HHS Public Access

Author manuscript

*Mov Disord*. Author manuscript; available in PMC 2020 September 01.

Published in final edited form as:

*Mov Disord*. 2019 September ; 34(9): 1279–1282. doi:10.1002/mds.27675.

# Parkinson's Disease Gene Therapy: Will Focused Ultrasound and Nanovectors Be the Next Frontier?

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Recently, one of us (JHK) published a review article in *Movement Disorders*[1] that attempted to concisely summarize current thinking on disease-modifying approaches for Parkinson's disease (PD). In that article, significant sections were devoted to the use of trophic factors, such as glial cell-line derived neurotrophic factor (GDNF) and neurturin (NTN), in gene therapy clinical trials for PD. Broadly speaking, it was concluded that, despite ample positive evidence from preclinical studies showing the ability of trophic factors to slow nigrostriatal degeneration, outcomes from PD clinical trials using trophic factors have been largely disappointing. Many important issues were raised that could explain the disconnect between preclinical and clinical outcomes, and these issues have also been covered comprehensively in other excellent reviews.[2,3] Here, we specifically focus on 3 of these key issues and discuss how they could potentially be addressed by an emerging, noninvasive, magnetic resonance (MR) image-guided, gene-therapy approach.

- Issue 1. The first issue pertains to limited volumetric coverage of the delivered gene therapy after direct injection. Poor volumetric coverage was observed, for example, in postmortem specimens from the CERE-120 (NTN) clinical trial, wherein only a small fraction of the putamen was covered with the transgene.[4] Moreover, although local sprouting was evident, it was still quite restricted.
- Issue 2. The second issue pertains to the poor retrograde transport of vector-delivered GDNF within the volume of tissue displaying transgene expression.[5] Again, this issue is derived from the analysis of postmortem specimens. Specifically, specimens from 2 patients with α-synucleinopathies that had been treated only a few months earlier with NTN gene therapy revealed that <1% of substantia nigra (SN) neurons were positively stained for NTN. Transfection

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**Relevant conflicts of interests/financial disclosures:** Nothing to report.

efficiency was greater in 2 patients treated more than 4 years earlier, but it was still <5%.

- Issue 3. The third issue, related to issue 2, pertains to the clear benefit of treating patients at a relatively early postdiagnosis, perhaps even prodromal, timepoint. Within about 4 to 5 years after PD diagnosis, tyrosine hydroxylase positive dopamine transporter fibers are almost entirely absent.[6] Thus, unless a patient is treated early enough after diagnosis, there is unlikely to be sufficient substrate for successful transfection.

There have been recent surgical and vector development advances that have partially addressed some of these issues. For example, the coadministration of gadolinium with the therapeutic vector in combination with intraoperative MRI permits real-time visualization of vector infusion to fill the intended target zones.[7] This allows for individualized patient dosing based on the size of the target. In a similar vein, a recent clinical trial combined a multicatheter convection enhanced delivery system with T1-weighted MR imaging of gadolinium-labeled artificial cerebrospinal fluid to verify the putamen-wide coverage of GDNF.[8] In addition, new vectors that can be delivered systemically or into the CSF-filled spaces may provide widespread transfection.[9] However, this approach lacks the regional specificity often required for trophic factor therapy, with widespread delivery being potentially dangerous.[10]

Yet another approach that we believe has promise for addressing these key issues combines (1) MR image-guided focused ultrasound (FUS) for noninvasive, regionally specific, targeting, and transient opening of the blood–brain barrier (BBB) with (2) systemically injected nanovectors that are specifically engineered to penetrate through brain tissue. One of us (RJP) has covered the underlying methodology and physical principles of FUS-mediated BBB opening in previous reviews,[11,12] and another review on this topic is provided in this issue of *Movement Disorders*.[13] We refer the reader to these articles for more comprehensive discussion. Briefly, such approaches first entail the intravenous injection of 1-μm to 4-μm diameter ultrasound contrast agent microbubbles (MBs), followed by the application of pulsed-FUS to the brain region of interest. The size of the ultrasound focus varies depending on the device being used, but it is usually relatively small (ie, ranging from about the size of a grain of rice to the size of a grape) and ellipsoidal in shape. The successful treatment of volumes larger than the focus can be achieved by applying multiple sonications, with treatment planning and monitoring typically performed under image guidance with MRI. As circulating MBs pass through the ultrasound focus, they oscillate and vibrate in response to the ultrasound pressure waves, thereby imparting fluid shear stresses on, and circumferential stresses within, the capillary wall. These stresses both disrupt tight junctions and elicit active transport processes, thereby providing a transient (ie, ~4-6 hours) window wherein circulating drugs and/or genes may cross the BBB. This phenomenon has been studied for more than a decade. Both small and large animal studies have shown it is safe, and the ability to monitor the behavior of the MBs via their acoustic emissions has proven instrumental in controlling the BBB opening[14-17] and even subsequent drug delivery.[18] Such approaches have also been used to successfully deliver neurotrophic factors and genes to the brains of rodents.[19-21] Importantly, the BBB opening with MBs and

ultrasound has entered clinical trials for patients with Alzheimer's disease and primary brain tumors[22-24] and shows an excellent safety profile thus far.

The second key component of this strategy entails the use of a gene-delivery vector that efficiently penetrates brain tissue. Brain tissue contains a dense network of extracellular matrix composed of adhesive macromolecules. Because of this, the penetration of conventional nanoparticles, including gene vectors, through brain tissue is impeded via both steric hindrance and multivalent adhesive interactions. During the past few years, 2 of us (JH and JSS) have engineered nanoparticles possessing small particle diameters and nonadhesive surface coatings that can spread through the brain tissue barrier relatively unhindered (ie, "brain-penetrating" nanoparticles [BPN]).[25-27] Key studies by Nance et al.[28] and Timbie et al.[29] have demonstrated that BPN on the order of 70 nm or less in diameter may be safely delivered across both the BBB and blood–tumor barriers. Furthermore, we have shown that nonviral gene vectors may be formulated with brain-penetrating properties[30] and delivered across the BBB with FUS to elicit robust and safe transgene expression in the ultrasound focal region.[31] Building off these results, we next developed a nonviral GDNF-BPN formulation and targeted its delivery to the striatum of PD rats [6-hydroxydopamine (6-OHDA)] with ~70% nigrostriatal degeneration. Of note, this strategy yielded substantial GDNF protein expression, restored tyrosine hydroxylase positive neurons in both the striatum and SN pars compacta, and markedly improved motor function.[32]

Ultimately, we believe this study[32] most effectively supports our contention that combining MR image-guided FUS with BPN offers an opportunity to overcome the major issues of gene delivery that were previously noted. First, in contrast to direct injection, which applies the gene therapy vectors via a single point source, the use of MR image-guided FUS permits delivery from potentially all capillary surfaces that fall within the ultrasound focus. Thus, it is capable of more fully covering a target volume in a noninvasive manner, without multiple brain penetrations and their associated adverse events. As such, it may address issue 1, namely, poor volume of transfection. Second, the penetrating properties of the nanoparticles may yield a more homogeneous distribution of transgene expression within the target volume, leading to enhanced transfection efficiency and a means to address issue 2. This is important because hotspots of dopamine caused by heterogeneous trophic factor delivery have been proposed to mediate off-medication dyskinesias.[33,34] Indeed, we have previously observed that, after mCherry-BPN delivery across the BBB, the distribution of the mCherry reporter was quite uniform through the focal region, and both neurons and glial cells were transfected.[31] Third, we postulate that the noninvasive nature of the FUS approach may eventually make it a more appealing option for prodromal PD patients seeking gene therapy, especially when considering that the only other option would be a highly invasive direct injection. In support of this argument, we note that several Alzheimer's patients have already undergone noninvasive BBB opening with MR image-guided FUS. Of note, given the documented beneficial influence of BBB opening alone on the clearance of plaques in Alzheimer's disease mouse models,[35,36] these patients did not receive a therapeutic agent.[22] We submit that the success of this trial speaks to the safety and noninvasive nature of the treatment. Treating patients at an early stage would address issue 3 and markedly raise the potential of a given gene-therapy treatment to yield a therapeutically significant result.

Looking ahead, we acknowledge that preclinical evidence supporting the combination of MR image-guided FUS with gene-bearing BPN as a means to overcome key challenges to effective gene therapy for PD is only still emerging. The potential toxicity and off-target transfection of systemically injected BPN still need further assessment before translation. That said, in studies conducted thus far, BPN containing dense polyethylene glycol "shielding" have demonstrated negligible levels of inflammation after FUS-mediated delivery to the brain and transfection has been confined to the focal region.[31,32] If safety concerns associated with the use of polyethylenimine-based BPN do arise, biodegradable poly(μ-amino ester) BPN, which has been shown to be safe and effective for gene therapy in the brain, provides an appealing alternative.[37,38]

In addition, although we have demonstrated efficacy and safety in the rat 6-OHDA PD model, the features of this model fail to capture and recapitulate various essential features of PD pathology, such as slowly progressive neuronal loss and α-synuclein aggregation. Thus, in the near term, it will be important to perform FUS-mediated, GDNF BPN–based, gene therapy studies in better suited models (ie, α-synuclein models with characteristic Lewy body pathology[39] and dopaminergic neuron loss[40]). Focused ultrasound (FUS) has been used previously to deliver viral vectors to mice overexpressing human α-synuclein,[41] and FUS may directly inhibit α-synuclein aggregation;[42] therefore, a clear precedent exists for these investigations. We recognize that the putative therapeutic efficacy of GDNF has been challenged based on evidence that *Ret* expression is reduced when α-synuclein is overexpressed in rats via a recombinant adeno-associated virus.[43,44] However, the relevance of this recombinant adeno-associated virus α-synuclein model to PD has been challenged recently using several lines of evidence.[45] Moreover, we submit that the ability of GDNF, when applied every 4 weeks via convection-enhanced delivery throughout the entire putamen, to elicit a positive PET signal in PD patients indicates that GDNF signaling is maintained.[8] That said, we also emphasize that GDNF-BPN are by no means the only PD therapeutic that may be delivered across the BBB with FUS and MBs. Our approach represents a platform technology amenable to the delivery of virtually any nucleic acid therapeutic, including RNA inhibitors and vectors for trophic factors other than GDNF.

Ultimately, if our preclinical studies in advanced α-synuclein–based mouse models of PD are successful, we are hopeful that large animal studies and, eventually, translation to clinical trials will follow. Such trials would likely use the same MR image-guided FUS system (ie, Exablate Neuro from Insightec, Tirat Carmel, Israel) that is being implemented in clinical BBB opening trials for Alzheimer's disease and primary brain tumors today;[22,23] however, other systems now being tested in clinical trials (eg, NaviFUS, Taipei City, Taiwan) could also be appropriate. The targeting capabilities of these systems would ensure that the delivery of therapeutic nucleic acid–bearing BPN across the BBB is restricted to the targets of interest, thereby obviating off-target effects.

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