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A phase1 study of stereotactic gene delivery of AAV2-NGF for Alzheimer's disease

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Abstract

Background: Nerve growth factor (NGF) is an endogenous neurotrophic-factor protein with the potential to restore function and to protect degenerating cholinergic neurons in Alzheimer's disease (AD), but safe and effective delivery has proved unsuccessful.

Methods: Gene transfer, combined with stereotactic surgery, offers a potential means to solve the long-standing delivery obstacles. An open-label clinical trial evaluated the safety and tolerability, and initial efficacy of three ascending doses of the genetically engineered gene-therapy vector adeno-associated virus serotype 2 delivering NGF (AAV2-NGF [CERE-110]). Ten subjects with AD received bilateral AAV2-NGF stereotactically into the nucleus basalis of Meynert.

Results: AAV2-NGF was safe and well-tolerated for 2 years. Positron emission tomographic imaging and neuropsychological testing showed no evidence of accelerated decline. Brain autopsy tissue confirmed long-term, targeted, gene-mediated NGF expression and bioactivity.

Conclusions: This trial provides important evidence that bilateral stereotactic administration of AAV2-NGF to the nucleus basalis of Meynert is feasible, well-tolerated, and able to produce long-term, biologically active NGF expression, supporting the initiation of an ongoing multicenter, double-blind, sham-surgery-controlled trial.

Keywords: Neurotrophic factors; Translational R&D; Gene therapy; Neuroprotection; Neurorestoration; Nucleus basalis of Meynert; Cholinergic neurons; Nerve growth factor

1. Introduction

Alzheimer's disease (AD) continues to represent a significant and serious unmet medical need. Cholinesterase inhibitors are the primary treatment option for patients with AD, although their effects are merely palliative, providing modest symptomatic improvement for some, but not all, patients. Moreover, no treatment to date has slowed the rate of disease progression of AD.

Nerve growth factor (NGF) is an endogenous neurotrophic factor that, based on extensive research involving a

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Conflicts of Interest: R.T. Bartus, T.L. Baumann, C.D. Herzog and J.M. Ostrove are consultants to Sangamo Biosciences, the company that acquired Ceregene, including all rights to AAV2-NGF (CERE-110). All other authors have nothing to disclose.

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wide range of animal models [1–13], is able to repair and restore function of basal forebrain cholinergic neurons—the same population of neurons the dysfunction of which has been linked to early cognitive impairment in AD [14–24], and on which the cholinesterase inhibitors exert their modest therapeutic effects. However, it was recognized during the early development of cholinesterase inhibitors that significant, dose-limiting toxicity occurs, compromising their potential efficacy to avoid unacceptable side effects [16,19]. More than two decades after their initial testing, this limitation has still not been resolved. Thus, a treatment such as NGF that, unlike cholinesterase inhibitors, improves selectively the function of the cholinergic basal forebrain neurons only, while also protecting them from further degeneration, would represent a major advance in the treatment of AD. Although attempts were made long ago to deliver NGF to patients with AD, sustained protein delivery targeted specifically to these neurons proved impractical [25,26]. This difficulty has since been observed similarly when other neurotrophic factors were administered to patients with Parkinson's disease [27–30]. Thus, many investigators in the field have concluded that if the therapeutic potential of neurotrophic factors like NGF is ever to be realized, more innovative and effective delivery methods must be developed [27,29–33].

During the past decade, gene transfer has emerged as a practical means of overcoming all the obstacles associated with delivering recombinant neurotrophic factor protein to the brain, thus possibly providing the "enabling" technology required for translating the use of these proteins into viable biotherapeutics for human neurodegenerative diseases (for comprehensive reviews, see [33,34]). Rather than attempting to deliver the large, three-dimensionally complex protein exogenously, the gene for the protein is delivered to the targeted site using a safe viral vector, thereby inducing local cells to manufacture and secrete the protein through their endogenous systems, potentially for the lifetime of the individual. Gene transfer is now able to provide controlled, predictable, and long-lasting biologically active protein to specific targeted brain sites (via stereotactic surgery) in a safe and effective fashion [35].

AAV2-NGF (CERE-110) is a gene transfer construct that was bioengineered and developed to deliver NGF to the degenerating cholinergic neurons of the nucleus basalis of Meynert (NBM) in AD. The NBM was chosen as the anatomic target because it is comprised of densely packed cholinergic neurons and is the major source of cholinergic input to the entire neocortex. AAV2-NGF is delivered by direct stereotactic injection in the NBM, programming those neurons genetically to express NGF continuously for their lifetime (for a video illustration, see http://www.adcs.org/studies/ngf.aspx). Although the reliance on standard stereotactic surgical techniques requires a paradigm shift from the way AD has been treated traditionally, the magnitude of the problems associated with AD, in combination with the lack of any other robustly effective treatment, demands that truly innovative approaches be given serious consideration. In fact, neurosurgical approaches for treating many other brain diseases (e.g., glioma, epilepsy, and Parkinson's disease) have become fairly common [36], and the prognosis of AD is no less tragic than those. The collective characteristics of safe, long-term, biologically active protein expression that can be targeted to specific brain sites for life after a single surgical procedure argues that the approach may be practical despite the surgical requirement. Thus, establishing the safety and feasibility of a surgically based NGF therapeutic approach would represent an important milestone in advancing the treatment for AD, and an essential prerequisite to performing a larger, multicenter, randomized controlled trial required for testing possible efficacy. Herein, we report results from 10 patients with mild to moderate AD who were treated with AAV2-NGF, providing evidence for the safety and tolerability of the procedure for at least 2 years posttreatment, and also providing preliminary evidence for persistent expression and bioactivity of NGF in autopsy tissue and the possibility of a delayed loss of neuronal function via fluorodeoxyglucose (FDG)–positron emission tomographic (PET) imaging.

2. Materials and methods

2.1. AAV2-NGF viral vector

AAV2-NGF is an adeno-associated serotype 2 viral vector engineered genetically to express only human NGF. The design and production has been described in detail previously [37]. Briefly, the genome consists of AAV2 inverted terminal repeats flanking an NGF expression cassette containing a CAG promoter, the human NGF complementary DNA, and a human growth hormone polyadenylation signal. Amino acid and DNA sequencing confirmed that only the intact NGF prepro sequence is expressed. Viral vectors are formulated in 2 mM magnesium chloride, in 1× phosphate-buffered saline (PBS; formulation buffer), stored frozen in sterile vials, from which vector can be removed by syringe after thawing.

2.2. Participants

The trial was registered at clinicaltrials.gov (NCT00087789) and conducted over a 6-year period. The study was approved by the human subjects review committees of Rush University Medical Center and the University of California at San Diego, where the clinical study took place, and was reviewed by the Food and Drug Administration and the National Institutes of Health Recombinant DNA Advisory Committee. Written informed consent was obtained from all participants. Participants were deemed capable of providing informed consent if, on