clinical trial. Patients with hATTR amyloidosis were enrolled at sites in New Zealand and the United Kingdom. Of the six patients enrolled, three had the p.T80A mutation, two had the p.S97Y mutation, and one had the p.H110D mutation. These patients had sensory polyneuropathy and mild or no cardiac involvement (New York Heart Association class 1 status). The first two dosage groups (0.1 mg/kg and 0.3 mg/kg) reported an excellent safety profile.

Remarkably, the 0.1 mg/kg dosage group demonstrated a mean reduction in circulating TTR of 52% (range 47% to 56%), while the 0.3 mg/kg group demonstrated reduced serum TTR by 87% (range 80% to 96%). This degree of reduction by i.v. injection is likely to be clinically important for the treatment of patients with ATTR amyloidosis. The fact that this level of response was seen at the 0.3 mg/kg dose most likely suggests that this therapy could be scaled sufficiently for the therapy to be offered to most or all patients with this diagnosis.

Importantly, if this were to translate to other genetic conditions, this level of efficiency could be quite important for these other conditions as well. One such condition, alpha-1 antitrypsin liver disease, would be expected to respond to 50% or greater reduction of the mutant protein.[10] While the single-edit gene knockout approach is designed only to treat toxic gain-of-function mutations, the surprisingly positive finding that the lipid nanoparticle (LNP) platforms is efficient enough to work for this condition bodes well for future therapies that require delivery of a homology-directed repair (HDR) template in addition to Cas9 and one or two sgRNAs.

However, as exciting as it is to see the huge success described in this report with a safe reduction of circulating TTR protein after liver gene editing, one cannot forget the additional sites of TTR production, such as the choroid plexus, and we cannot deem this approach as a cure for hATTR. *In vivo* CRISPR-Cas9 gene editing is a powerful tool, showing here that its potential is more than just a promise on paper or in small animals. But the scientific community still needs to find the way to further improve the technology and make it a reality to treat other organs.

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CDNF: An innovative actor in disease-modifying approaches for Parkinson's disease

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Although cerebral dopamine neurotrophic factor (CDNF) is considered a "neurotrophic factor (NF)," its mechanism of action differs from classic NFs that increase cell survival through intracellular signaling. CDNF is located in the endoplasmic reticulum (ER), is secreted in response to ER stress, and reduces ER stress after internalization.[1] In this issue of *Molecular Therapy*, Albert et al.[2] demonstrate that CDNF directly binds to α-synuclein *in vitro* and *in vivo*. They further show that CDNF reduced α-synuclein aggregation as well as internalization of α-synuclein preformed fibrils (PFFs) in neuronal cultures. Delivery of CDNF alleviated behavioral deficits induced by α-synuclein PFFs. Interestingly, in this model, the behavioral abnormalities appeared in the absence of DA neuronal cell death. The

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data suggest that CDNF can reverse neuronal dysfunction at a very early stage, before the appearance of evident neurodegeneration.

The etiology of Parkinson's disease (PD) is multifactorial and possibly differs among patients. Various phenomena have been implicated, such as mitochondrial defects, neuroinflammation, and disturbed proteostasis resulting in α-synuclein aggregation. These phenomena are interrelated, thus probably generating a self-amplifying progression of the disease.

Thanks to the discovery of the important role of dopamine depletion in PD, a breakthrough in the pharmacological treatment was made in the 1960s, in the form of the oral administration of L-dopa, the dopamine precursor.[3] Exogenous L-dopa is taken up into remaining DA neurons where it is converted into dopamine by aromatic acid decarboxylase. A second breakthrough in the history of PD treatment is deep brain stimulation, which involves implanting and adjusting electrodes in the brain in order to reduce the activity of the subthalamic nucleus (STN), an overactive nucleus of the motor loop.[4]

Gene transfer approaches using AAV and LV vectors were subsequently designed based on similar rationales as the pharmacological or the neurosurgical approaches, i.e., dopamine replacement and compensation of the motor loop's circuitry, respectively. The first clinical trials demonstrated safety and tolerability as well as clinical benefits, and larger studies have been launched (ClinicalTrials.gov: NCT03562494, NCT03720418, and NCT03562494). However, none of these treatments could reduce progressive loss of DA neurons.

Counteracting neurodegeneration using NFs could constitute the first disease-modifying approach. Glial cell line-derived neurotrophic factor (GDNF) and Neurturin, both belonging to the GDNF family of ligands (GFLs), protect dopaminergic (DA) neurons and reduce motor symptoms in toxin-induced animal models of PD. However, these factors failed to demonstrate significant clinical benefit in clinical trials, following intracerebral delivery of either recombinant protein or AAV2-mediated gene therapy.[5] Interestingly, post-mortem analyses revealed neuronal fiber regrowth and functional improvements evidenced by PET-scan imaging. Similar outcomes were obtained with protein and AAV-mediated gene delivery, suggesting that the limiting factors are related to GFL biology rather than to the therapeutic platform. The reasons for these failures have been discussed by a panel of experts[5] who suggested that the too-far advanced stage of the patients' pathology and insufficient coverage of the putamen were the main reasons for the poor outcome of these clinical trials. The hope is that enrolling earlier-stage patients combined with an improved neurosurgical method will allow this approach to achieve ultimate clinical efficacy (ClinicalTrials.gov: NCT04167540).

However, a crucial aspect of PD neuropathogenesis was not addressed in the pre-clinical studies underlying the design of the GFLs clinical trials: α-synucleinucleopathy. Regardless of the etiology, α-synuclein aggregation is thought to play a central role in the initiation and/or in the progression of PD.[6] Anders Björklund's group questioned whether GDNF/NTRN could protect DA neurons in the presence of α-synucleinucleopathy (this issue is reviewed by Manfredsson et al.[5]). Indeed, in a local transgenic model exhibiting AAV-mediated human α-synuclein overexpression in the rat substantia nigra, AAV-GDNF failed to protect the DA neurons, which was attributed to α-synuclein-induced downregulation of GDNF pro-survival signaling. The authors speculated that, since α-synuclein aggregation is a major hallmark of PD, the failure of GDNF to reduce it would preclude a beneficial outcome. However, Krys Bankiewicz's showed that α-synuclein is not overexpressed in patients with sporadic PD, thus questioning the usefulness of the AAV-α-synuclein model used. Indeed, to drive neuronal cell death and behavioral deficits within a short time, the AAV vector had been optimized for efficient expression in DA neurons, resulting in supraphysiological α-synuclein overexpression. Interestingly, Chmielarz et al.,[7] using the α-synuclein PFF model, showed that GDNF reduced α-synuclein accumulation, via mTOR/Akt signaling, supporting the potential of GDNF to treat PD. Thus, the reasons for GDNF failure in the clinical trials await further investigations.

The same research group, Albert et al., previously evaluated CDNF recombinant protein in toxin-induced models and showed that it was as potent as GDNF in reducing neuronal cell death. Interestingly, the effects of GDNF and CDNF were additive, further suggesting that they act on different aspects of PD pathology.[8] In conclusion, like GDNF, CDNF potently interferes with neuronal cell death and α-synuclein aggregation. However, CDNF—but not GDNF—directly interacts with α-synuclein and modulates ER stress, which is thought to contribute to PD.[9] Thus, CDNF is a multifunctional therapeutic that is possibly more potent than classic NFs in tackling the multifactorial causes of PD.

Finally, a drawback of NFs acting through receptors and signal transduction is the possibility of off-target and saturating effects after long-term treatments.[10] If CDNF does not induce such undesired effects, it could be more easily translated to the clinics than GFLs.

A phase I safety clinical study using CDNF recombinant protein infusion is ongoing (ClinicalTrials.gov: NCT03295786). If successful, CDNF protein or gene therapy could constitute a breakthrough and become the first disease-modifying treatment for PD. If long-term CDNF safety is established, a viral vector-mediated delivery could be implemented to avoid repeated intracerebral infusions and device-related adverse effects.

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