# α-synuclein immunotherapy as a suitable treatment for Parkinson disease

Author: Ilona Schuldink (S4577183)
Supervisor: Prof. Dr. ULM Eisel
2de assessor: Prof. Dr. A.M. Dolga

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# Table of content

## Abstract

At the current moment in time, the only way to treat Parkinson's disease (PD) is via symptomatic treatments that involve the dopaminergic pathway. This way of treatment only affects part of the motor symptoms of the patients. It is currently investigated if other types of treatment could potentially stop or halt the progression of PD. Via the idea of clinical trials that have been done with immunotherapy for Alzheimer's disease, the same type of treatment is being tested for PD. For PD the immunotherapy mostly focuses on the aggregated α-synuclein formation in the neurons. The aggregated α-synuclein can transfer from neurons to other neurons via the cell-to-cell transmission. This aggregated α-synuclein is an important hallmark of PD as it is thought to be the potential cause of PD. The research question that is going to be investigated in this essay is about if α-synuclein immunotherapy is a suitable treatment for PD.

α-synuclein immunotherapy is tested in animal models, and is currently tested in clinical trials. The results thus far have been promising in reducing the aggregated α-synuclein from the brain with the use of both active and passive immunization. But there are still some concerns revolving around this new type of immunotherapy. These concerns are about the specific targeting of aggregated α-synuclein, and not the native monomeric form of α-synuclein. Other concerns are about the effectiveness of the antibodies that need to cross the blood brain barrier, and the chance of inducing an autoimmune T-cell response. To give a possible solution for these concerns, more information needs to be gathered about the mechanisms and the antibodies that are used for α-synuclein immunotherapy. But overall, the future prospects of α-synuclein look positive in the sense that in the future there might be a suitable therapy for the treatment of PD.

# Introduction

Parkinson's disease (PD) affects 1-3% of adults over the age of 60 years worldwide. This means that millions of people in the entire world have daily problems with tremors, bradykinesia, rigidity, and postural instability together with more non-motor symptoms. The main characterization of PD is the selective loss of dopaminergic neurons in the Substantia nigra and the aggregation of misfolded α-synuclein (Samii et al., 2004). Aggregated α-synuclein is thought to be a potential cause of PD (Polymeropoulos et al., 1997). These aggregates are formed from the native monomeric form of α-synuclein. It is proposed that α-synuclein can transfer between neurons to spread the aggregation of α-synuclein to other neurons (Henderson et al., 2019).

Currently, the only approved treatments of PD focus on symptom treatment and not on the treatment of the cause of the PD itself. The treatment that is most used for PD is Levodopa. Levodopa is an effective dopamine-based treatment that has a positive effect on the motor symptoms. But the downside of Levodopa is that it can work less effectively if this medicine is taken up for a longer period of time. If this is the case, then there is also the option for deep brain stimulation (DBS). This is a surgical procedure that can help with the motor symptom problems of patients (Capriotti & Terzakis, 2016).
But the end goal to treat a disease is to treat the cause of the disease and not only the symptoms, so that the progressive loss of motor and non-motor functions is halted or stopped. This is one of the reasons that new treatments are being tested. A promising form of treatment that is recently being tested for Alzheimer's disease is the use of immunotherapy. Because of the positive test results in clinical trials, this style of treatment is at the current moment also being tested for PD. This immunotherapy for PD focuses on generating antibodies against aggregated α-synuclein. This immunotherapy can be done in two ways, with the use of active immunization and with the use of passive immunization. Active immunization uses the individual's immune system to generate antibodies against α-synuclein, and passive immunization uses antibodies that are directly injected into an individual (George & Brundin, 2015).

In this essay, the immunotherapy that focuses on antibodies targeting aggregated α-synuclein is being discussed. The research question that is being investigated in this essay is:
"Is α-synuclein immunotherapy a suitable treatment for Parkinson's Disease?"

Information has been gathered about the current state of the progression of immunotherapy as a treatment for Parkinson's disease, as well as information about the possible concerns that are still surrounding this new form of treatment.

# Parkinson Disease

Parkinson's disease (PD) is a progressive neurodegenerative disease that affects between 1-3% of the world's population at the current moment. PD is hereby a movement disorder that affects mostly older adults with an age over 60 years (Samii et al., 2004). Within these elderly groups there are no racial differences, but it has been noted that men are more likely to get PD than women (Khan et al., 2019). Research revealed that PD is a mostly age-related disease that can be influenced by environmental factors and genetic factors (Antony et al., 2013).

PD can be characterized by selective loss of dopaminergic neurons in the substantia nigra and by the accumulation of misfolded α-synuclein that can form into intra-cytoplasmic structures called Lewy bodies that are located in specific brain regions (Lee & Gilbert, 2016). The formation of Lewy bodies is considered a neuronal degeneration marker because the neuronal loss is primarily found in sites of these Lewy bodies (Wakabayashi et al., 2013). Lewy bodies are originally present in the nucleus of the vagus nerve and the olfactory bulb. After this it can spread to different areas of the brain and nervous system in patients with PD (Braak et al., 2003). Hypothesized is that PD starts in the peripheral nervous system and then slowly moves to the central nervous system (Müller et al., 2005).

Misfolded α-synuclein is the primary component of Lewy bodies. Different missense mutations in the α-synuclein gene can be associated with the autosomal dominant form of PD, whereby some single-nucleotide polymorphisms in the α-synuclein gene can be associated with an increased risk of PD. Furthermore, duplications and triplications in the α-synuclein gene are associated with atypical parkinsonian syndromes (George & Brundin, 2015).

The main risk for PD that cannot be prevented is age and family history. Other risk factors can be pesticide exposure and exposure to environmental chemicals. But the ultimate cause of PD is not yet known (Beitz, 2014). In around 5-10% of PD patients, several genetic factors can be identified (Tysnes & Storstein, 2017). These cases are mostly of familial PD, but the studies on these families can be used to gain insight into the pathogenesis and genetics of the other forms of PD to identify genes and gain information about the mechanisms of PD (Balestrino & Schapira, 2020).

## Symptoms

The symptoms that are prominent in PD can be sorted into two groups; motor symptoms and non-motor symptoms. The main motor symptoms consist of tremor, bradykinesia, rigidity, and postural instability. Other motor symptoms consist of slowness and stiffness (Beitz, 2014). The non-motor symptoms can be a varying array of symptoms, like anxiety, depression, sleep disorders, fatigue, and mood changes. A lot of patients usually suffer largely from these non-motor symptoms (Jagadeesan et al., 2017). Other non-motor symptoms include cognitive impairment, examples of this are dementia and autonomic dysfunction (Beitz, 2014). Other symptoms include gastrointestinal symptoms such as nausea, vomiting, and constipation (Liddle, 2018).

The diagnosis of PD is clinical, but specific other investigations can help to make a differentiation between PD and other atypical parkinsonism forms (Balestrino & Schapira, 2020). Different tests can help with the clinical diagnosis of PD, such as genetic testing, MRI, olfactory testing, dopamine-transporter-single-photon-emission-computed-tomography imaging (DaT-SPECT) (Tolosa et al., 2006). Early diagnosis of PD is very important, because by the time the patient notices the decrease in motor functions, around 60% of the dopaminergic neurons of the patient can already have been lost. This is because the neuronal damage happens sooner than the motor dysfunctions, sometimes these differences can be more than twenty years (Wang et al., 2019).

## Treatment

Currently, the treatment of PD is focused on the symptoms and not on the actual cause of the disease itself. This is because it is very difficult to find an effective and safe treatment to slow or halt the progression of PD. The used treatments are thus symptomatic, and are mostly focused on the dopaminergic pathway. These treatments have for the most part only an effect on the motor symptoms (Charvin et al., 2018).

The first treatment for PD is Levodopa. This is a drug that is most effective for the motor symptoms. Levodopa can cross the blood-brain barrier, where it is then converted into dopamine in the dopaminergic neurons. However, Levodopa can cause side effects like hypotension, nausea, confusion, impulse control disorders, and hallucinations (Beaulieu-Boire & Lang, 2015). Levodopa can also cause the development of motor complications (Olanow et al., 2006). It is important to not give patients Levodopa too early in the disease progression because the effectiveness of Levodopa becomes less with time (Capriotti & Terzakis, 2016).
The second treatment is dopamine agonists. Dopamine agonists are not as effective against the motor symptoms as Levodopa, but dopamine agonists can be used with a lower risk to induce dyskinesia. The downside of dopamine agonists is that it can cause more impulse control disorders (Blandini & Armentero, 2014).
The third treatment makes use of inhibitors, like the Monoamine oxidase B (MAO-B) inhibitor and the Catechol-O-methyl transfers (COMT) inhibitor. These inhibitors are usually prescribed when Levodopa and dopamine agonists have lost their main effectiveness. The inhibitors can then enhance the effectiveness of Levodopa and dopamine agonists again (Conolly & Lang, 2014). The MAO-B inhibitor has the ability to decrease the dopamine metabolism whereby the dopaminergic stimulation is extended (Robakis & Fahn, 2015). The COMT inhibitor is administered together with Levodopa to increase the half-life of Levodopa (Müller, 2015).

If Levodopa or the other possible treatments are not effective anymore, surgery in the form of deep brain stimulation (DBS) can be proposed. Hereby a pulse generator is implanted in the brain that delivers electrical stimulation to the brain areas where movement is controlled. Deep brain stimulation can block the abnormal nerve signals that can be the cause of the motor symptoms in PD (Olanow et al., 2009). Important aspects that need to be considered for DBS are for example the age of the patient, the disease duration, the responsiveness to Levodopa and the severity for this, and the cognitive state of the patients. There are also side effects present in DBS. These side effects can worsen the cognitive functions, can cause psychiatric symptoms and speech and ocular disturbances. Furthermore, DBS showed no improvement in the motor symptoms that are not responsive to Levodopa, these include events such as falling, freezing, and axial signs (Balestrino & Schapira, 2020).

## Parkinsonism

There are also different types of neurological diseases that look symptomatically like PD, but that are categorized as different diseases. These different diseases are categorized as atypical parkinsonian syndromes or parkinsonism (Levin et al., 2016). It can be difficult to determine whether the patient has PD or parkinsonism at an early stage of the disease progression. One way to determine the difference is to look at the response of the patient to Levodopa. While levodopa works well for PD, for parkinsonism the patients do not respond well to Levodopa therapy.
The types of atypical parkinsonian syndromes that are most typical are:

- Dementia with Lewy bodies (DLB)
- Multiple system atrophy (MSA)
- Progressive supranuclear palsy (PSP)
- Corticobasal degeneration (CBD)

The different atypical parkinsonian syndromes are mostly synucleinopathies or tauopathies, these syndromes are characterized with an unusual deposition of α-synuclein and tau, respectively.
PD is also categorized as a synucleinopathy disease. Synucleinopathies is an umbrella-term for diseases where α-synuclein is involved in (Choi et al., 2020). One thing that the different synucleinopathies have in common is that all these neurodegenerative diseases are defined as having an accumulation of oligomers that are misfolded together with the accumulation of α-synuclein aggregates (Burré et al., 2018).

## Lewy bodies & α-synuclein

The pathological hallmark of PD are Lewy bodies. The main components of these Lewy bodies are α-synuclein aggregates. A hypothesis was therefore proposed that α-synuclein aggregation can be associated with the pathogenesis of PD. This hypothesis is also supported with the discovery that point mutations in the α-synuclein protein are associated with the rarer forms of familial PD (Mehra et al., 2019). Within PD there is familial PD and sporadic PD. Familial PD is linked to α-synuclein gene mutations. The α-synuclein gene is also a risk factor of sporadic PD (Choi et al., 2020).

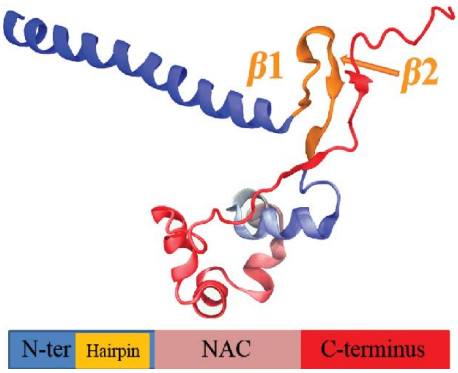


Figure 1. A schematic image of a structural α-synuclein monomer. It shows the N-terminus region, NAC region, and C-terminus region in the colors blue, pink, and red, respectively. The structure also contains β-hairpin formations. This β-hairpin region is colored yellow. *Modified from Yu et al. (2015)*

A-synuclein is translated from the SNCA gene and is part of the synuclein family. The SNCA gene is located on chromosome 4q21-23 (Polymeropoulos et al., 1997). The sequence of α-synuclein consists of 140 amino acids and it contains 3 main domains: the N-terminal domain, the C-terminal domain, and the non-amyloid-component (NAC) domain (Dehay et al., 2015). It is known that the native monomeric α-synuclein form has a close interaction with the SNARE complex, which in its turn has a major role in the neurotransmitter release. There have also been studies where it was suggested that α-synuclein can play an important role in the release of dopamine (Shin et al., 2020).
A-synuclein can form aggregates that are toxic in the brain tissue. These toxic aggregates can come from multiple different ways. There can be a mutation in the SNCA gene, the protein can become shortened, and it can be because of protein phosphorylation (Games et al., 2014). These modification on the α-synuclein protein can cause the transformation between different conformations such as monomers, oligomers, and fibrils. But it can also transform into aggregates which can cause different synucleinopathies including PD (Wong & Krainc, 2017).

## Transmission of α-synuclein

It has been discovered that the progressive pattern of α-synuclein pathology correlates with the symptoms of PD. In the early stages of PD the patients have motor dysfunction, at the same time α-synuclein is mainly found in the brain regions that control the motor functions. At the later stages of PD when the cognitive functions of patients worsen, α-synuclein is mainly found in cortical structures that are responsible for the higher cognitive processing (Braak & Del Tredici, 2008).
This progression of α-synuclein through the brain can be a suggestion that there is a physical transmission of α-synuclein from and to the different brain areas. This hypothesis is called the transmission hypothesis or the cell-to-cell hypothesis, and this hypothesis states that an initial "seed" of α-synuclein in a neuron can be released and taken up by other neurons that are potentially vulnerable. This then can cause the initiation of the misfolding and aggregation of α-synuclein in the receiving neurons (Henderson et al., 2019).

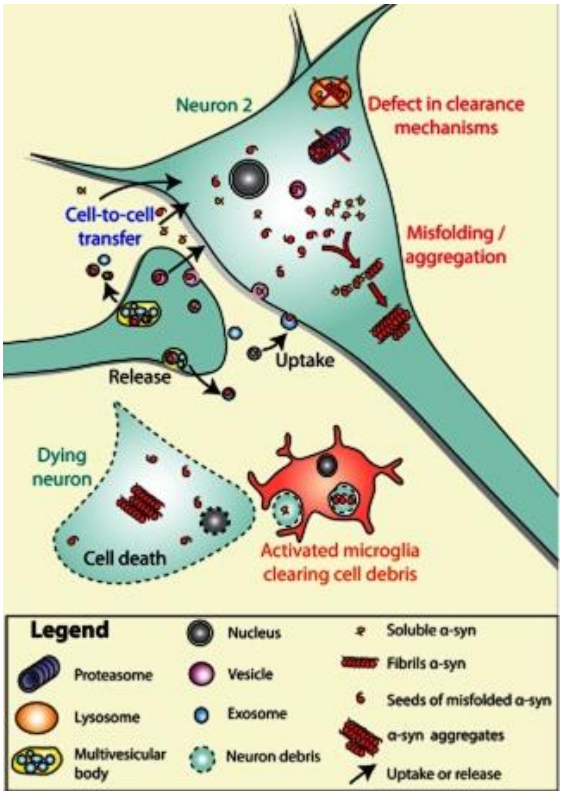


Figure 2. The schematic process of the cell-to-cell mechanism of transmission of α-synuclein. This process consists of the uptake, processing, and release of aggregated α-synuclein from neurons to other receiving neurons. *Modified from George et al. (2013).*

Various recent studies have found evidence that this α-synuclein protein transmission can indeed occur in the human brain. Because of these discoveries, the transmission hypothesis can also be an idea for a future treatment for PD or other neurodegenerative diseases (Henderson et al., 2019). With this finding there is a thought if the spread of aggregated α-synuclein is stopped, that this may stop the progression of PD as well (Lee et al., 2014).

## Uptake

When α-synuclein is secreted from neuronal cells, it becomes extracellular α-synuclein that resides in the cerebrospinal fluid. This extracellular α-synuclein can then go to other neuronal cells where it is taken up and internalized. There are multiple ways in how the α-synuclein can be internalized into cells. These ways include receptor-mediated endocytosis and phagocytosis (Choi et al., 2020).

## Processing

After the α-synuclein is internalized, it then undergoes endosomal trafficking and they are then degraded by lysosomes. The α-synuclein can survive this degradation, and can then induce the aggregation of α-synuclein proteins in the invaded neurons. The reason why it can survive the degradation might be because of lysosomal dysfunction (Lee & Lee, 2016). Due to the worsening of lysosomal function with age, the misfolded α-synuclein can escape the lysosomes whereby it can enter the cytoplasm (Henderson et al., 2019). Another mechanism where α-synuclein can get into the cytoplasm is via breakage of the lysosomes. Hereby the α-synuclein fibrils can get access to the cytosol. In the cytosol these α-synuclein fibrils act as a template for aggregation of monomeric α-synuclein (Flavin et al., 2017). The aggregated α-synuclein is then released from the neuron and is then spreading through the brain. The aggregated α-synuclein fibrils can cause toxicity (Hijaz et al. 2020).

## Release

This aggregated α-synuclein can then exit the neurons and target other neurons. Hereby the neurons that are synaptically connected and vulnerable are the most at risk to be targeted by this aggregated α-synuclein (Henderson et al., 2019). These aggregated α-synuclein fibrils can then be transported to the interconnected neurons to cause aggregation of the monomeric α-synuclein in these neurons as well. This is called the release and uptake mechanism (Polanco et al., 2018).

Another way that extracellular α-synuclein can act, is on glial cells. When α-synuclein is released from neurons, it is transported to astrocytes, resulting in a pro-inflammatory response (Lee et al., 2010). Furthermore, extracellular α-synuclein can activate microglia. This extracellular α-synuclein that activates microglia can have interactions with Toll-like receptor 2 and therefore activate the corresponding TLR2 signaling pathway. This contact can also produce an inflammatory response, which also has a major role in the degeneration of neurons (Kim et al., 2013).

It is true that the extracellular α-synuclein is only a small portion of all the α-synuclein that resides in the brain. But because of the steps this extracellular α-synuclein makes on the neighboring neuronal cells and glial cells, it can possibly be a good target for the treatment of PD (Lee & Lee, 2016). But also, the mechanism of release and uptake of this α-synuclein can be potential targets for treatment (Uemura et al., 2020). Another potential target for the treatment of PD comes from the mechanism whereby the aggregated α-synuclein can escape to the cytosol after being taken up by the cell (Choi et al., 2020).
New treatments are being proposed when these cell-to-cell transmission mechanisms were discovered. These treatments are focused on the prevention of the formation and the spreading of Lewy bodies and aggregated α-synuclein. The end goal of this is to halt and stop the progression of PD. At the current moment, different clinical trials are performed for treatments like immunotherapy. This kind of immunotherapy has to stop the spread of extracellular α-synuclein between neurons (Hijaz et al., 2020).

## α-synuclein immunotherapy

At the current moment in time, there is no treatment for PD and other synucleinopathies that target the aggregated α-synuclein (Shin et al., 2020). Recently there have been experimental immunotherapy studies done for Alzheimer's disease (AD), which gave a positive result for the possible treatment of the disease. For AD, several clinical trials have been performed with the use of both active and passive immunization. Because of this success with immunotherapy for AD, a search has been started to find a likewise solution for PD (George & Brundin, 2015). There have already been several anti-α-synuclein vaccines tested in early phases of clinical trials whereby positive results were obtained for patients with PD (Nimmo et al., 2020).

When using immunotherapy for neurodegenerative diseases, there is the obstacle of the blood brain barrier that can get in the way of effective treatment. The transport of different substances, in the case of immunotherapy these are antibodies, are limited from the peripheral circulation to the central nervous system. The movement of these substances are regulated by transporters and enzymes that are present on both sides of the blood brain barrier (George & Brundin, 2015).

In the case of PD, microglia and astrocytes are activated as a part of the neuroinflammatory response. Both the microglia and astrocytes are also immune cells that reside in the brain. It is thought that this can play a critical role in a positive result of immunotherapies for PD and other neurodegenerative diseases (George & Brundin, 2015). The most popular approach for immunotherapy against PD is to target the extracellular α-synuclein to reduce the total amount of α-synuclein and to block the cell-to-cell transmission of this α-synuclein to other neurons (Henderson et al., 2019).

It is important to know which conformation of α-synuclein is the target for immunotherapy. Some therapies may target the α-synuclein fibrils, while other therapies may target monomeric α-synuclein. When this monomeric α-synuclein is targeted, it might suppress the functions of normal α-synuclein. This is the reason why the most effective way of immunotherapy to treat PD is to target the aggregated α-synuclein, because this causes the least amount of side effects (Hijaz et al., 2020).

Extracellular aggregated α-synuclein can be taken up by neuronal and glial cells. During a trial it was noted that the antibodies can help in clearing the extracellular α-synuclein which leads to a reduction of aggregation α-synuclein in neuronal and glial cells. Furthermore, it was stated that antibodies can also directly block the cell-to-cell intercellular transmission of aggregated α-synuclein. Hereby the antibodies interfere with the transfer of extracellular aggregated α-synuclein to other neuronal and glial cells (Lee & Lee 2016).

Immunotherapy can be done with the use of two techniques. It can be done by using active immunization, whereby the individual's own immune system is used and where antibodies can be generated against α-synuclein. It can also be done by using passive immunization. This uses a direct injection of antibodies that can be used against the different domains of α-synuclein (George & Brundin, 2015). Which of the two techniques are being chosen can vary depending on what result is wanted. These results can for example include the increase of microglial clearance and inhibiting the neuronal uptake of aggregated α-synuclein (Uemura et al., 2020). A current hypothesis is that these antibodies against α-synuclein can stimulate the microglia to prevent the extracellular α-synuclein transfer from neurons to other neurons (George & Brundin, 2015).

## Active immunization

Active immunization tries to create an immune response against α-synuclein by producing protective antibodies (Uemura et al., 2020). The use of active immunization is already tested in animal models. Hereby mice can be injected with α-synuclein that originates from humans to promote the clearing of aggregated α-synuclein (Wang et al., 2019).
A way of using active immunization is with the use of DNA vaccines. With these DNA vaccines the antibodies recognize the misfolded α-synuclein that appear in the brain. Another way of using active immunization are cell-based vaccines. These cell-based vaccines can produce specific antibodies, they can improve the motor functions, and they will not induce an inflammatory response (Wang et al., 2019).

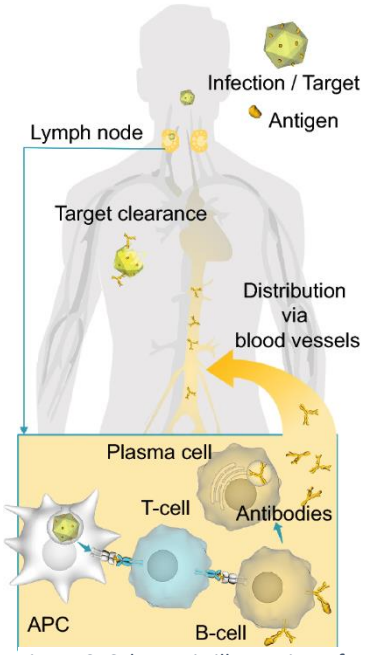

Figure 3. Schematic illustration of active immunization. When active immunization is happening in reaction to a vaccination after immunotherapy, the injected material is taken up and presented in the lymph nodes by antigen-presenting cells. This causes the activation of antigen-specific B cells which are mediated by the T-cells. The B-cells will turn into plasma cells, whereby antigen-specific antibodies will be produced that lead to the clearance of aggregated α-synuclein. *Modified from Schlake et al. (2019).*

Advantages of active immunization are that it is not a costly treatment for PD, it can be easily used for larger populations, and only a few injections are required (Schneeberger et al., 2016).
Active immunization therapy also has some disadvantages. There has been a variability found in the antibody response, especially by older patients. The reason for this can be because of the fact that PD is an age-related disease, and the immune system of elderly people cannot generate antibodies that efficient anymore, and then there is a chance that these people may get autoimmune side effects of the immunotherapy (George & Brundin, 2015). Another disadvantage of active immunotherapy is that only 0.1-1.0% of the antibodies that are produced by active immunization can cross the blood brain barrier. Furthermore, can active immunization induce an inflammatory response. Because of these reasons, active immunization is better fitting for patients who are in an early stadium of PD (Arevalo-Villalobos et al., 2017).

Currently, two short peptide vaccines are tested in phase II clinical trials. These vaccines are PD01A and PD03A. These vaccines can imitate original antigens, but they don't initiate a T-cell response because these peptides are too short. In mouse model trials it was noted that these short peptide vaccines can effectively clear the aggregated α-synuclein from the brains of mice (Henderson et al., 2019).

## Affitope PD01A and PD03A

At the current moment there is one company (AFFiRis) that is highly focused on the active immunization for targeting α-synuclein. This immunization is on a vaccine basis, and makes use of the so-called α-synuclein AFFITOPE (AFF). This AFF is a short peptide that looks like α-synuclein, but that has a different amino acid sequence than the native α-synuclein protein. This AFF peptide was made this way to produce antibodies by inducing a B-cell response without having a possible autoimmune T-cell response. In trials that focused on synucleinopathies in animal models, the vaccination with PD01A and PD03A caused a reduction of aggregated α-synuclein and neurodegeneration, whereby motor functions of the mice were saved, and AFF did not cause neural damage or neuroinflammation. At this moment AFF has successfully completed phase 1 trials (Shin et al., 2020).

## Passive immunization

Passive immunization tries to create engineered antibodies against α-synuclein (Uemura et al., 2020). The use of passive immunization has been done in animal models. Hereby the mice will be injected with α-synuclein antibodies which can cause the formation of antigen-antibody complexes with α-synuclein whereby these complexes can then be cleared by microglia and lysosomes (Wang et al., 2019).

The antibodies that are used for passive immunotherapy are mostly single-chain antibodies. These antibodies have the advantage that they are able to cross the blood brain barrier easier than with the use of active immunotherapy because these antibodies make use of receptor-mediated transport. A disadvantage to passive immunotherapy is however that their half-life in blood is short, and because of this the effectiveness of this therapy decreases (Sweeney et al., 2018). Passive immunization is suitable for both mild and severe cases of PD (Arevalo-Villalobos et al., 2017).

There are different approaches to passive immunization. The antibodies that can be generated can be antibodies which are focused on the C-terminus of α-synuclein, but they can also be focused on the N-terminal of α-synuclein (George & Brundin, 2015).
At the current moment it is unclear if particular sites are more effective to target for the best treatment of PD, more studies have to be done to determine this (Henderson et al., 2019).

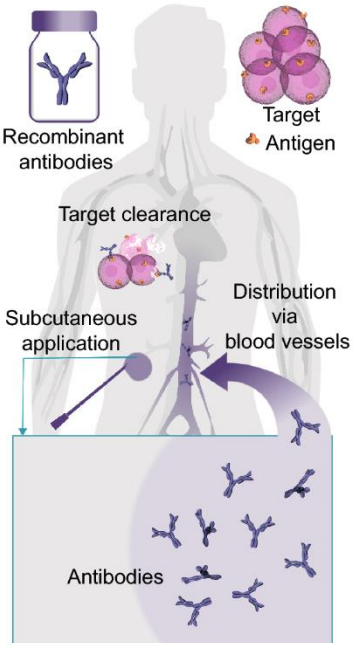


Figure 4. Schematic illustration of passive immunization. With passive immunization, the antibodies are not produced by plasma B-cell, but they are manufactured outside of the patient's body and then be injected. After this injection, the antibodies diffuse and enter the circulation. They then start to act like endogenous antibodies. *Modified from Schlake et al. (2019).*

## Cinpanemab

Cinpanemab (BIIB054) is an anti-α-synuclein IgG1 monoclonal antibody from human origin that targets the N-terminal region of α-synuclein. This monoclonal antibody has high selectivity for aggregated α-synuclein, and it is currently in phase II clinical trial (Shin et al., 2020). This antibody is targeting the α-synuclein aggregates. The antibody has a high specificity for the fibrillar form of α-synuclein, and because of this makes it a promising treatment for PD (Weihofen et al., 2019).

## Prasinezumab

Prasinezumab (PRX002) is an antibody that has been developed by the company Prothena. This antibody specifically targets the C-terminal region of α-synuclein. A clinical study which used an animal model showed that this way of passive delivery of PRX002 has blocked the cell transmission, it minimized the pathology of intracellular α-synuclein, and it restored cognitive and motor functions in various PD mouse models. Because of these positive results, PRX002 is currently in phase II clinical trial (Shin et al., 2020).
These kinds of antibodies are used to target different domains of α-synuclein. PRX002 is a monoclonal antibody that can bind to misfolded and aggregated α-synuclein, and then clear this α-synuclein through the blood brain barrier. It is tested that PRX002 is non-immunogenic and that it has no notable side effects (Jankovic et al., 2018).

## Other methods of passive immunization

Another method of passive immunotherapy are antibodies that can recognize abnormal sites of α-synuclein. The phosphorylation of Ser129 of α-synuclein can induce aggregation of this α-synuclein. Because of this reason, an antibody that can recognize Ser129 can be used as a potential for immunotherapy against PD (Wang et al., 2019).
Another promising vaccine that is currently being tested in phase I clinical trials is VX-03. This vaccine binds high levels of aggregated α-synuclein in the affected brain regions of PD patients (Nimmo et al., 2020).

Both the results of active and passive immunization in the preclinical studies thus far have been positive. It is noted that with both the active and passive immunization, the α-synuclein pathology is reduced and the motor functions and cognitive behavior is improved (George & Brundin, 2015).
A general fact about immunotherapy against α-synuclein is that it currently can only target the extracellular α-synuclein. But this targeting can increase the degradation of extracellular α-synuclein through microglia, and it can increase the clearing of extracellular α-synuclein into the cerebrospinal fluid (Henderson et al., 2019).
A new method of immunotherapy is making use of nanoantibodies. The advantage of nanobodies is that these antibodies are intracellularly expressed as intrabodies. Whereas normal antibodies only can target the extracellular targets (Wang et al., 2019)

## Discussion

This essay touched upon the question if immunotherapy could be a suitable treatment for PD. Because the currently used treatments for PD are only treating the symptoms and not the cause of the disease itself, there have been new strategies investigated. A new strategy that is currently being tested in clinical trials is immunotherapy, whereby the focus is on the reduction of aggregated α-synuclein with the use of antibodies (Wang et al., 2019). This type of therapy would be highly effective in PD patients who are in the early stages of the disease, whereby the aggregation of α-synuclein is not yet as prominent as by PD patients who are in later stages of the disease. A lot of information that is used currently in the testing of immunotherapy for PD, has roots in the immunotherapy for Alzheimer's disease. (George & Brundin, 2015).

Already there have been phase I and II clinical trials done for different antibodies for α-synuclein in active and passive immunotherapy. The results of these trials have been largely positive, whereby there were limited side effects noted. Because the results of these clinical trials were mostly positive, this gives good expectations that α-synuclein immunotherapy is an effective method for the treatment of PD. The most prominent antibodies that are being tested at the current moment are PD01A and PD03A for active immunotherapy, and BIIB054 and PRX002 for passive immunotherapy (Shin et al., 2020).

The studies that are performed for immunotherapy against PD have mostly successful results. But there are some possible problems that can arise by using immunotherapy against PD.
It has been noted is that the antibodies sometimes cannot differentiate between the monomeric form of α-synuclein and the aggregated fibril form of α-synuclein. Because of this there is a chance that the normal α-synuclein levels are being decreased, and that the normal α-synuclein cannot perform its function effectively anymore (Lee & Lee, 2016). Therefore, the antibodies need to be designed to recognize the different conformation of α-synuclein, and where they only target the aggregated fibril forms of α-synuclein so that the normal α-synuclein level are still at a normal functional level (Shin et al., 2020).

An important concern of immunotherapy that targets aggregated α-synuclein is that it can cause inflammatory autoimmunity that is caused by Th17 cells. The reason why there is a T-cell response is because the antibodies for the immunotherapy target a self-peptide, and Th17 cells are involved in the neuroinflammatory process of neurodegenerative diseases. Therefore, it is important to consider the adaptive immune system when designing antibodies to target the self-protein α-synuclein (George & Brundin, 2015). Therefore, the antibody also needs to be able to differentiate between the different conformation of α-synuclein. Hereby it is important to minimize the inflammatory response to limit the chance of causing autoimmunity (Nimmo et al., 2020).

Another possible concern is the crossing of the antibodies through the blood brain barrier. Hereby antibodies need to be developed to be able to cross this barrier more easily. It is stated that antibodies from passive immunotherapy can cross the blood brain barrier more efficiently than the antibodies from active immunotherapy. But still, the number of actual antibodies that can cross this blood brain barrier is low. Alternatively, the use of nanoantibodies can be investigated further to be able to effectively target the intracellular α-synuclein of the neurons. Not a lot is known about the use of nanobodies in immunotherapy at the current moment, and more research needs to be done on this topic (Lee & Lee, 2016).

A different potential concern is that some of the immunotherapies tests are performed in animal models, and thus the specific working on humans is sometimes not clearly known yet. Furthermore, some studies test the antibodies in vitro, while the actual working needs to be done in vivo in the intended patient (Nimmo et al., 2020). Especially for passive immunization, where the antibodies need to be engineered to be "humanized", is it important for the testing of the specific effectivity and possible toxic effects (Lee & Lee, 2016).

The last possible concern that might happen during clinical trials for α-synuclein immunotherapy is the same problem that happened in multiple clinical trials for AD immunotherapy that targeted amyloid-β. Within these trials the working of the antibodies stopped or halted, whereby the symptoms and progression of AD started again. A possible reason for this could be that the progression of AD in patients was already too advanced to be able to recover from immunotherapy. These problems have not yet arisen in immunotherapy for PD, but it is good to know that this might be a potential problem that can also happen with immunotherapy for PD.
Other lessons can also be learned from the studies that have been done for immunotherapy for AD. Herein it is important that PD patients who are in the early stages of PD are selected for clinical trials as well to see the difference in effect of the immunotherapy. It is difficult to select this group because PD diagnosis is mostly clinical, and there is a lot of room for misdiagnosis. Because motor symptoms can be noted in PD patients even after around twenty years, the aggregation of α-synuclein is already happening for a long time. Therefore, and with the added reason that in earlier stages of PD there is a shortfall of biomarkers, the diagnosis of PD in very early stages of the disease is complicated (Shin et al. 2020).

It is also noted that some patients have not only PD, but also a combination of other forms of proteinopathies. If this is the case, then it is important to know what the effect on the patient will be if α-synuclein is targeted whereby the α-synuclein levels are reduced. But it can also be that only targeting α-synuclein is not enough to stop or halt the progression of PD in combination with other proteinopathies in the patients (Shin et al., 2020).
Another interesting point to consider when doing studies with α-synuclein immunotherapy is that the aggregated α-synuclein can differ depending on the disease. This means that the aggregated α-synuclein between PD and other synucleinopathies can differ, and that specialized immunotherapy for PD might not optimally work for other synucleinopathies. More research has to be done to gather more information about the different types of aggregated α-synuclein and how α-synuclein immunotherapy can be designed for these differences (Uemura et al., 2020).

To conclude this essay with the research question if α-synuclein immunotherapy is a suitable treatment for PD, the answer to this question is that this type of immunotherapy could become a suitable treatment for PD in a few years when more research and clinical trials have been done to take away some of the concerns that surround the use of α-synuclein immunotherapy for patients with PD. α-synuclein immunotherapy is proving to be a promising treatment for PD and other synucleinopathies. And the clinical trials that are performed, show the reduction of aggregated α-synuclein in patients. But there are still some potential concerns and problems that need attention in future studies.

Overall, a lot of the possible concerns surrounding α-synuclein immunotherapy revolve around the fact that certain important information is still unknown. Therefore, it is important that the antibodies that are used need to be specific and reliable for the targeting of aggregated α-synuclein, and if these measures are taken then there is a very good chance that in the near future there is a definite treatment for PD in the form of α-synuclein immunotherapy.

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