. 2012; 18(Suppl 1):S11–S13. [PubMed: 22166404]
- Schulz-Schaeffer WJ. The synaptic pathology of alpha-synuclein aggregation in dementia with Lewy bodies, Parkinson's disease and Parkinson's disease dementia. *Acta Neuropathol*. 2010; 120:131–143. [PubMed: 20563819]
- Seelaar H, Rohrer JD, Pijnenburg YA, Fox NC, van Swieten JC. Clinical, genetic and pathological heterogeneity of frontotemporal dementia: a review. *J Neurol Neurosurg Psychiatr*. 2011; 82:476–486. [PubMed: 20971753]
- Shi J, Shaw CL, Du Plessis D, Richardson AM, Bailey KL, Julien C, et al. Histopathological changes underlying frontotemporal lobar degeneration with clinicopathological correlation. *Acta Neuropathol*. 2005; 110:501–512. [PubMed: 16222525]
- Sigurdsson EM. Immunotherapy targeting pathological tau protein in Alzheimer's disease and related tauopathies. *J Alzheimers Dis*. 2008; 15:157–168. [PubMed: 18953105]
- Sigurdsson EM, Brown DR, Daniels M, Kascsak RJ, Kascsak R, Carp R, et al. Immunization delays the onset of prion disease in mice. *Am J Pathol*. 2002; 161:13–17. [PubMed: 12107084]
- Snowden J, Neary D, Mann D. Frontotemporal lobar degeneration: clinical and pathological relationships. *Acta Neuropathol*. 2007; 114:31–38. [PubMed: 17569065]
- Spillantini MG. Parkinson's disease, dementia with Lewy bodies and multiple system atrophy are alpha-synucleinopathies. *Parkinsonism Relat Disord*. 1999; 5:157–162. [PubMed: 18591134]
- Spillantini MG, Goedert M. Tau protein pathology in neurodegenerative diseases. *Trends Neurosci*. 1998; 21:428–433. [PubMed: 9786340]
- Spillantini MG, Goedert M. The alpha-synucleinopathies: Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy. *Ann N Y Acad Sci*. 2000; 920:16–27. [PubMed: 11193145]
- Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. Alpha-synuclein in Lewy bodies. *Nature*. 1997; 388:839–840. [PubMed: 9278044]
- Stefanova N, Hainzer M, Stemberger S, Couillard-Després S, Aigner L, Poewe W, et al. Striatal transplantation for multiple system atrophy--are grafts affected by alpha-synucleinopathy? *Exp Neurol*. 2009; 219:368–371. [PubMed: 19460374]
- Streit WJ, Mrak RE, Griffin WS. Microglia and neuroinflammation: a pathological perspective. *J Neuroinflammation*. 2004; 1:14. [PubMed: 15285801]
- Sutherland GT, Halliday GM, Silburn PA, Mastaglia FL, Rowe DB, Boyle RS, et al. Do polymorphisms in the familial Parkinsonism genes contribute to risk for sporadic Parkinson's disease? *Mov Disord*. 2009; 24:833–838. [PubMed: 19224617]
- Takeda A, Mallory M, Sundsmo M, Honer W, Hansen L, Masliah E. Abnormal accumulation of NACP/alpha-synuclein in neurodegenerative disorders. *Am J Pathol*. 1998; 152:367–372. [PubMed: 9466562]
- Takeuchi S, Fujiwara N, Ido A, Oono M, Takeuchi Y, Tateno M, et al. Induction of protective immunity by vaccination with wild-type apo superoxide dismutase 1 in mutant SOD1 transgenic mice. *J Neuropathol Exp Neurol*. 2010; 69:1044–1056. [PubMed: 20838241]
- Tamura Y, Hamajima K, Matsui K, Yanoma S, Narita M, Tajima N, et al. The F(ab)'2 fragment of an Abeta-specific monoclonal antibody reduces Abeta deposits in the brain. *Neurobiol Dis*. 2005; 20:541–549. [PubMed: 15908227]
- Taniguchi S, McDonagh AM, Pickering-Brown SM, Umeda Y, Iwatsubo T, Hasegawa M, et al. The neuropathology of frontotemporal lobar degeneration with respect to the cytological and biochemical characteristics of tau protein. *Neuropathol Appl Neurobiol*. 2004; 30:1–18. [PubMed: 14720172]

- Tarawneh R, Holtzman DM. Critical issues for successful immunotherapy in Alzheimer's disease: development of biomarkers and methods for early detection and intervention. *CNS Neurol Disord Drug Targets*. 2009; 8:144–159. [PubMed: 19355934]
- Thal DR. The role of astrocytes in amyloid β -protein toxicity and clearance. *Exp Neurol*. 2012; 236:1–5. [PubMed: 22575598]
- Thomas MP, Chartrand K, Reynolds A, Vitvitsky V, Banerjee R, Gendelman HE. Ion channel blockade attenuates aggregated alpha synuclein induction of microglial reactive oxygen species: relevance for the pathogenesis of Parkinson's disease. *J Neurochem*. 2007; 100:503–519. [PubMed: 17241161]
- Town T. Alternative Abeta immunotherapy approaches for Alzheimer's disease. *CNS Neurol Disord Drug Targets*. 2009; 8:114–127. [PubMed: 19355932]
- Troquier L, Caillierez R, Burnouf S, Fernandez-Gomez FJ, Grosjean ME, Zommer N, et al. Targeting phospho-Ser422 by active Tau Immunotherapy in the THYTau22 mouse model: a suitable therapeutic approach. *Curr Alzheimer Res*. 2012; 9:397–405. [PubMed: 22272619]
- Tsigelny IF, Bar-On P, Sharikov Y, Crews L, Hashimoto M, Miller MA, et al. Dynamics of alpha-synuclein aggregation and inhibition of pore-like oligomer development by beta-synuclein. *The FEBS journal*. 2007; 274:1862–1877. [PubMed: 17381514]
- Tsigelny IF, Sharikov Y, Miller MA, Masliah E. Mechanism of alpha-synuclein oligomerization and membrane interaction: theoretical approach to unstructured proteins studies. *Nanomedicine*. 2008; 4:350–357. [PubMed: 18640077]
- Tsuboi K, Grzesiak JJ, Bouvet M, Hashimoto M, Masliah E, Shults CW. Alpha-synuclein overexpression in oligodendrocytic cells results in impaired adhesion to fibronectin and cell death. *Mol Cell Neurosci*. 2005; 29:259–268. [PubMed: 15911350]
- Ubhi K, Masliah E. Recent advances in the development of immunotherapies for tauopathies. *Exp Neurol*. 2011; 230:157–161. [PubMed: 20970422]
- Ubhi K, Rockenstein E, Mante M, Inglis C, Adame A, Patrick C, et al. Neurodegeneration in a transgenic mouse model of multiple system atrophy is associated with altered expression of oligodendroglial-derived neurotrophic factors. *J Neurosci*. 2010; 30:6236–6246. [PubMed: 20445049]
- Ubol S, Levine B, Lee SH, Greenspan NS, Griffin DE. Roles of immunoglobulin valency and the heavy-chain constant domain in antibody-mediated downregulation of Sindbis virus replication in persistently infected neurons. *J Virol*. 1995; 69:1990–1993. [PubMed: 7853547]
- Uéda K, Fukushima H, Masliah E, Xia Y, Iwai A, Yoshimoto M, et al. Molecular cloning of cDNA encoding an unrecognized component of amyloid in Alzheimer disease. *Proc Natl Acad Sci USA*. 1993; 90:11282–11286. [PubMed: 8248242]
- Uro-Coste E, Russano de Paiva G, Guilbeau-Frugier C, Sastre N, Ousset PJ, da Silva NA, et al. Cerebral amyloid angiopathy and microhemorrhages after amyloid beta vaccination: case report and brief review. *Clin Neuropathol*. 2010; 29:209–216. [PubMed: 20569670]
- Urushitani M, Ezzi SA, Julien JP. Therapeutic effects of immunization with mutant superoxide dismutase in mice models of amyotrophic lateral sclerosis. *Proc Natl Acad Sci USA*. 2007; 104:2495–2500. [PubMed: 17277077]
- Valente EM, Arena G, Torosantucci L, Gelmetti V. Molecular pathways in sporadic PD. *Parkinsonism Relat Disord*. 2012; 18(Suppl 1):S71–S73. [PubMed: 22166460]
- van Duijn CM, Dekker MC, Bonifati V, Galjaard RJ, Houwing-Duistermaat JJ, Snijders PJ, et al. Park7, a novel locus for autosomal recessive early-onset parkinsonism, on chromosome 1p36. *Am J Hum Genet*. 2001; 69:629–634. [PubMed: 11462174]
- Vekrellis K, Stefanis L. Targeting intracellular and extracellular alpha-synuclein as a therapeutic strategy in Parkinson's disease and other synucleinopathies. *Expert Opin Ther Targets*. 2012; 16:421–432. [PubMed: 22480256]
- Wakabayashi K, Hayashi S, Yoshimoto M, Kudo H, Takahashi H. NACP/alpha-synuclein-positive filamentous inclusions in astrocytes and oligodendrocytes of Parkinson's disease brains. *Acta Neuropathol*. 2000; 99:14–20. [PubMed: 10651022]

- Wakabayashi K, Matsumoto K, Takayama K, Yoshimoto M, Takahashi H. NACP, a presynaptic protein, immunoreactivity in Lewy bodies in Parkinson's disease. *Neurosci Lett*. 1997; 239:45–48. [PubMed: 9547168]
- Wakabayashi K, Takahashi H. Cellular pathology in multiple system atrophy. *Neuropathology*. 2006; 26:338–345. [PubMed: 16961071]
- Wakabayashi K, Yoshimoto M, Tsuji S, Takahashi H. Alpha-synuclein immunoreactivity in glial cytoplasmic inclusions in multiple system atrophy. *Neurosci Lett*. 1998; 249:180–182. [PubMed: 9682846]
- Wang YJ, Zhou HD, Zhou XF. Modified immunotherapies against Alzheimer's disease: toward safer and effective amyloid clearance. *J Alzheimers Dis*. 2010; 21:1065–1075. [PubMed: 21504118]
- White AR, Enever P, Tayebi M, Mushens R, Linehan J, Brandner S, et al. Monoclonal antibodies inhibit prion replication and delay the development of prion disease. *Nature*. 2003; 422:80–83. [PubMed: 12621436]
- Wiessner C, Wiederhold KH, Tissot AC, Frey P, Danner S, Jacobson LH, et al. The second-generation active A β immunotherapy CAD106 reduces amyloid accumulation in APP transgenic mice while minimizing potential side effects. *J Neurosci*. 2011; 31:9323–9331. [PubMed: 21697382]
- Wilcock DM, Colton CA. Anti-amyloid-beta immunotherapy in Alzheimer's disease: relevance of transgenic mouse studies to clinical trials. *J Alzheimers Dis*. 2008; 15:555–569. [PubMed: 19096156]
- Winblad B, Andreasen N, Minthon L, Floesser A, Imbert G, Dumortier T, et al. Safety, tolerability, and antibody response of active A β immunotherapy with CAD106 in patients with Alzheimer's disease: randomised, double-blind, placebo-controlled, first-inhuman study. *Lancet Neurol*. 2012; 11:597–604. [PubMed: 22677258]
- Winner B, Jappelli R, Maji SK, Desplats PA, Boyer L, Aigner S, et al. In vivo demonstration that alpha-synuclein oligomers are toxic. *Proc Natl Acad Sci USA*. 2011; 108:4194–4199. [PubMed: 21325059]
- Wisniewski T, Boutajangout A. Vaccination as a therapeutic approach to Alzheimer's disease. *Mt Sinai J Med*. 2010; 77:17–31. [PubMed: 20101719]
- Wolfgang WJ, Miller TW, Webster JM, Huston JS, Thompson LM, Marsh JL, et al. Suppression of Huntington's disease pathology in *Drosophila* by human single-chain Fv antibodies. *Proc Natl Acad Sci USA*. 2005; 102:11563–11568. [PubMed: 16061794]
- Wolozin BL, Pruchnicki A, Dickson DW, Davies P. A neuronal antigen in the brains of Alzheimer patients. *Science*. 1986; 232:648–650. [PubMed: 3083509]
- Yoshida M. Multiple system atrophy: alpha-synuclein and neuronal degeneration. *Neuropathology*. 2007; 27:484–493. [PubMed: 18018485]
- Yu YJ, Zhang Y, Kenrick M, Hoyte K, Luk W, Lu Y, et al. Boosting brain uptake of a therapeutic antibody by reducing its affinity for a transcytosis target. *Sci Transl Med*. 2011; 3 84ra44.
- Zhou C, Emadi S, Sierks MR, Messer A. A human single-chain Fv intrabody blocks aberrant cellular effects of overexpressed alpha-synuclein. *Mol Ther*. 2004; 10:1023–1031. [PubMed: 15564134]
- Zimprich A, Benet-Pagès A, Struhal W, Graf E, Eck SH, Offman MN, et al. A mutation in VPS35, encoding a subunit of the retromer complex, causes late-onset Parkinson disease. *Am J Hum Genet*. 2011; 89:168–175. [PubMed: 21763483]
- Zimprich A, Biskup S, Leitner P, Lichtner P, Farrer M, Lincoln S, et al. Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. *Neuron*. 2004; 44:601–607. [PubMed: 15541309]

Figure 1. Passive immunization against α -syn induces the clearance of intraneuronal α -syn aggregates

A. A transgenic mouse model expressing human α -syn under the control of the PDFG β promoter was immunized with antibodies against different regions of α -syn, and the effect of immunization was analyzed. B. Antibodies designed for passive immunization and α -syn amino acids that they recognize. C. Passive immunization with antibody 9E4 clears intracellular α -syn aggregates in the neocortex and hippocampus, compared to IgG1-immunized mice. For immunolabeling, an antibody against calpain-cleaved α -syn was used (Modified from Masliah et al., 2011). NAC, Non-A β component; Tg, transgenic.

Figure 2. Mechanism of α -syn clearance induced by passive immunization

A. Circulating antibodies may recognize α -syn oligomers accumulating in the plasma membrane. Binding of antibodies to membrane-bound α -syn induces receptor-mediated endocytosis ($\text{Fc}\gamma$) and degradation by autophagy. Membrane budding and formation of multivesicular bodies (ESCRT pathway) is depicted. B. Representative electron micrographs of sections from an α -syn transgenic mouse immunized with the 9E4 antibody or the control IgG1 antibody, and immunolabeled with gold-tagged anti-mouse antibody. The 9E4 antibody localizes in autophagosome-like structures (arrows) (Modified from Masliah et al., 2011).

Figure 3. Mechanisms of α -syn clearance in immunotherapy

Antibodies against α -syn, produced after active immunization or introduced by passive immunization, can target α -syn at several points of the progression of the pathology. A. Antibodies can recognize α -syn oligomers accumulating in the neuronal membrane and induce their degradation via autophagy. B. Antibody binding blocks trans-synaptic dissemination of α -syn from postsynaptic to presynaptic terminals. C. Binding of circulating antibodies has the potential to halt extracellular propagation of α -syn oligomers. D. Microglia-mediated clearance of antibody-bound α -syn is a potential mechanism to remove toxic extracellular α -syn oligomers. Finally, propagation of α -syn oligomers to grafted neuronal stem cells is also a process that can be potentially prevented by immunotherapy.

Table 1
Clinical studies on immunotherapies for neurodegenerative diseases
Information regarding clinical trials was found at clinicaltrials.gov and alzforum.org. NP, not published; aa, amino acids.

Drug name	Phase	Phase	Sponsor	References
Alzheimer's disease				
Active immunotherapy: vaccines				
AFFITOPE AD02	II	1–6 aa	Affiris	Schneberger et al., 2010
AFFITOPE AD03	I	NP	Affiris	
ACC-001	II	1–6 aa	Pfizer/Janssen	
CAD106	II	1–6 aa	Novartis	Winblad et al., 2012
UB 311	I	1–14 aa	United Biomedical	
V950	I	N-terminus	Merck	
Passive immunotherapy: antibodies				
Bapineuzumab	III	1–5 aa	Janssen/Elan/Pfizer	Rinne et al., 2010 Blenow et al., 2012
Crenelumab	II	12–23 aa	Genentech	Adolfsson et al., 2012
Gantenerumab	III	Conformational	Hoffman-La Roche	Bohrmann et al., 2012 Ostrowitzki et al., 2012
GSK933776	I	N-terminus	GlaxoSmithKline	
Ponezumab	II	33–40 aa	Pfizer	La Porte et al., 2012
Solanezumab	III	13–28 aa	Eli Lilly	Farlow et al., 2012
Gammagard	III	Immunoglobulin	Baxter Healthcare corporation	Magga et al., 2010
Octagam	II	Immunoglobulin	Octapharma	
Parkinson's disease				
Active immunotherapy: vaccines				
AFFITOPE PD01	I	NP	Affiris	Schneberger et al., 2012