CHAPTER

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Novel disease-modifying treatments for synucleinopathies

Wolfgang Singer[1], *Jose-Alberto Palma*[2], *Horacio Kaufmann*[2] *and Phillip A. Low*[1]

[1]Department of Neurology, Mayo Clinic, Rochester, MN, United States [2]Department of Neurology, New York University School of Medicine, New York, NY, United States

Introduction

Synucleinopathies are disorders characterized by the abnormal accumulation of the misfolded protein α-synuclein (αSyn) in neurons and glia that affect over 1 million people in the United States and have no cure. Parkinson disease is the most common of the synucleinopathies whereas multiple system atrophy (MSA) is an orphan (i.e., less than 200,000 cases in the United States.), but more aggressive disease. The phenotype and natural history of MSA are described in Chapter 95, and pathogenesis in Chapter 94. Relevant fluid biomarkers are described in Chapter 141. There are no currently available treatments that can halt or slow disease progression, and most patients with MSA die within 7−10 years from symptom onset.

Several pharmaceutical companies are working toward targeted disease-modifying therapies based on two premises. The first is that a better understanding of the mechanistic steps in the pathogenesis of the disease would allow interventions to interrupt disease development and progression. The second is that recognition of early or even preclinical disease would allow the most effective intervention.

Pathophysiology of synucleinopathies

The pathogenesis of the synucleinopathies appears causally linked to accumulation, aggregation, and spreading of misfolded αSyn in the central and peripheral nervous system. There is αSyn misfolding, forming oligomers which are thought to be toxic. Why αSyn oligomerizes remains poorly understood. This process appears related to a number of factors including but not limited to mitochondrial dysfunction, hyperphosphorylation, neuroinflammation, lipid metabolism abnormalities, increased production of αSyn, and defective αSyn clearance and autophagy. Neurotrophic factor deficits, especially brain-derived neurotrophic factor (BDNF), glial-derived neurotrophic factor (GDNF), and nerve growth factor (NGF), appear to facilitate this process. Misfolded αSyn oligomers undergo a cell-to-cell prionlike spread, which may further result in neurotrophic factor deficiency, neuroinflammation (microglial activation), and oxidative stress. There is also a breakdown of the blood−brain barrier. At the cellular level, changes include calcium flooding of neurons and cell death. While the major pathophysiology events appear to be common to all synucleinopathies, differences in αSyn oligomer conformation underlie αSyn distribution and disease phenotype. Namely, αSyn oligomers in MSA are different from those of Lewy body disorders (PD and DLB), resulting in a different neuropathological distribution. The determinants of each αSyn oligomer conformation are not known, although iron metabolism abnormalities might be involved, and the distribution of iron dysmetabolism might relate to the lesion sites.

Disease-modifying therapeutic approaches

Dissecting the multiple pathogenic pathways involved in the synucleinopathies provides options for potential intervention. Fig. 142.1 shows a translational

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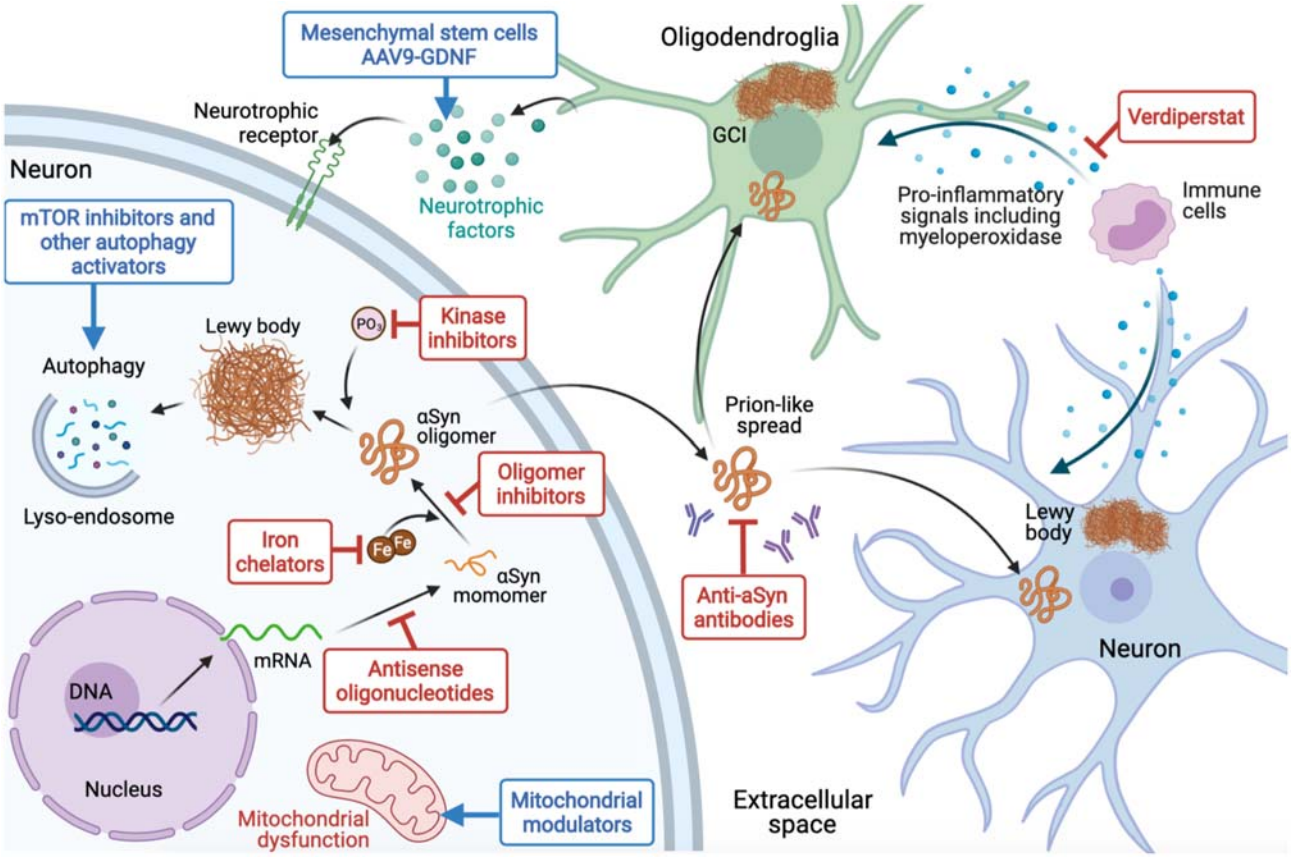

FIGURE 142.1 Pathogenetic mechanisms in MSA and potential therapeutic targets.

approach to pathogenesis and lists steps potentially amenable to clinical intervention. The specific approaches are:

(a) αSyn lowering and blocking

Strategies to reduce αSyn production include the intrathecal administration of antisense oligonucleotides blocking targeting αSyn nRNA (BIIB101), currently being tested in a phase 1 clinical trial for MSA (NCT04165486). Strategies to block αSyn misfolding include small molecules that inhibit oligomer formation, such as the orally active anle138b (currently being tested in a phase 1 clinical trial for PD, NCT04685265) and UCB0599 (currently being tested in a phase 2 clinical trial for PD, NCT04658186), and immune system activation with intravenous administration of αSyn-mimicking peptides (AFFITOPE PD01A and PD03A), currently in early clinical development for PD and MSA (NCT02270489). Many other companies are investigating small molecules inhibiting αSyn in preclinical stages (e.g., ICBI and AC Immune). Approaches to develop targeted αSyn degradation are in preclinical stages (Arvinas Therapeutics).

(b) αSyn spread

Multiple pharmaceutical companies are developing antibodies targeting extracellular αSyn in an attempt to reduce or stop the prionlike spread. While anti-αSyn antibodies could theoretically block the prionlike spread, they will have no effect on αSyn production, as antibodies cannot bind to intracellular proteins. The paradigm would thus need to be combined with anti-αSyn antibodies, to stop the prionlike spread, with therapies that can reduce αSyn production and intracellular accumulation.

Currently developed antibodies vary in terms of modality (humanized vs. human), epitope (C-terminus vs. N-terminus), and selectivity (oligomers vs. monomers). Recent clinical trials have been disappointing. Prasinezumab (PRX002, Roche/Prothena), a monoclonal humanized antibody targeting the C-terminus, failed to meet the primary endpoint in a phase 2a trial in PD, in spite of which a phase 2b trial is currently ongoing. Cinpanemab (BIIB054, Biogen), a human-derived monoclonal antibody targeting the N-terminus, was discontinued in early 2021 after a phase-2 trial for PD missed its primary and secondary endpoints. ABBV-0805, a humanized monoclonal antibody developed by

Abbvie for PD, was withdrawn in mid-2020 for strategic reasons. Other companies, such as Takeda/AstraZeneca and Lundbeck, continue their early clinical development efforts of human-derived monoclonal anti-αSyn antibodies for PD and MSA (NCT04449484 and NCT03611569, respectively).

While this approach appears safe, a significant concern with intravenously administered anti-αSyn antibodies is their poor penetration into the CNS. Some potential strategies to overcome this include intrathecal administration, vectorization of the antibody into an adeno-associated virus with CNS tropism (preclinical development, Voyager Therapeutics), and antibody engineering to enhance their ability to cross the blood–brain barrier (preclinical development, Denali Therapeutics).

(c) αSyn clearance and autophagy

Pathways involved in autophagy and lysosomal function have received increased attention because their modulation could be a potential therapeutic strategy for synucleinopathies. One of these pathways is the mammalian target of rapamycin complex (mTOR) signaling pathway. Activation of the mTOR pathway promotes cell survival, growth, and proliferation via increased protein synthesis, whereas inhibition of the mTOR pathway results in increased autophagy, among other biological effects. Thus, increased activation of autophagy via mTOR inhibition may result in increased αSyn clearance and reduced neurodegeneration. Treatment with rapamycin (also known as sirolimus, a potent inhibitor of the mTOR pathway) in animal models of synucleinopathy reduced the amount of intracellular aggregated αSyn. Similarly, treatment with rapamycin for 16 weeks in a transgenic mouse model of MSA resulted in decreased αSyn levels in the substantia nigra.

Sirolimus has been approved by most regulatory agencies as an orally active immunosuppressant to prevent organ transplant rejection, and its adverse event profile is well known. An ongoing investigator-sponsored single-center phase-2 double-blind randomized placebo-controlled futility trial at New York University aims to determine whether sirolimus is of sufficient promise to slow the progression of MSA (NCT03589976). In this trial, patients receive oral sirolimus at dosages from 2 to 6 mg/day, or matching placebo, for a year. These dosages are well tolerated and associated with relatively low immunologic risk. The trial, which is also collecting neuroimaging and biomarker data, will be completed in 2021.

RTB101, a selective mTOR complex 1 (mTORC1) oral inhibitor, underwent a phase 1/2b clinical trial for PD. The trial was suspended during the COVID-19 pandemic, the results were never published, and the sponsor (resTORbio) merged with another company (Adicet Bio) in 2020. The status of this program is unknown.

(d) Neurotrophic factor deficiency

There are reduced growth factors including NGF, BDNF, and GDNF in experimental and human MSA. One approach to correct this deficiency is the use of neurotrophic factor producing-mesenchymal adult stem cells.

Mesenchymal adult stem cells (MSCs) are capable of differentiating into different cell types dependent on their microenvironment, have been shown to secrete and restore neurotrophic factors in neural tissue exerting neuroprotective effects, including in the mouse model of MSA, and also have well-described immunomodulatory properties. Consequently, clinical trials have been conducted to explore the therapeutic potential of autologous MSCs in MSA. Following a positive open-label trial, a randomized, double-blind, placebo-controlled single-center trial was conducted in Korea. Patients were randomized to receive active treatment (intraarterial autologous bone marrow–derived MSCs into bilateral carotid and dominant vertebral arteries at baseline, followed by intravenous administrations at 30, 60, and 90 days) vs. sham procedures. The study met its primary and several secondary endpoints and showed significantly slower disease progression in the active limb. However, a number of concerns were expressed about this study, including the small number of patients enrolled, its single-center nature, and importantly significant safety concerns about the intraarterial approach resulting in ischemic lesions in a significant percentage of patients. A different approach was subsequently explored in a phase 1/2 trial at Mayo Clinic, using intrathecal MSC delivery in a dose-escalation design. This approach was found to be safe and well tolerated, apart from low back and lower extremity discomfort along with imaging evidence of reactive changes about the cauda equina at higher dose levels, which were subsequently abandoned. The generally well-tolerated low and medium dose levels were associated with dose-dependent, significantly slowed rates of disease progression. A marked rise of neurotrophic factors was found in CSF following MSC administrations providing support for the hypothesized underlying mechanisms. A double-blind placebo-controlled trial of intrathecal MSC administration has now been designed and is expected to launch in late 2021.

Other approaches aiming at growth factor deficiency include increasing GDNF via in-vivo gene replacement therapy by intraparenchymal delivery via a viral vector,

as well as FTY720-Mitoxy which increases expression of various neurotrophic factors.

(e) Neuroinflammation

Evidence from preclinical and human postmortem studies suggests that misfolded αSyn accumulation is associated with oxidative stress, and neuroinflammation with astroglial and microglial activation. The reactive oxygen-generating enzyme myeloperoxidase (MPO) is a key driver of oxidative and neuroinflammatory processes underlying neurodegeneration. Preclinical and clinical data have shown that MPO produces cytotoxic oxidizing and nitrosylating compounds. MPO expression and activity are significantly increased in activated immune cells, such as microglia, at the sites of neuroinflammation and neurodegeneration in the brains from patients with PD and MSA. This upregulation of MPO is believed to promote neurodegeneration.

Verdiperstat (previously known as BHV-3241 or AZ3241) is an oral selective and irreversible MPO inhibitor. Studies in animal models of PD and MSA demonstrated that MPO inhibition with verdiperstat suppresses microglial activity and neuroinflammation, though neuroprotective effects were only demonstrated when animals were treated before the disease was fully established. A phase 2 trial in PD showed that verdiperstat was safe and elicited a significant reduction of TSPO activity as measured by ^{11}C-PBR28 PET imaging. A phase 2 trial in MSA showed no target engagement (i.e., no differences in ^{11}C-PBR28 PET imaging in the verdiperstat group vs. placebo), and no significant differences in clinical outcomes, although the trial was not powered to detect clinical statistical differences (NCT02388295). The compound is currently being tested in patients with MSA in a large multicenter phase-3 clinical trial (NCT03952806), the results of which are expected by the end of 2021.

The inflammasome is a multiprotein, cytosolic complex that functions as sensor in the innate immune system. Its activation by pathologic proteins and other stressors triggers the production and secretion of proinflammatory cytokines, resulting in neuroinflammation and cell death. One specific inflammasome, NLRP3, appears to be particularly linked to neurodegeneration. Preclinical evidence suggests that inhibition of the NLRP3 inflammasome prevents αSyn and neurodegeneration. Inzomelid, developed by the company Inflazome, is an oral, brain-penetrant inhibitor of the NLRP3 inflammasome. The company disclosed plans to start clinical development of inzomelid for PD with an agreement with the Michael J. Fox Foundation to develop NLRP3 PET imaging. In 2020, however, Inflazome was acquired by Roche, and the status of inzomelid for PD is currently unknown.

(f) Dysfunctional iron metabolism

Increasing evidence suggests that the redox active metal iron promotes αSyn aggregation. αSyn binds iron with micromolar affinity, and both iron and dopamine promote αSyn aggregation, and can react together to form damaging peroxides. There is evidence of a regional increase of iron in the substantia nigra of PD patients, as well as a rapid accumulation of nigral iron in animals following intoxication with the Parkinsonian agents 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Moreover, iron is needed for numerous catalytic and metabolic processes including as a cofactor for tyrosine hydroxylase, the enzyme catalyzing the conversion of tyrosine to L-DOPA.

Deferiprone (3-hydroxy-1,2-dimethyl-4-pyridinone) is a high-affinity iron chelator, which has been used in several clinical trials for PD, with promising results. Data from two placebo-controlled studies have been published. In a 40-patient phase 2/3 trial completed in 2011, a 6-month course of 30 mg/kg daily deferiprone reduced substantia nigra iron levels and slowed decline on the UPDRS (NCT00943748). A phase 2 trial in 22 patients with PD reported a reduction in brain iron and a trend toward improvement in motor scores and quality of life (NCT01539837). A dose-ranging phase 2 trial evaluating a delayed-release formulation in 140 people with PD, conducted at 20 sites, finished in 2019; the results are not published (NCT02728843). In 2016, a large multicenter trial began recruiting 372 patients with PD at 25 sites, to evaluate a 9-month regimen of 30 mg/kg per day of the same formulation. The trial is expected to finish in 2021 (NCT02655315).

Other iron chelators with improved characteristics are in clinical development. ATH-434 (formerly known as PBT434) is a novel, orally bioavailable, moderate iron affinity 8-hydroxyquinazolinone which is being developed by Alterity Therapeutics. ATH-434 binds iron sufficiently to abolish pathological reaction with αSyn, but with an affinity that is designed not to disrupt physiological iron homeostasis and, therefore, avoid related side effects. A phase 1 study performed in healthy volunteers showed that ATH-434 is safe and crosses the blood–brain barrier (ACTRN12618000541202). The company has plans to begin a phase 2 trial for MSA in late 2021.

(g) Dysfunctional glucose metabolism

Epidemiology, molecular genetics, and cell biology studies have identified links between synucleinopathies and type 2 diabetes mellitus. Several recent discoveries have highlighted common cellular pathways that potentially relate neurodegenerative processes with abnormal mitochondrial function and glucose metabolism. This

evidence supports further study of these pathways, most importantly to identify neuroprotective agents for the synucleinopathies, and/or establish more effective prevention or treatment for type 2 diabetes mellitus. In parallel with these advances, there are already randomized trials evaluating several established treatments for insulin resistance as possible disease modifying drugs in PD, with only preliminary insights regarding their mechanisms of action in neurodegeneration.

A placebo-controlled trial with pioglitazole, an antidiabetic peroxisome proliferator-activated receptor gamma activator, failed to show efficacy in patients with PD (NCT01280123). Exenatide, a glucagonlike peptide-1 agonist antidiabetic, significantly reduced deterioration in motor symptoms in patients with PD disease in a randomized, placebo-controlled trial. In addition, there were trends favoring the exenatide group in assessments of nonmotor symptoms, cognition, and quality of life. Multiple clinical trials with different formulations of exenatide for PD and MSA are ongoing (NCT04431713; NCT04232969, NCT04305002, NCT04269642, NCT04154072). A phase-2 placebo-controlled trial for PD with liraglutide, a glucagonlike peptide-1 receptor agonist approved as antidiabetic medication, is ongoing (NCT02953665). While the multiplicity of clinical trials highlights the current interest of glucagonlike peptide-1 receptor agonist for the potential treatment of the synucleinopathies, it remains uncertain whether these compounds affect the underlying disease pathophysiology or simply induce long-lasting symptomatic effects.

(h) Mitochondrial dysfunction

SBT-272, in clinical development by Stealth Therapeutics, is a peptide compound that targets the inner mitochondrial membrane where it binds reversibly to cardiolipin improving electron transport chain function and ATP production and reducing the formation of pathogenic reactive oxygen species. SBT-272 is currently being evaluated in healthy subjects in a phase 1 clinical trial. If successful, the company has disclosed their intention to test it in patients with MSA.

(i) Non-aSyn genetically validated targets

Mutations in *GBA*, *LRRK2,* and *Parkin* genes have been associated with genetic forms of PD. Some of these mutations have been detected in pathologically confirmed MSA cases, suggesting that both disorders may have similar genetic forms. Consequently, targeted therapies addressing the function of these genes and proteins are in development.

Mutations in *GBA1*, which encodes the lysosomal enzyme glucocerebrosidase enzyme, are the leading genetic risk factors for PD and for DLB. Insufficient GBA1 function causes a buildup of glucosylceramide. Venglustat is a brain-penetrant inhibitor of the enzyme glucosylceramide synthase, thus reducing the accumulation of glucosylceramide. Venglustat is being developed by Sanofi-Genzyme for the treatment of lysosomal storage diseases, including Gaucher and Fabry. The fact that *GBA1* mutations are the most frequently found in patients with PD raised the possibility that venglustat could be also useful in PD. Supporting this hypothesis, some pieces of research suggest that glucosylceramide may promote αSyn oligomerization. Venglustat had excellent tolerability and safety profile in a phase 1 study in healthy volunteers, and went on to a placebo-controlled phase 2 in involving 270 participants with PD who were heterozygous for a *GBA* mutation as well as asymptomatic prodromal (i.e., REM sleep behavior disorder) carriers of a *GBA* mutation. In 2021, the trial missed its primary endpoint, and development of venglustat for PD was halted.

Prevail Therapeutics is developing PR001, a gene-replacement therapy that uses adeno-associated virus 9 (AAV9) to deliver a functional copy of the *GBA1* gene to the brain. The therapy comprises a one-time injection into the cerebrospinal fluid in the cisterna magna at the base of the brain. A phase 1/2 sham-controlled trial in patients with PD and a GBA mutation began in January 2020. In August 2020, Prevail announced that serious adverse events had occurred 3 months after injection in the first treated PD-GBA patient, possibly due to immunogenicity to the AAV9, as the unspecified events reportedly resolved with immunosuppression. The protocol was amended to an open-label design, and with concomitant administration of immunosuppressants. The trial end date is 2027. In January 2021, Prevail was acquired by Eli Lilly.

An oral, brain-penetrant LRRK2 kinase inhibitor (DNL151) being developed by Denali had safe and promising phase 1 results (NCT04056689). In August 2020, Denali announced it would advance clinical development of DNL151 in collaboration with Biogen. Two trials are planned for 2021, one in PD patients with *LRRK2* mutations, and one in patients with sporadic PD. Biogen is currently testing an intrathecal antisense oligonucleotide blocking the production of LRRK2 protein (BIIB094) in a phase 1 trial, expected to finish in 2022 (NCT03976349).

Mutations in *PRKN* (*PARK2*) cause autosomal recessive PD. PRKN encodes the E3 ubiquitin ligase Parkin, which plays a key role in mitochondrial quality control and turnover. Loss of activity of Parkin E3 ligase appears to play a pathogenic role in both inherited and sporadic PD. PINK1 (PTEN-induced putative kinase 1) encodes a mitochondrially tethered kinase that regulates Parkin activity through phosphorylation events. Mutations in

PINK1, although rare, are associated with autosomal recessive PD similar to PD with PRKN mutations. Several companies have candidates modulating this pathway in PD including Progenra and Vincere Biosciences.

Conclusion

The availability of multiple potential disease-modifying therapies in early clinical development is exciting. While the hope is that these pathophysiology-based interventions, alone or in combination, administered early in the disease process, may slow or halt its progression, many challenges remain. These include the heterogeneity of the clinical course of the synucleinopathies and the lack of validated fluid and imaging biomarkers for diagnosis and disease progression, but there have been important developments recently (e.g., aSyn protein misfolding cyclic amplification assay, neurofilament light chain, aSyn-containing exosomes). These developments are crucial when studying disease-modifying compounds with modest but clinically significant effects. Moreover, the possibility of applying disease-modifying therapies in patients who are still in the prodromal stages of the disease raises the possibility of preventing the development of PD, MSA, and DLB.

Further reading

[1] Athauda D, Maclagan K, Skene SS, Bajwa-Joseph M, Letchford D, Chowdhury K, et al. Exenatide once weekly versus placebo in Parkinson's disease: a randomised, double-blind, placebo-controlled trial. Lancet 2017;390:1664–75.

[2] Bennett CF, Kordasiewicz HB, Cleveland DW. Antisense drugs make sense for neurological diseases. Annu Rev Pharmacol Toxicol 2021;61:831–52.

[3] Finkelstein DI, Billings JL, Adlard PA, Ayton S, Sedjahtera A, Masters CL, et al. The novel compound PBT434 prevents iron mediated neurodegeneration and alpha-synuclein toxicity in multiple models of Parkinson's disease. Acta Neuropathol Commun 2017;5:53.

[4] Jankovic J, Goodman I, Safirstein B, Marmon TK, Schenk DB, Koller M, et al. Safety and tolerability of multiple ascending doses of PRX002/RG7935, an anti-α-synuclein monoclonal antibody, in patients with Parkinson disease: a randomized clinical trial. JAMA Neurol 2018;75:1206–14.

[5] Jucaite A, Svenningsson P, Rinne JO, Cselényi Z, Varnäs K, Johnström P, et al. Effect of the myeloperoxidase inhibitor AZD3241 on microglia: a PET study in Parkinson's disease. Brain 2015;138:2687–700.

[6] Lemos M, Venezia S, Refolo V, Heras-Garvin A, Schmidhuber S, Giese A, et al. Targeting α-synuclein by PD03 AFFITOPE and Anle138b rescues neurodegenerative pathology in a model of multiple system atrophy: clinical relevance. Transl Neurodegener 2020;9:38.

[7] Ndayisaba A, Wenning GK. Inhibition of the mammalian target or rapamycin (mTOR): a potential therapeutic strategy for multiple system atrophy. Clin Auton Res 2020;30:7–8.

[8] Palma JA, Kaufmann H. Novel therapeutic approaches in multiple system atrophy. Clin Auton Res 2015;25:37–45.

[9] Singer W, Dietz AB, Zeller AD, Gehrking TL, Schmelzer JD, Schmeichel AM, et al. Intrathecal administration of autologous mesenchymal stem cells in multiple system atrophy. Neurology 2019;93:e77–87.

[10] von Linstow CU, Gan-Or Z, Brundin P. Precision medicine in Parkinson's disease patients with LRRK2 and GBA risk variants—let's get even more personal. Transl Neurodegener 2020;9:39.