SCIENTIFIC ABSTRACT

Alzheimer's disease is a neurodegenerative disorder characterized by gradual cognitive decline, followed by loss of basic neural functions, and eventually death. Among the neuronal populations that degenerate and die in Alzheimer's disease are the basal forebrain cholinergic neurons (BFCNs) of the nucleus basalis of Meynert (NBM). The loss of the NBM cholinergic neurons may underlie a significant component of the pathogenesis of mild to moderate Alzheimer's disease. In particular, the degree of loss of BFCN cholinergic activity has been correlated with the severity of dementia in Alzheimer's disease. Pharmacological blockade of cholinergic transmission has been shown to impair cognitive functions in both animals and humans, and conversely, augmentation of cholinergic transmission with cholinesterase inhibitors partially restores cognitive function in Alzheimer's disease patients. Thus it is logical to expect that both protecting against the loss of BFCNs and enhancing the vitality of remaining BFCNs may improve cognitive function and/or reduce the rate of cognitive deterioration in Alzheimer's disease.

It is well established that the neurotrophin (β-nerve growth factor (NGF) can protect BFCNs against and reverse the effects of a number of degenerative stimuli including axotomy and natural aging. Numerous published non-clinical studies with rats and monkeys have shown that NGF protects BFCN cell number and cell size from fimbria-fornix lesion. Similarly, the loss of choline acetyltranferase expression in BFCNs, the shrinkage of BFCNs, and the cognitive decline that occur in aged rats and monkeys can all be reversed by NGF administration. NGF should exert the same trophic influence on degenerating BFCNs in subjects with Alzheimer's disease. Therefore, NGF might be expected to reverse or slow the neurodegeneration as well as the cognitive decline characteristic of Alzheimer's disease. However, to date, the challenges associated with delivery of NGF to the BFCNs in humans have precluded meaningful clinical examination of this possibility.

Since NGF is a protein, it does not readily cross the blood brain barrier, and therefore it cannot effectively access central nervous system (CNS) targets, such as the NBM, when delivered systemically. When the blood brain barrier was bypassed in a clinical trial using infusion of NGF into the CNS ventricular system, adverse effects arose. Since then, it has been established that the adverse effects of NGF are specific to ventricular administration and can be avoided by direct administration to the brain parenchyma. Therefore, for the clinical trial in subjects with Alzheimer's, targeted delivery of NGF to the NBM is required.

The ability to have NGF produced in situ, precisely at the target BFCN population, can be achieved by gene transfer following administration via a single neurosurgical procedure. Although there are many methods of gene transfer being developed, adeno-associated virus (AAV)-based vectors are arguably the safest and most effective means currently available for gene delivery to the brain. AAV-based vectors have been and continue to be tested in clinical trials, including several utilizing gene transfer to the brain. In non-clinical studies, AAV-based vectors have been shown to effectively deliver NGF to both protect BFCNs from fimbria-fornix lesion and reverse the effects of natural aging. Thus, delivery of NGF to the BFCNs could be an effective treatment for Alzheimer's disease, and AAV-based gene transfer may represent a safe and effective method for delivering NGF to the BFCNs.

CERE-110 is an AAV serotype 2 (AAV2)-based gene transfer vector for the delivery of human NGF. It consists of an AAV2 capsid carrying a single stranded DNA molecule (vector genome), which includes AAV2 inverted terminal repeats (ITRs) and an NGF expression cassette. No genes encoding viral proteins are included in the vector genome. The NGF expression cassette consists of the 'CAG' promoter and enhancer sequence, the full-length human NGF cDNA, and the human growth hormone polyadenylation signal. The two ITRs flank the NGF expression cassette and are required for proper vector DNA replication and packaging in CERE-110 production. Non-clinical efficacy studies in rats have confirmed that, like other methods of NGF delivery, CERE-110 both protects BFCNs from fimbria-

fornix lesion and reverses the effects of natural aging on BFCNs. Additionally non-clinical safety studies in rats have shown that CERE-110 is well tolerated when administered to the NBM. Non-clinical studies in monkeys have confirmed both the biological activity and safety of CERE-110 administered to the NBM.

The proposed clinical trial, "*A Phase I/II Dose-Escalating, Randomized, and Controlled Study to Assess the Safety, Tolerability, and Efficacy of CERE-110 [Adeno-Associated Virus-Based Gene Transfer Vector for the Delivery of beta-Nerve Growth Factor (NGF)] in Subjects with Mild to Moderate Alzheimer's Disease*" will investigate the safety and efficacy of CERE-110-mediated gene transfer to, and consequent expression of NGF in, the region of the basal forebrain containing the NBM in subjects with mild to moderate Alzheimer's disease. The objective of the open-label Phase I portion of the trial is to determine the safety profile of each of two doses of CERE-110. In the first dose cohort, $2x10^9$ vector genomes (vg) of CERE-110 will be surgically delivered to each of two sites in the basal forebrain of each of the two brain hemispheres. Thus, between the four injections, each subject will receive a total of $8x10^9$ vg of CERE-110. Three subjects will be administered this dose, and subjects will be staggered by four weeks to allow for safety observation in each subject prior to dosing the next. Stopping rules will be based upon the Dose-Limiting Toxicity (DLT) guidelines. If there has been no DLT, and the safety profile of the drug is favorable, a second cohort of subjects will receive a five-fold higher dose. Each subject in this second cohort will receive a total dose of $4x10^{10}$ vg of CERE-110, also divided equally into four discrete injections; surgery will be timed in the same staggered fashion as that of the first cohort. The potential for dose de-escalation exists, and additional subjects may receive the lower dose as necessary. All subjects will be closely monitored at regular intervals during the study.

The objectives of the double-blind, randomized, and controlled Phase II portion of the trial are to assess the efficacy as well as safety of CERE-110 when delivered bilaterally to the basal forebrain of subjects with mild to moderate Alzheimer's disease, as compared to control subjects receiving a sham surgical procedure. Thirty subjects (20 CERE-110; 10 control) will be randomly assigned to receive either sham surgery (which will not involve breach of the dura matter) or CERE-110 at the highest safe dose, as determined from the Phase I portion of the study, in a double-blind fashion. The primary measure of CERE-110 efficacy will be the change in Alzheimer's Disease Assessment Scale-Cognitive Score (ADAS-COG) over time as compared to control. Safety will be closely monitored. Secondary study objectives will be to further evaluate preliminary efficacy using other measures, and to assess the distribution of, and potential immune response to, both CERE-110 and NGF.

Both portions of the study will include additional evaluation of functional outcomes as measured by the following assessments: the Mini-Mental State Evaluation (MMSE), the Instrumental Activities of Daily Living (ADCS-ADL), the Clinical Dementia Rating Scale (CDR), the Clinical Global Impression of Change (ADAS-CGIC), the Beck Depression Inventory (Beck), the Hamilton Depression Scale, and the Neuropsychiatric Inventory (NPI). To assess effects of CERE-110 on brain structure and function, subjects will undergo positron emission tomography (PET) scanning and magnetic resonance imaging (MRI) periodically following the procedure.

Subjects will be aged between 50 and 80 years old and able to provide informed consent. The primary enrollment criteria will be a diagnosis of mild to moderate Alzheimer's disease as determined by the NINCDS/ADRDA criteria and an MMSE score in the range of 16 to 24, reflecting mild to moderate dementia. Subjects will have no other significant neurological or medical abnormalities contraindicating surgery or study participation. The duration of the study will be 24 months, and after completion, each subject will undergo annual clinical evaluation.