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Review

Clinical tests of neurotrophic factors for human neurodegenerative diseases, part 2: Where do we stand and where must we go next?

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ABSTRACT

The therapeutic potential of neurotrophic factors has been recognized for decades, with clinical trials in human neurodegenerative diseases extending back at least 25 years. While improvements in clinical dosing paradigms have reduced the side effects commonly seen in the earlier trials, efficacy has remained a serious disappointment (reviewed in Bartus and Johnson, 2016). This lengthy clinical effort stands in contrast to robust effects consistently achieved from different neurotrophic factors in a variety of animal models of neurodegeneration. This review discusses the prevailing assumption and supporting data that the major reason for the disappointing efficacy of past clinical trials is related to suboptimal dosing methods. It is concluded that while further improvements in dosing parameters might be useful, a much greater problem centers around a number of specific morphologic and functional changes in neurons in human neurodegenerative disease that mitigate the ability of neurotrophic factors to exert their effects. Moreover, the biological substrate which neurotrophic factors depend upon to exert their effects continues to erode as time progresses, due to the progressive nature of these diseases. For this reason, most of the empirically-supported reasons contributing to the weak neurotrophic responses in human patients can be mitigated by enrolling less severely advanced cases. It is further concluded that recent clinical trials of neurotrophic factors have generated important evidence that shifts risk: benefit assessments to support enrolling earlier-stage patients. While the Alzheimer's field has begun to shift attention toward much earlier-stage (even prodromal) patients in trials intended to modify disease progression, other neurodegenerative diseases (e.g., Parkinson's, ALS and possibly HD) must now consider similar changes in approach.

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1. Introduction

Despite many decades of animal research supporting the therapeutic potential of neurotrophic factors for treating neurodegenerative diseases, 25 years of clinical trials to establish their utility as novel therapies for

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diseases has not produced conclusive evidence of efficacy (Bartus and Johnson, 2016). Despite this, enthusiasm persists for testing neurotrophic factors in humans as potential therapeutic candidates. A multi-center, randomized controlled trial testing NGF in AD recently reported disappointing efficacy (Rafii et al., 2015), and yet the authors discussed approaches for continued testing in AD. Two Phase 1 clinical trials testing GDNF (glial-cell derived neurotrophic factor) in advanced PD patients have begun to enroll subjects, one applying gene therapy techniques (NINDS, 2015) and the other infusion of human recombinant protein (http://www.medgenesis.com/news.htm). Finally, several relatively recent review articles proposed the continued utility of neurotrophic factors for treating several neural-related disorders, including AD (Alves et al., 2016; O'Connor and Boulis, 2015), PD (Sullivan and Toulouse, 2011; O'Connor and Boulis, 2015), ALS (Henriques et al., 2010; Genç and Özdinler, 2014; O'Connor and Boulis, 2015), optic nerve degeneration (Shum et al., 2016) and hearing loss (Sun et al., 2011; Fukui and Raphael, 2013), typically using gene therapy as an enabling delivery technology.

Given the continued interest in neurotrophic factors as potential therapeutic agents, along with the continuing discrepancies that exist between the biological effects reported in animals versus the clinical outcomes in humans, it would seem timely and valuable to evaluate the current thinking in the field, along with the empirical data supporting alternative viewpoints. As chronicled in the previous paper (Bartus and Johnson, 2016) many of the scientific advances and clinical trials in the field over the past decades were based on the prevailing assumption that that degenerating diseased human brain is capable of responding to exogenous trophic factors in a manner reasonably comparable to that seen in animal models and that better clinical outcomes would occur if superior delivery and dosing methods were developed and implemented. While changes in dosing paradigms did reduce side effects, they have not yet produced the intended improvements in efficacy (Bartus and Johnson, 2016). Investigators continue to pursue the hypothesis that still-further improvements in dosing will likely provide a sufficient difference in biologic response to neurotrophic factors to produce the desired clinical improvement (Gill, 2014; Bankiewicz, 2014, 2015; Fibiger, 2014), though others have suggested that far different variables, particularly involving the stage of disease deserve much more attention (Olanow et al., 2015; Kordower et al., 2013; Bartus, 2015; Bartus et al., 2015; Sullivan and O'Keeffe, 2016).

2. The primary hypothesis of ongoing clinical trials: the need to further change dosing parameters

The major hypothesis of the two ongoing GDNF trials (NINDS, 2015; http://www.medgenesis.com/news.htm) is that the prior controlled trials with AAV-NRTN (Marks et al., 2010; Olanow et al., 2015) and GDNF protein (Lang et al., 2006) did not produce robust efficacy due inadequate dosing parameters. Specifically, the primary objective of the ongoing studies is to increase brain-target (putaminal) coverage in an effort to improve upon that disappointing efficacy. Among the primary variables that can be manipulated to achieve improvements in dosing are the dose, the volume (or percentage) of target covered by factor and the concentration of factor exposed to neurons in the targeted parenchyma. In the case of the ongoing GDNF gene therapy trial, the vector doses to be delivered and intended volume of expression have been disclosed. These values can be compared to those from the most recent AAV2-NRTN Phase 2b trial, shown in Table 1. The current AAV2-GDNF trial will test a range of ascending doses, which are projected to provide wide-spread putaminal coverage (up to 90%, compared to approximately 30% for the past AAV2-NRTN Phase 2b trial). While all four doses are therefore intended to cover a much larger area of the putamen, the three lower doses will provide levels and concentrations (vg/mm3) of vector and protein that are lower than the prior AAV2-NRTN Phase 2b trial; only highest dose is estimated to provide a concentration of vector and protein roughly equivalent to the previous AAV-NRTN trial. It is not possible to state whether the same concentration of vector in the parenchyma of the covered putamen yields precisely the same concentration of neurotrophic factor from the respective AAV2 vectors, but they are likely comparable. Thus, the ongoing AAV2-GDNF trial will provide a test of the hypothesis that an increase in volume of putaminal coverage in these similar subject populations will provide efficacy not achieved by the lower coverage of the completed AAV2-NRTN trials.

The authors are unaware of any publically disclosed information regarding the doses of GDNF to be used in the ongoing recombinant GDNF infusion trial (http://www.medgenesis.com/news.htm). However, the information provided regarding the trial focuses on the use of enhanced convection to provide increased coverage of the putaminal target as the primary reason for its anticipated success. Therefore, unless very substantial increases in dose are planned to account for the stated goal of increasing putaminal coverage, this trial may actually provide much lower concentrations of GDNF to degenerating nerve terminals, compared to the earlier GDNF infusion studies (Gill et al., 2003; Slevin et al., 2005; Lang et al., 2006) and the AAV2-NRTN studies, but over a presumably larger region of the putamen.

The question of what concentration of protein-expressing-vector, or recombinant protein is required for the desired biological activity is likely important, albeit difficult to answer. A critical question is whether the relationship between the concentration of neurotrophic factor and the magnitude of biological response is comparable between diseased human brain versus normal humans and brains from animal models of disease. Autopsy data from neurotrophic clinical trials in PD and AD provide insight into these questions. For example, Parkinson's patients given AAV2-NRTN (Bartus et al., 2011a, 2015) and recombinant GDNF (Love et al., 2005) consistently show that the only observable biological response to the neurotrophic factor occurs in the relatively small region

Table 1
Past AAV2-NRTN and ongoing AAV2-GDNF Parkinson's trials: absolute doses and relative concentrations of vector in putamen.

Study article	Human dose: putamen (vg)	Vol putamen targeted	Concentration (vg)/mm^3	Efficacy outcome
AAV2-NRTN (Phase 2b)	1.0×10^{12}	30%: 1200 mm^3	8.3×10^{8}	Inadequate
AAV2-GDNF (dose 1)	0.09×10^{12}	90%: 3600 mm^3	0.025×10^{8}	TBD
AAV2-GDNF (dose 2)	0.3×10^{12}	90%: 3600 mm^3	0.83×10^{8}	TBD
AAV2-GDNF (dose 3)	0.9×10^{12}	90%: 3600 mm^3	2.7×10^{8}	TBD
AAV2-GDNF (dose 4)	3.0×10^{12}	90%: 3600 mm^3	8.3×10^{8}	TBD

Three dosing parameters important to gene therapy studies are listed: (1) dose level (vg: vector genomes, which directly impacts the amount of neurotrophic factor expressed), (2) volume of putamen targeted (and therefore the percent of putamen likely exposed to neurotrophic factor), and (3) concentration of vector achieved within localized target area (which affects both the number of vg copies per cell and the amount of neurotrophic factor exposed to the targeted neuronal terminals).
The AAV2-NRTN dose listed was used in the most recent, double-blind trial (i.e., the Phase 2b CERE-120 trial; Olanow et al., 2015). The volume of putamen targeted was derived from the 18% coverage empirically determined from autopsy tissue (Bartus et al., 2015) from four subjects enrolled into the prior AAV2-NRTN Phase 2a trial (Marks et al., 2010). It was then estimated that the 300% increase in dose (between that tested in the initial the Phase 2a versus the more recent Phase 2b trial) would minimally produce a 66% increase in putaminal coverage.
The AAV2-GDNF values were based on information obtained from the most recent update posted on Clinical Trials.Gov as well as information and projections provided in oral presentations on that trial (Bankiewicz, 2014, 2015).
For both AAV2-GDNF and AAV2-NRTN, estimates of vector (and therefore corresponding transgene) concentrations were achieved by accounting for the dose delivered and the projected volume of distribution; i.e., concentration (vg/cm^3) equals dose delivered (vg) divided by volume of expression (cm^3).