Passive Immunization Strategies in Animal Models

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Alpha-synucleinopathies are progressive neurodegenerative diseases that are characterized by pathological misfolding and accumulation of the protein alpha-synuclein (αsyn) in neurons, axons or glial cells in the brain, but also in other organs. The abnormal accumulation and propagation of pathogenic αsyn across the autonomic connectome is associated with progressive loss of neurons in the brain and peripheral organs, resulting in motor and non-motor symptoms. To date, no cure is available for synucleinopathies, and therapy is limited to symptomatic treatment of motor and non-motor symptoms upon diagnosis. Recent advances using passive immunization that target different αsyn structures show great potential to block disease progression in rodent studies of synucleinopathies. However, passive immunotherapy in clinical trials has been proven safe but less effective than in preclinical conditions.

alpha-synuclein passive immunization disease stratification

1. Introduction

Twenty-five years ago, it was found that aggregated alpha-synuclein (αsyn) is the major protein component of Lewy pathology [1]. Subsequent studies discovered that point mutations within or duplications/triplications of the αsyn gene (*SNCA*) are linked to familial PD [2][3][4]. These findings indicate a central role of αsyn in Lewy body diseases (LBD). Since then, Parkinson's disease (PD), dementia with Lewy bodies (DLB), pure autonomic failure (PAF) and multiple system atrophy (MSA) are classified as synucleinopathies, also called α-synucleinopathies, as they all are characterized by pathological accumulation of the protein αsyn. PD, DLB and PAF predominantly present with intraneuronal and neuritic deposits of misfolded αsyn, i.e., Lewy bodies and Lewy neurites. Furthermore, the accumulation of pathogenic αsyn is associated with progressive disrupted cellular function, neuronal death and subsequent dysfunction in the central and peripheral nervous system [5]. MSA is a distinct case of α-synucleinopathies, as it is characterized by predominant glial cytoplasmic inclusions (GCIs) [6], later also called Papp-Lantos bodies [7].

Patients are classified as PD, DLB, PAF or MSA based on their clinical symptoms and later, post-mortem by the spatiotemporal distribution of pathogenic αsyn [8]. The spatiotemporal distribution is likely dependent on a combination of different factors, disease onset site and neuroanatomical connections as well as cellular vulnerability and the presence of concomitant tau and/or Aβ pathology. The clinical representation of PD, DLB, PAF and MSA patients is highly heterogeneous esp. in early disease stages, and displays a large clinical overlap, as each α-synucleinopathy may include a wide range of motor, cognitive, gastrointestinal and/or other autonomic

disturbances, complicating early and accurate diagnosis. For example, DLB merely differentiates from PD diagnosis by the occurrence of cognitive dysfunction prior to motor dysfunction by only one year [9], which is very short, considering that non-motor symptoms occur up to 20 years prior to motor symptoms in PD [10]. PD, DLB and MSA show both central and peripheral nervous system involvement of αsyn pathology [11][12]. In PAF, αsyn pathology is confined within the autonomic nervous system (ANS) without motor dysfunction [13]. These patients also have an increased risk to pheno-convert into other α-synucleinopathies later in life, possibly indicating a pathophysiological disease continuum [12]. Furthermore, MSA patients with autonomic-only presentation in the early disease stage can be misdiagnosed as PAF. Moreover, MSA patients presenting with parkinsonism may be misdiagnosed as PD [14]. These α-synucleinopathies progress at different velocities with different intensities, but may evolve to similar advanced disease stages over time where the entire body is affected [15][16].

Currently, there is no cure for any of these α-synucleinopathies; hence, there is a great interest in targeting pathogenic αsyn as a strategy to halt disease progression. To reduce levels of harmful misfolded αsyn, a clearing process of the protein has to be established. This can be achieved with immunotherapies using vaccination strategies with antibodies directed against harmful αsyn [17]. The aim of a particular immunotherapy is to reduce the amount of misfolded αsyn in the body, and thereby block the spread of pathogenic αsyn, consequently reducing progressive neurodegeneration and, therefore, symptoms [18]. Passive immunization with naturally occurring autoantibodies (nAbs) that are part of the innate immune system is considered more safe than active immunization or vaccination where an antigen is injected to induce the production of antibodies [19]. Preclinical studies using nAbs have shown reduced trans-synaptic spread of pathogenic αsyn, as well as improved motor and cognitive deficits in PD mouse models. In contrast, preliminary data from on-going clinical phase I and phase II trials using passive immunotherapies targeting different forms of αsyn are unable to demonstrate efficacy in reducing disease progression [20]. Whether nAbs provide protection against developing PD, increasing evidence suggests that anti-αsyn nAbs may have a protecting effect in inhibiting αsyn seeding and can recognize Lewy body pathology [21]. nAbs have been extensively evaluated in PD as reviewed by Scott et al. [22]; however, most studies have been restricted to assessing total IgG nAbs levels. A few studies have evaluated IgG nAb subclasses, IgM nAbs and the binding properties of these nAbs, showing a switched immunological response in PD and MSA patients and further a reduced binding towards αsyn [23][24][25]. A more thorough evaluation is needed to fully map the immunological responses in PD and other synucleinopathies.

Discrepancy between animal and patient studies might be explained by a combination of poor αsyn targeting and poor patient selection. The strain hypothesis in α-synucleinopathies postulates that each disease entity is characterized by a distinct conformation of pathogenic αsyn; therefore, each α-synucleinopathy could be caused by a unique αsyn structure or strain. This implies that different α-synucleinopathies require different nAbs targeting a specific αsyn strain. Unfortunately, clinical trials lack accurate patient stratification and individual disease heterogeneity is often not considered during patient recruitment, as trials assume a common pathogenetic mechanism of disease across patients. The highly heterogeneous profile of the prodromal disease phase of α-synucleinopathies make early and accurate stratification very challenging. Consequently, patients are often misdiagnosed at early disease stages and may not benefit from a certain immunotherapy. Further, patients in advanced disease stages with established major neurodegeneration might benefit less compared to prodromal

patients. It remains to be elucidated whether the formation of mature dense αsyn or Lewy pathology aggravates or protects against neurodegeneration [26]. It is hypothesized that endogenous αsyn goes through four stages to ultimately form mature Lewy pathology: misfolding of endogenous αsyn, oligomerization, formation of fibrils and, finally, development of dense inclusions. The immature oligomeric and fibrillary αsyn appear to be most toxic compared to mature Lewy pathology [27], indicating such conformers could be particularly attractive as therapeutic targets instead of mature Lewy pathology. Lack of these considerations might have contributed to disappointing results. Future trials should focus on enrolment of prodromal patients after detailed stratification into different disease subtypes by using disease- and strain-specific biomarkers. Additionally, target biology should be optimized towards immature strain-specific pathology. For this purpose, it is crucial to gain insight in the earliest physiological to pathological events underlying αsyn misfolding and abnormal aggregation using animal models of α-synucleinopathies.

2. C-Terminal Targeting Approaches

The first candidate antibody tested in preclinical models was the monoclonal antibody (mAb) clone 9E4 targeting the C-terminal of human αsyn [28][29]. The 9E4 murine mAb recognizes the amino acids (aa) 118–126 of human αsyn (hαsyn) and has been shown to reduce toxic truncated species of αsyn, rescued behavioral deficits in PD-GFβ-αsyn transgenic mice and co-localizes with pathology in several brain regions [28]. These results were confirmed again by Masliah's group [18] in the Thy1 αsyn (line 61) mice, further expanded to investigate the 9E4 analogs, the 5C1 and 5D12, and the 1H7 targeting the overlapping region of NAC and C-terminal. The 1H7 and 5C1 showed comparable decreased toxic αsyn truncated species, proposed to be reduced by internalization and lysosomal degradation [28], as well as improved behavioral deficits and protected tyrosine hydroxylase (TH) cell loss [18]. The 1H7 mAb was further investigated in Thy1 αsyn (line 61) mice, laterally injected with human αsyn expressing Lentivirus [30]. The 1H7 reduced axonal aggregation of αsyn and protected axonal integrity, as well as improved memory deficits and increased colocalization of αsyn and Iba-1 positive microglia, suggestive for microglia phagocytosis of extracellular αsyn [30]. The main difference is that 1H7 preferably binds aggregated αsyn at the C-terminus but also monomers. Following the results of Masliah and colleagues [30][29], targeting the C-terminal has become an optimistic immunization targeting strategy. Thus, several other antibodies have been produced targeting the C-terminus of αsyn. Parallel to the 9E4 mAb, another mAb targeting the C-terminal, the Ab274, was additionally investigated in collaboration between Masliah, Seung-Jae Lee and colleagues [31]. The Ab274, a IgG2a murine mAb, was investigated in PD-GFβ-αsyn mice (line M) showing reduced αsyn in cortical and limbic brain regions by microglial phagocytosis, additionally improving behavioral deficits [31]. Two other mAbs have been produced to target the C-terminal of αsyn, the Syn211 [32] and AB2 [33]. The Syn211 was tested in wild-type (wt) mice with intrastriatal injection of preformed αsyn fibrils (PFFs) and reduced insoluble αsyn and phosphorylated αsyn aggregates [32]. The AB2 mAb similarly reduced αsyn in brain homogenates in nigral αsyn-overexpressing wt rats [33].

3. N-Terminal and NAC Targeting Approaches

Interestingly, Tran and Shahaduzzaman tested an N-terminal-targeting antibody in parallel: Syn303 (aa 1–5) [32] and AB1 (aa 16–35) [33]. It seemed that the mAbs targeting the N-terminal surpassed the effects of the C-terminal targeting mAbs. In addition to overall reduced αsyn levels, the Syn303 reduced αsyn spread in the SNpc with 30% and in the ipsilateral and contralateral amygdala with 40%, and further improved motoric deficits [32]. However, in a later study, Syn303 was found inferior to their novel syn9048 mAb targeting the C-terminal and preferably binding aggregated αsyn structures [34]. The N-terminal-targeting AB1 additionally reduced DA and NeuN cell loss [33]. Very recently Chen and colleagues [35] (Chen et al., 2021) conducted a preclinical study using a NAC-targeting mAb (NAC32), which showed reduced αsyn pathology in the SN (25%), prevented TH+ neuron degradation and further reduced behavioral deficits [35]. Targeting monomeric (soluble non-toxic) αsyn proposes a different challenge, as reduction of functional αsyn potentially could harm normal physiological properties. After all, studies investigating αsyn knock-out or knock-down have shown aberrant dopamine synthesis and release, and even dopaminergic degeneration [36], and potential other physiological functions. It is therefore of utmost importance to ensure that mAbs targeting monomeric non-toxic αsyn do not negatively affect normal dopamine synthesis and/or its release. A way to circumvent this challenge is to target extracellular toxic αsyn conformers.

4. Conformational Targeting Approaches

Numerous antibodies have been developed targeting different αsyn conformational structures, from small oligomeric to larger fibrillary structures. Lindstrøm and colleagues [37] were the first to report on a mAb selective for conformational αsyn structures, this mAB47 is an IgG1 mAb which only reduces αsyn protofibrils in the spinal cord, but not in the brain, of Thy-1-H[A30P] mice [38]. Kallab and colleagues [39] later worked with a different clone of mAB47, called Rec47, in an MSA mouse model, the PLP-αsyn tg mouse model, which, in contrast to Lindstrøm and colleagues [37], showed reduced microglia signal and reduced activated microglial cells, correlated to reduced oligomeric αsyn. Furthermore, they observed reduced GCIs in the spinal cord, colocalization of phosphorylated αsyn pathology and correlation between Iba-1 positive microglia and oligomeric αsyn. They suggested an autophagy-directed elimination of αsyn [39]. Very recently, Nordström and colleagues thoroughly investigated the mAb47 (murine version of ABBV-0805), firstly establishing the binding region of the mAb to the C-terminal (121–127 aa) of αsyn, but more selective for aggregated αsyn species [40]. Nordström and colleagues extensively evaluated mAb47 in three different PD mice models with and without injection of preformed fibrils (to induce seeding) in both a prophylactic and therapeutic manner. They observed in wt mice, as well as in Thy-1-h[A30P] mice injected with 10 µg fibrils in the gastrocnemius muscle, a prolonged survival with the mAb47 treatment. In a Thy-1-h[A30P] mice injected with 1 µg fibrils, they further observed a reduced soluble and insoluble αsyn in the brain and reduced levels of phosphorylated αsyn in the CSF in both a prophylactic and therapeutic regime. Moreover, both soluble and insoluble levels were reduced in the brain in a dose-dependent administration of mAb47, more effective towards soluble αsyn. Lastly, they investigated the efficacy of mAb47 in an A53T+/− intracerebral fibril-seeding mice model with fibril injection into the anterior olfactory nucleus. After 16 weeks of weekly mAb47 intraperitoneal administration, spreading of phosphorylated αsyn was reduced in the CA1 hippocampal region [40]. El-Agnaf and colleagues studied three antibodies selective for oligomers and aggregates (Syn-01, Syn-02 and Syn-04) and two for mature aggregates (Syn-F1 and Syn-F2) [41]. Weekly injections over a 3-

month period in mThy1 αsyn (line 61) mice showed that the Syn01, Syn-04 and Syn-F1 exhibit an overall similar effect by reducing αsyn in central brain regions (striatum, SN, and neocortex). Moreover, they reduced total αsyn, oligomeric αsyn and Syn-01, Syn02 and Syn-04 also reduced 5G4-aggregated αsyn. Only the Syn-01, Syn-04 and Syn-F1 rescued neuronal degradation and behavioral deficits. Syn-01 and Syn-04 further reduced astro- and microgliosis [41]. As for the 1H7, Schofield and colleagues from AstraZeneca among others developed a high-affinity monoclonal anti-αsyn antibody, MEDI1341, which binds the C-terminal monomeric form and aggregated αsyn [42]. Weekly administration of MEDI1341 in mThy1 αsyn mice with intra-hippocampal αsyn injections [30], reduced αsyn in hippocampal and neocortical areas [42]. As mentioned, Henderson and colleagues [34] tested the preferred binding of the novel Syn9048 mAb. Comparable to the previously tested mAb, Syn303 [32], Henderson et al. demonstrated reduced spread of αsyn pathology in the brain and attenuated dopamine reductions in the striatum of wt mice with PFF unilateral injection in the dorsal striatum [34]. Huang and colleagues used a different approach, isolating anti-αsyn nAbs from IViG using column chromatography, and administered them weekly at low (0.8 mg/kg) and at high (2.4 mg/kg) dosages in a A53T transgenic PD mouse model [43]. In both low and high dosages Huang and colleagues showed that nAbs reduced phosphorylated αsyn and soluble αsyn in the brainstem. Both dosages reduced astrocytes in the striatum and increased αsyn and microglia co-localization, as well as rescued motoric deficits. The rescuing effects were shown to be effective in a dose-dependent manner, with further reduced phosphorylated αsyn in cortical areas and reduced total human insoluble, soluble and oligomeric αsyn as in the brainstem. The effect of higher dosage further rescued behavioral deficits, in addition to the rescuing effect of pathological alterations e.g., reduced activated microglia and rescued TH+ positive neurons among others [43]. The BIIB054, also called cinpanemab, is a monoclonal mAb targeting the N-terminal (aa 1–10) with 800-fold greater affinity towards aggregated αsyn produced by Weihofen and colleagues in collaboration between Biogen Ltd. and Neurimmune AG Ltd. [44]. Weihofen and colleagues tested the BIIB054 in three different mouse models: (1) in female wt seeded contralateral with fibrils, they observed reduced truncated αsyn at 100 days and improved hangwire test at 60 days; (2) in male transgenic A53T mice (M83) seeded with fibrils in the striatum, they showing less severe paralysis at day 5, reduced paralysis at day 7 and weight loss at day 9; and (3) in male and female fibril-seeded BAC αsyn A53T mice [45], they reported rescuing effects of the contralateral DAT signal at 90 days post seeding [44].

Huang and Weihofen investigated αsyn-specific IViG nAbs and the BIIB054 mAb respectively [43][44], and both incorporate the idea that healthy individuals have antibodies resisting pathology. Huang and colleagues isolated anti-αsyn nAbs from IViG, containing immunoglobulins gathered from a large healthy population [43]. Weihofen and colleagues went a step further, investigating the paratopes from a repertoire of B cell receptors (BCRs) from healthy individuals and produced αsyn-specific nAbs from the repertoire [44]. In both studies, the nAbs showed significant rescuing effects in preclinical animal PD models. **Table 1** shows an overview of PD animal studies investigating the different passive immunization strategies.

5. Passive Candidates Translated into Clinical Trials

Of the preclinical evaluated passive immunization candidates, a few have been translated into clinical trials (**Table 1**). *Prasinezumab* (PRX002) is a humanized IgG1 antibody from the murine version of 9E4 [18][28]. Although it did not meet its primary outcome (MDS-UPDRS), the antibody significantly showed decline on the UPDRS-III and patients with fast progressive and severe symptoms benefited more from the treatment and is currently running phase II, the PASADENA study. The second antibody tested in clinical trials is the mAB47or rec47 [37][39], now called ABBV-0805, however, the company AbbVie cancelled the phase Ib trial due to strategic reasons. MEDI1341 from AstraZeneca and Takeda Pharmaceuticals are currently running its phase Ib in early PD patients; the study will run into 2022. BIIB054, also called *Cinpanemab*, classified as a human-derived mAb made through reverse translational engineering, started a large phase II study, SPARK, but halted the development of *Cinpanemab* after it missed its primary and secondary endpoint. A fourth mAb, called LU AF82422, a humanized IgG1 monoclonal antibody, did not report any preclinical report, and no results from its phase I study are available yet. However, they recently released a phase II initiation press release.

Table1. Passive immunization candidates currently in clinical trials.

Target (αsyn)	Name	Companies	Antibody/Clone	Binding Site (aa)	Clinical Groups	Current Clinical Phase	Clinical Trial ID
Aggre.	PRX002/(*Prasinezumab*)–PASADENA study	Hoffman-La Roche; Prothena Biosciences Limited.	Humanized IgG1 mab version of murine 9E4	Preferable aggregated αsyn within the C-terminal at aa 118–126 (VDPDNEAYE)	PD patients (H&Y < 2)	Phase II; active; recruitment completed.	NCT03100149
Aggre. (Oligo/proto-fibrils)	ABBV-0805	AbbVie; BioArctic Neuroscience AB	Humanized mAB47 mab	Preferable aggregated αsyn within the C-terminal at aa 121–127 (DNEAYEM)	PD patients (<5 years from diagnosis and H&Y < 3)	Phase I; recruiting.	NCT04127695
Aggre.	MEDI1341	Astra Zeneca; Takeda Pharmaceuticals	Humanized IgG1 mab	Preferable aggregated αsyn within the C-terminal (within the aa 103–129 region)	Healthy individuals (MEDI1341 vs. placebo)	Phase I; recruitment completed.	NCT03272165
Aggre.	BIIB054 (*Cinpanemab*)–SPARK study	Biogen; Neuroimmune	Healthy human memory B cells derived mab	Preferable aggregated αsyn, oxidized at N-terminal aa: 4–10 (FMKGLSK)	PD patients (<3 years from diagnosis and H&Y < 2.5)	Phase II; Terminated	NCT03318523

Target (αsyn)	Name	Companies	Antibody/Clone	Binding Site (aa)	Clinical Groups	Current Clinical Phase	Clinical Trial ID
Aggre.	Lu AF82422–AMULET study	H. Lundbeck A/S; Genmab A/S	Humanized IgG1 mab	Preferable aggregated αsyn within the C-terminal at aa 112–117 (ILEDMP)	MSA-P and MSA-C patients (<5 years from diagnosis, UMSARS ≤ 16, MoCA ≥ 22)	Phase II; recruiting	NCT05104476

Abbreviations: αsyn: alpha-synuclein; mab: monoclonal antibodies; aggre: aggregates/aggregated; aa: amino acids; oligo: oligomers/oligomeric.

6. Towards Personalized Immunotherapy

Several mechanisms have been implicated to trigger the initiation of pathogenic αsyn in the gut. Besides regulating the uptake of nutrients and water, the gut also provides an essential barrier against harmful or toxic substances from the external environment entering the body. About 400 m^2 of gut internal membranes are exposed to environmental factors, compared to ~2 m^2 of total skin surface area, meaning the gut is the main organ protecting against ingested toxic, pathogenic [46] or foreign substances and the pathogenesis of multiple system atrophy can originate in the gut [47], alter the gut microbiome [48] and cause mucosal inflammation and oxidative stress [49][50]. A complex interplay of these factors are then able to trigger αsyn misfolding in the gut plexi, and an increased permeability of the intestinal barrier or 'leaky gut' will ultimately provide a route of transmission for the gut-formed αsyn seeds to the brain [51]. These findings indicate the gut as an important target for passive immunization therapy for two reasons. Early intervention in prodromal disease stages of gut-first cases may halt formation of pathogenic αsyn and subsequent gut-to-brain propagation. Second, only 0.1–0.2% of nAbs cross the blood–brain barrier. Therefore, it is conceivable that immunotherapy in prodromal patients with 'leaky gut' could be more effective. Increased gut-permeability in prodromal patients with leaky gut might yield a better uptake of the administered nAbs near the source of pathogenic αsyn, resulting in a better treatment efficacy, as opposed to brain-first cases where the source is located in the brain (see **Figure 1**). Body-first PD patients are characterized by a more rapidly progressing phenotype, with faster motor and non-motor progression and more rapid cognitive decline, compared to brain-first PD patients [15][52]. This might explain why patients with fast progressive and severe symptoms benefited most from the treatment with Prasinezumab in the clinical trial. The validity of the SOC model requires further investigation also in the prodromal phase. Detailed phenotyping of non-iRBD prodromal (i.e., brain-first subtype) patients is not yet available. Therefore, fundamental questions remain to be addressed: how these subtypes differ in their disease initiation mechanisms and progression patterns (esp. in the prodromal phase), and how such knowledge could be exploited for tailored subtype-specific immunotherapy. Future animal models should take into account varying disease onset sites to

3. Chartier-Harlin, M.-C.; Kachergus, J.; Roumier, C.; Mouroux, V.; Douay, X.; Lincoln, S.; Levecque, C.; Larvor, L.; Andrieux, J.; Hulihan, M.; et al. Alpha-synuclein locus duplication as a cause of familial Parkinson's disease. Lancet 2004, 364, 1167–1169.
4. Singleton, A.B.; Farrer, M.; Johnson, J.; Singleton, A.; Hague, S.; Kachergus, J.; Hulihan, M.; Peuralinna, T.; Dutra, A.; Nussbaum, R.; et al. alpha-Synuclein locus triplication causes Parkinson's disease. Science 2003, 302, 841.
5. Visanji, N.P.; Lang, A.E.; Kovacs, G.G. Beyond the synucleinopathies: Alpha synuclein as a driving force in neurodegenerative comorbidities. Transl. Neurodegener. 2019, 8, 28.
6. Papp, M.I.; Kahn, J.E.; Lantos, P.L. 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.
7. Jellinger, K.A.; Lantos, P.L.; Papp, M.I. Lantos inclusions and the pathogenesis of multiple system atrophy: An update. Acta Neuropathol. 2019, 119, 657–667.
8. Kovacs, G.G. Molecular Pathological Classification of Neurodegenerative Diseases: Turning towards Precision Medicine. Int. J. Mol. Sci. 2016, 17, 189.
9. Armstrong, M.J.; Emre, M. Dementia with Lewy bodies and Parkinson disease dementia: More different than similar? Neurology 2020, 94, 858–859.
10. Borghammer, P. How does parkinson's disease begin? Perspectives on neuroanatomical pathways, prions, and histology. Mov. Disord. 2018, 33, 48–57.
11. Beach, T.G.; Adler, C.H.; Sue, L.I.; Vedders, L.; Lue, L.; White Iii, C.L.; Akiyama, H.; Caviness, J.N.; Shill, H.A.; Sabbagh, M.N.; et al. Multi-organ distribution of phosphorylated alpha-synuclein histopathology in subjects with Lewy body disorders. Acta Neuropathol. 2010, 119, 689–702.
12. Mendoza-Velásquez, J.A.; Flores-Vázquez, J.F.; Barrón-Velázquez, E.; Sosa-Ortiz, A.L.; Illigens, B.M.W.; Siepmann, T. Autonomic Dysfunction in α-Synucleinopathies. Front. Neurol. 2019, 10, 363.
13. Thaisetthawatkul, P. Pure Autonomic Failure. Curr. Neurol. Neurosci. Rep. 2016, 16, 74.

14. Palma, J.-A.; Norcliffe-Kaufmann, L.; Kaufmann, H. Diagnosis of multiple system atrophy. Auton. Neurosci. 2018, 211, 15–25.
15. Borghammer, P. The α-Synuclein Origin and Connectome Model (SOC Model) of Parkinson's Disease: Explaining Motor Asymmetry, Non-Motor Phenotypes, and Cognitive Decline. J. Parkinsons. Dis. 2021, 11, 455–474.
16. Borghammer, P.; Horsager, J.; Andersen, K.; Van Den Berge, N.; Raunio, A.; Murayama, S.; Parkkinen, L.; Myllykangas, L. Neuropathological evidence of body-first vs. brain-first Lewy body disease. Neurobiol. Dis. 2021, 161, 105557.
17. Albus, A.; Jördens, M.; Möller, M.; Dodel, R. Encoding the Sequence of Specific Autoantibodies Against beta-Amyloid and alpha-Synuclein in Neurodegenerative Diseases. Front. Immunol. 2019, 10, 2033.
18. Games, D.; Valera, E.; Spencer, B.; Rockenstein, E.; Mante, M.; Adame, A.; Patrick, C.; Ubhi, K.; Nuber, S.; Sacayon, P.; et al. Reducing C-terminal-truncated α-synuclein by immunotherapy attenuates neurodegeneration and propagation in Parkinson's disease-like models. J. Neurosci. 2014, 34, 9441–9454.
19. Bergström, A.-L.; Kallunki, P.; Fog, K. Development of Passive Immunotherapies for Synucleinopathies. Mov. Disord. 2015, 31, 16–21.
20. Antonini, A.; Bravi, D.; Sandre, M.; Bubacco, L. Immunization therapies for Parkinson's disease: State of the art and considerations for future clinical trials. Expert Opin. Investig. Drugs 2020, 29, 685–695.
21. Li, X.; Koudstaal, W.; Fletcher, L.; Costa, M.; van Winsen, M.; Siregar, B.; Inganäs, H.; Kim, J.; Keogh, E.; Macedo, J.; et al. Naturally occurring antibodies isolated from PD patients inhibit synuclein seeding in vitro and recognize Lewy pathology. Acta Neuropathol. 2019, 137, 825–836.
22. Scott, K.M.; Koui, A.; Yeoh, S.L.; Clatworthy, M.R.; Williams-Gray, C.H. A Systematic Review and Meta-Analysis of Alpha Synuclein Auto-Antibodies in Parkinson's Disease. Front. Neurol. 2018, 9, 815.
23. Brudek, T.; Winge, K.; Folke, J.; Christensen, S.; Fog, K.; Pakkenberg, B.; Pedersen, L.O. Autoimmune antibody decline in Parkinson's disease and Multiple System Atrophy; a step towards immunotherapeutic strategies. Mol. Neurodegener. 2017, 12, 44.
24. Folke, J.; Rydbirk, R.; Løkkegaard, A.; Salvesen, L.; Hejl, A.-M.; Starhof, C.; Brooh, S.; Winge, K.; Christensen, S.; Pedersen, L.Ø.; et al. Distinct Autoimmune Anti-α-Synuclein Antibody Patterns in Multiple System Atrophy and Parkinson's Disease. Front. Immunol. 2019, 10, 2253.
25. Folke, J.; Rydbirk, R.; Løkkegaard, A.; Hejl, A.-M.; Winge, K.; Starhof, C.; Salvesen, L.; Pedersen, L.Ø.; Aznar, S.; Pakkenberg, B.; et al. Cerebrospinal fluid and plasma distribution of anti-α-

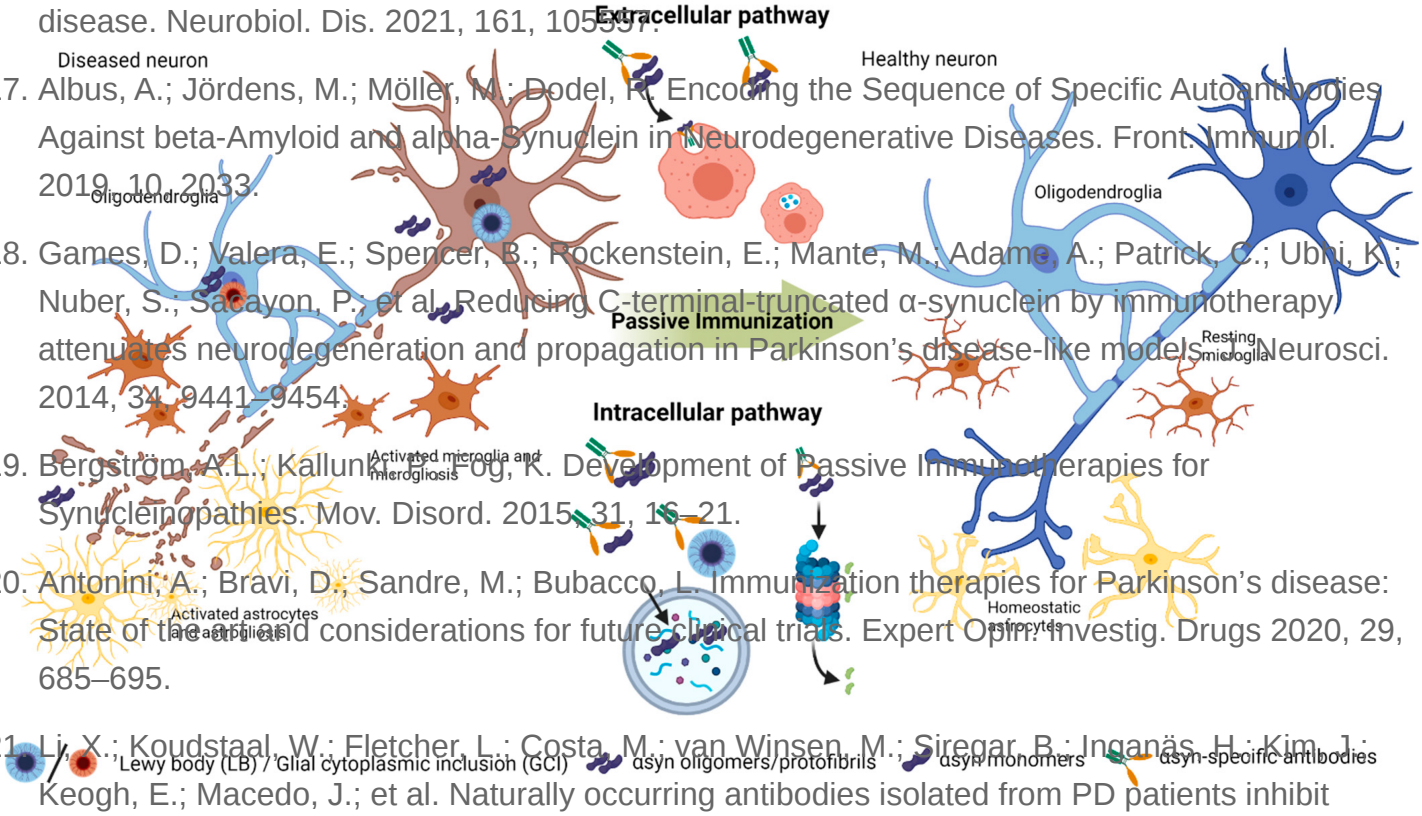

Figure 1. Passive immunization of pre-motor body-first PD patients enhances dopamine survival. Patients with probable prodromal body-first PD could be identified by a combination of several early biomarkers, such as the presence of pathological alpha-synuclein (αsyn) in skin and/or gut biopsies, polysomnography-verified RBD, cardiac sympathetic denervation on MIBG scintigraphies, but normal or near-normal nigrostriatal dopaminergic innervation on DaT SPECT. Such detailed phenotyping in the pre-motor phase might reveal body-first PD, allowing early intervention and optimal patient selection for clinical trials. Pre-motor start of nAbs treatment increases treatment efficacy by delaying or blocking peripheral-to-brain propagation of pathology, before any irreversible damage to the dopamine system is done, hereby enhancing the probability of dopamine survival in body-first PD. Furthermore, increased gut permeability in prodromal body-first patients with leaky gut or increased intestinal permeability might yield a better uptake of the administered nAbs near the source of pathogenic αsyn conformers, resulting in a better treatment efficacy, as opposed to brain-first cases where the source is located in the brain and only 0.1–0.2% of nAbs cross the blood–brain barrier. Abbreviations: nAbs: naturally occurring autoantibodies; DMV: dorsal motor nucleus of the vagus; LC: locus coeruleus; SN: substantia nigra, PAF: pure autonomic failure, PSG: polysomnography, BBB: blood brain barrier. Created using Biorender.com (accessed on 30 November 2021).

Future clinical studies should focus on detailed imaging-based phenotyping for accurate stratification of prodromal disease subtypes, as careful patient selection for clinical trials will likely increase treatment efficacy and translation ability of preclinical studies. An αsyn PET tracer would allow for early stratification and detailed investigation and follow-up of synucleinopathy subtypes. Until that is discovered, a combination of other biomarkers should be used. The gut and skin, as well as blood and CSF, are easily accessible for biopsy studies to detect and quantify (subtype-specific) αsyn. Using ultra-sensitive methods, such as PMCA or RT-QuIC, on these biopsies, could contribute to an a priori screening of patients with toxic prion-like αsyn phenotype. This could provide not only more personalized interventions, but also plan for more effective clinical trials with minus-αsyn PD patients, as proposed to be the case in Parkin and LRRK2 mutation carriers [53]. Furthermore, in combination with imaging techniques, such as a DaT brain scan, MIBG heart scan and donepezil gut scan, this may enable prodromal diagnosis, together with quantification of non-motor symptoms such as RBD (polysomnography), gastrointestinal transit time (radio opaque markers [54]), orthostatic hypotension and dementia (cognition test). Detailed imaging-based and αsyn templating-positive phenotyping is of significant importance to identify patients in the earliest phase of the disease, but also to evaluate treatment effects of immunotherapy (see **Figure 2**). Nevertheless, the road to use immune-based therapies on the basis of *a priori* preselected individuals is still long and cumbersome.

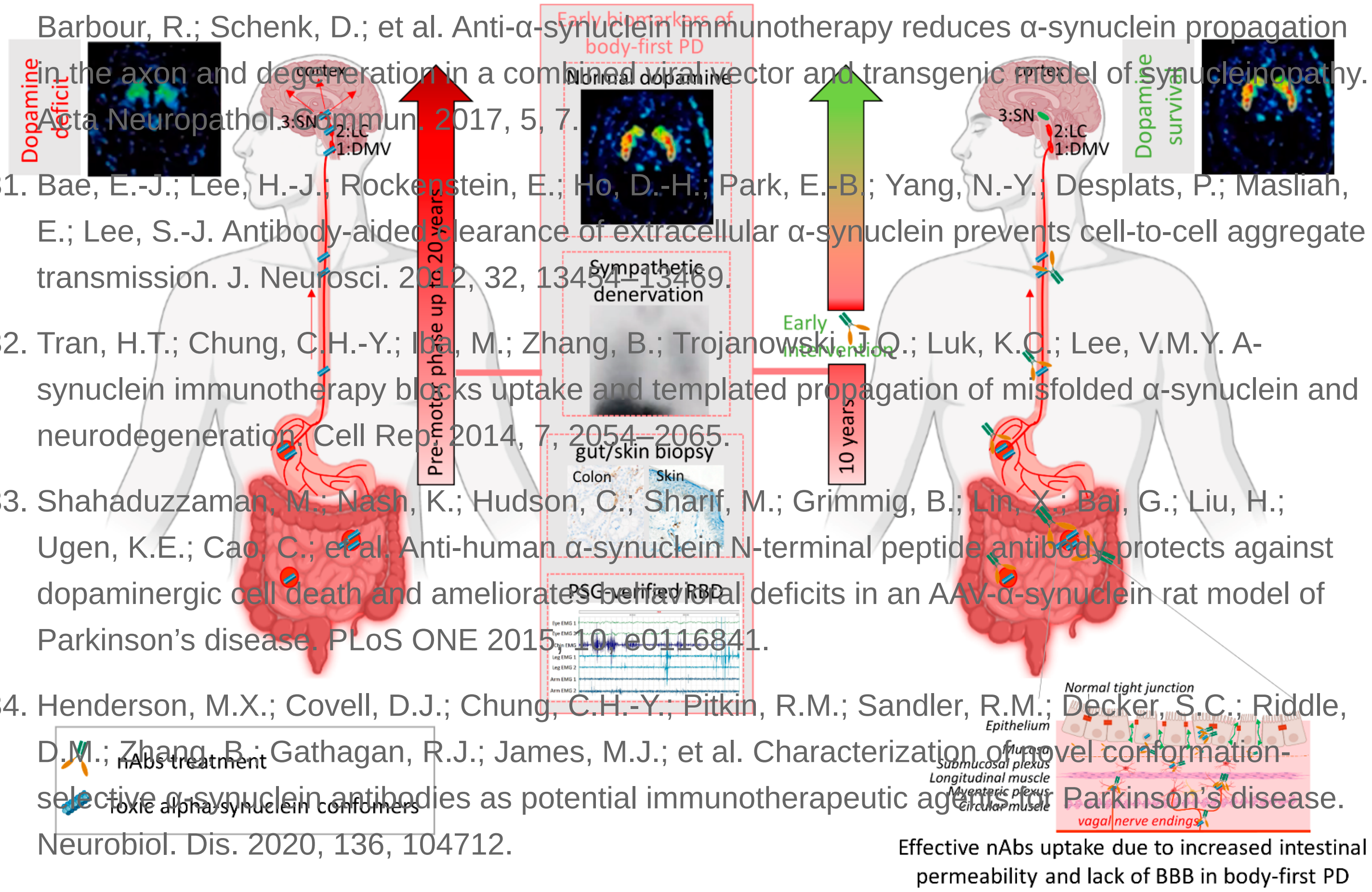

Figure 2. Passive immunization of pre-motor body-first PD patients enhances dopamine survival. Patients with probable prodromal body-first PD could be identified by a combination of several early biomarkers, such as the presence of pathological alpha-synuclein (αsyn) in skin and/or gut biopsies, polysomnography-verified RBD,

synuclein IgMs and IgGs in multiple system atrophy and Parkinson's disease. Parkinsonism Relat. Disord. 2021, 87, 98–104.
26. Chartier, S.; Duyckaerts, C. Is Lewy pathology in the human nervous system chiefly an indicator of neuronal protection or of toxicity? Cell Tissue Res. 2018, 373, 149–160.
27. Ingelsson, M. Alpha-Synuclein Oligomers-Neurotoxic Molecules in Parkinson's Disease and Other Lewy Body Disorders. Front. Neurosci. 2016, 10, 408.
28. Masliah, E.; Rockenstein, E.; Mante, M.; Crews, L.; Spencer, B.; Adame, A.; Patrick, C.; Trejo, M.; Ubhi, K.; Rohn, T.T.; et al. Passive immunization reduces behavioral and neuropathological deficits in an alpha-synuclein transgenic model of lewy body disease. PLoS ONE 2011, 6, e19338.
29. Masliah, E.; Rockenstein, E.; Adame, A.; Alford, M.; Crews, L.; Hashimoto, M.; Seubert, P.; Lee, M.; Goldstein, J.; Chilcote, T.; et al. Effects of alpha-synuclein immunization in a mouse model of Parkinson's disease. Neuron 2005, 46, 857–868.
30. Spencer, B.; Valera, E.; Rockenstein, E.; Overk, C.; Mante, M.; Adame, A.; Zago, W.; Seubert, P.; Barbour, R.; Schenk, D.; et al. Anti-α-synuclein immunotherapy reduces α-synuclein propagation in the axon and degeneration in a combined viral vector and transgenic model of synucleinopathy. Acta Neuropathol. Commun. 2017, 5, 7.
31. Bae, E.-J.; Lee, H.-J.; Rockenstein, E.; Ho, D.-H.; Park, E.-B.; Yang, N.-Y.; Desplats, P.; Masliah, E.; Lee, S.-J. Antibody-aided clearance of extracellular α-synuclein prevents cell-to-cell aggregate transmission. J. Neurosci. 2012, 32, 13454–13469.
32. Tran, H.T.; Chung, C.H.-Y.; Iba, M.; Zhang, B.; Trojanowski, J.Q.; Luk, K.C.; Lee, V.M.Y. A-synuclein immunotherapy blocks uptake and templated propagation of misfolded α-synuclein and neurodegeneration. Cell Rep. 2014, 7, 2054–2065.
33. Shahaduzzaman, M.; Nash, K.; Hudson, C.; Sharif, M.; Grimmig, B.; Lin, X.; Bai, G.; Liu, H.; Ugen, K.E.; Cao, C.; et al. Anti-human α-synuclein N-terminal peptide antibody protects against dopaminergic cell death and ameliorates behavioral deficits in an AAV-α-synuclein rat model of Parkinson's disease. PLoS ONE 2015, 10, e0116841.
34. Henderson, M.X.; Covell, D.J.; Chung, C.H.-Y.; Pitkin, R.M.; Sandler, R.M.; Decker, S.C.; Riddle, D.M.; Zhang, B.; Gathagan, R.J.; James, M.J.; et al. Characterization of novel conformation-selective α-synuclein antibodies as potential immunotherapeutic agents for Parkinson's disease. Neurobiol. Dis. 2020, 136, 104712.
35. Chen, Y.-H.; Wu, K.-J.; Hsieh, W.; Harvey, B.K.; Hoffer, B.J.; Wang, Y.; Yu, S.-J. Administration of AAV-Alpha Synuclein NAC Antibody Improves Locomotor Behavior in Rats Overexpressing Alpha Synuclein. Genes 2021, 12, 948.

36. Gorbatyuk, O.S.; Li, S.; Nash, K.; Gorbatyuk, M.; Lewin, A.S.; Sullivan, L.F.; Mandel, R.J.; Chen, W.; Meyers, C.; et al. In vivo RNAi-mediated alpha-synuclein silencing induces nigrostriatal degeneration. Mol. Ther. 2010, 18, 1450–1457.

37. Lindström, V.; Fagerqvist, T.; Nordström, E.; Eriksson, F.; Lord, A.; Tucker, S.; Andersson, J.; Johannesson, M.; Schell, H.; Kahle, P.J.; et al. Immunotherapy targeting α-synuclein protofibrils reduced pathology in (Thy-1)-h α-synuclein mice. Neurobiol. Dis. 2014, 69, 134–143.

38. Kahle, P.J.; Neumann, M.; Ozmen, L.; Muller, V.; Jacobsen, H.; Schindzielorz, A.; Okochi, M.; Leimer, U.; van Der Putten, H.; Probst, A.; et al. Subcellular localization of wild-type and Parkinson's disease-associated mutant alpha-synuclein in human and transgenic mouse brain. J. Neurosci. 2000, 20, 6365–6373.

39. Kallab, M.; Herrera-Vaquero, M.; Johannesson, M.; Eriksson, F.; Sigvardson, J.; Poewe, W.; Wenning, G.K.; Nordström, E.; Stefanova, N. Region-Specific Effects of Immunotherapy with Antibodies Targeting α-synuclein in a Transgenic Model of Synucleinopathy. Front. Neurosci. 2018, 12, 452.

40. Nordström, E.; Eriksson, F.; Sigvardson, J.; Johannesson, M.; Kasrayan, A.; Jones-Kostalla, M.; Appelkvist, P.; Söderberg, L.; Nygren, P.; Blom, M.; et al. ABBV-0805, a novel antibody selective for soluble aggregated α-synuclein, prolongs lifespan and prevents buildup of α-synuclein pathology in mouse models of Parkinson's disease. Neurobiol. Dis. 2021, 161, 105543.

41. El-Agnaf, O.; Overk, C.; Rockenstein, E.; Mante, M.; Florio, J.; Adame, A.; Vaikath, N.; Majbour, N.; Lee, S.-J.; Kim, C.; et al. Differential effects of immunotherapy with antibodies targeting α-synuclein oligomers and fibrils in a transgenic model of synucleinopathy. Neurobiol. Dis. 2017, 104, 85–96.

42. Schofield, D.J.; Irving, L.; Calo, L.; Bogstedt, A.; Rees, G.; Nuccitelli, A.; Narwal, R.; Petrone, M.; Roberts, J.; Brown, L.; et al. Preclinical development of a high affinity α-synuclein antibody, MEDI1341, that can enter the brain, sequester extracellular α-synuclein and attenuate α-synuclein spreading in vivo. Neurobiol. Dis. 2019, 132, 104582.

43. Huang, Y.-R.; Xie, X.-X.; Ji, M.; Yu, X.-L.; Zhu, J.; Zhang, L.-X.; Liu, X.-G.; Wei, C.; Li, G.; Liu, R.-T. Naturally occurring autoantibodies against α-synuclein rescues memory and motor deficits and attenuates α-synuclein pathology in mouse model of Parkinson's disease. Neurobiol. Dis. 2019, 124, 202–217.

44. Weihofen, A.; Liu, Y.; Arndt, J.W.; Huy, C.; Quan, C.; Smith, B.A.; Baeriswyl, J.-L.; Cavegn, N.; Senn, L.; Su, L.; et al. Development of an aggregate-selective, human-derived α-synuclein antibody BIIB054 that ameliorates disease phenotypes in Parkinson's disease models. Neurobiol. Dis. 2019, 124, 276–288.

45. Kuo, Y.-M.; Li, Z.; Jiao, Y.; Gaborit, N.; Pani, A.K.; Orrison, B.M.; Bruneau, B.G.; Giasson, B.I.; Smeyne, R.J.; Gershon, M.D.; et al. Extensive enteric nervous system abnormalities in mice

transgenic for artificial chromosomes containing Parkinson disease-associated alpha-synuclein gene mutations precede central nervous system changes. Hum. Mol. Genet. 2010, 19, 1633–1650.

46. Ghosh, S.S.; Wang, J.; Yannie, P.J.; Ghosh, S. Intestinal Barrier Dysfunction, LPS Translocation, and Disease Development. J. Endocr. Soc. 2020, 4, bvz039.
47. van IJzendoorn, S.C.D.; Derkinderen, P. The Intestinal Barrier in Parkinson's Disease: Current State of Knowledge. J. Parkinsons. Dis. 2019, 9, S323–S329.
48. Boertien, J.M.; Pereira, P.A.B.; Aho, V.T.E.; Scheperjans, F. Increasing Comparability and Utility of Gut Microbiome Studies in Parkinson's Disease: A Systematic Review. J. Parkinsons. Dis. 2019, 9, S297–S312.
49. Brudek, T. Inflammatory Bowel Diseases and Parkinson's Disease. J. Parkinsons. Dis. 2019, 9, S331–S344.
50. Lee, H.-S.; Lobbestael, E.; Vermeire, S.; Sabino, J.; Cleynen, I. Inflammatory bowel disease and Parkinson's disease: Common pathophysiological links. Gut 2021, 70, 408–417.
51. Jan, A.; Gonçalves, N.P.; Vaegter, C.B.; Jensen, P.H.; Ferreira, N. The Prion-Like Spreading of Alpha-Synuclein in Parkinson's Disease: Update on Models and Hypotheses. Int. J. Mol. Sci. 2021, 22, 8338.
52. Borghammer, P.; Van Den Berge, N. Brain-First versus Gut-First Parkinson's Disease: A Hypothesis. J. Parkinsons. Dis. 2019, 9, S281–S295.
53. Kalia, L.V.; Lang, A.E. Parkinson's disease. Lancet 2015, 386, 896–912.
54. Knudsen, K.; Haase, A.-M.; Fedorova, T.D.; Bekker, A.C.; Østergaard, K.; Krogh, K.; Borghammer, P. Gastrointestinal Transit Time in Parkinson's Disease Using a Magnetic Tracking System. J. Parkinsons. Dis. 2017, 7, 471–479.