Ex vivo gene therapy and Parkinson’s Disease

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ND13.300
In Vivo Gene Therapy

- the genetic material is transferred directly into cells within a patient
- the process is direct and involves less manipulation
- may also be the only viable option in tissues where individual cells cannot be cultured in sufficient quantities or the culture cells cannot be re-implanted.
Ex Vivo Gene Therapy

- the genetic material is first inserted into cells grown in vitro
- the transfected cells are then selected, expanded, and introduced into a patient
- autologous cells (obtained from the same patient prior to the procedure) are normally used
In Vivo and Ex Vivo Gene Therapy

Cloned gene X

Gene transfer

Patient cells $X^-$

Some cells now $X^+$

Select $X^+$ cells

Amplify

$X^+$ cells

Cells removed

Return genetically modified cells to patient
Stable Transmission of Integrated Genes to Daughter Cells

Cloned Gene → Porous nuclear envelope

Integrated gene → Cell Division
Episomal gene
Episomal Transgene

Advantage:
- long-term effect of the transgene and insertional mutagenesis are non-issues

Disadvantage:
- transient expression
- repeated treatments may be necessary
Integrated Transgene

Advantage:
- perpetual
- may provide a stable expression and a cure

Disadvantage:
- random insertion may lead to silencing of the transgene or inactivation or dysregulated activation of host genes
- unknown, long-term effect of the transgene
Adenoviral Vectors

Favorable features:

- causes benign infection
- safety, lack of association with oncogenicity
- well characterized and easily manipulated
- stability and high titers of recombinant vectors
- ability to infect a broad range of cell types, including dividing and nondividing cells
- high efficiency of cellular uptake
- large insert capacity (up to 37 kbp)
- little risk of random chromosomal integration
Adeno-Associated Viruses (AAVs)

- a group of small DNA viruses which usually requires adenovirus or herpesvirus as a helper to replicate efficiently
- a non-pathogenic human parvovirus that is widespread in the human population (about 80% is seropositive)
- in the absence of co-infection by a helper virus, unmodified AAV usually integrates stably at a high frequency into a specific site on chromosome 19q13.3-qter (AAVS1)
- subsequent superinfection with an adenovirus or herpesvirus can activate the integrated virus DNA, resulting in progeny virions
Retroviral Vectors

Favorable features:
- well characterized and easily manipulated
- stability of recombinant vectors
- can be pseudotyped to infect a broad range of cell types
- high efficiency of gene transfer
- stable and precise integration of the transgene
- low immunogenicity
Lentiviral vectors

Favorable features:
• Infects quiescent (non-dividing) cells efficiently
• Extended expression
• Integration
• Efficiently infects cell *in vivo*
In vivo gene therapy:

GDNF in Parkinson’s Disease
Towards a neuroprotective gene therapy for Parkinson’s disease: use of adenovirus, AAV and lentivirus vectors for gene transfer of GDNF to the nigrostriatal system in the rat Parkinson model

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<table>
<thead>
<tr>
<th>Vector</th>
<th>Host [Ref.]</th>
<th>Injection volume (μl)</th>
<th>Site of injection</th>
<th>GDNF content in striatum (ng/mg tissue)</th>
<th>GDNF content in substantia nigra (ng/mg tissue)</th>
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<tbody>
<tr>
<td>Adenovirus</td>
<td>Rat [13]</td>
<td>2</td>
<td>str (one site)</td>
<td>1–2*</td>
<td>ND</td>
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<tr>
<td></td>
<td>2</td>
<td></td>
<td>sn (one site)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monkey [9]</td>
<td>30</td>
<td></td>
<td>str (one site)</td>
<td>0.09*</td>
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<tr>
<td>rAAV</td>
<td>Rat [34]</td>
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<td>str (three sites)</td>
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<td>0.1</td>
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<tr>
<td></td>
<td>Rat [34]</td>
<td>2</td>
<td>sn (two sites)</td>
<td>1.58</td>
<td>1.41</td>
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<tr>
<td></td>
<td>Rat [45]</td>
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<td>ND</td>
<td>0.2–0.6*</td>
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<tr>
<td>Lentivirus</td>
<td>Rat</td>
<td>3</td>
<td>str (three sites)</td>
<td>2.3–6.3</td>
<td>0.2–0.9</td>
</tr>
<tr>
<td>Mouse [4]</td>
<td>1</td>
<td></td>
<td>sn (one site)</td>
<td>ND</td>
<td>0.5–0.6*</td>
</tr>
<tr>
<td>Monkey [37]</td>
<td>45</td>
<td></td>
<td>str (five sites)</td>
<td>0.22–0.35*</td>
<td>ND</td>
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Reading #2: Bjorklund et al.,
Fig. 5. Over-expression of GDNF in the striatum by intrastratal injections of the AAV-GDNF vector can block dopamine neuron degeneration induced by the intrastratal 6-OHDA lesion, as illustrated in B and C. Importantly, the axons along the nigrostriatal pathway are partially preserved. These rescued axons provide the substrate for sprouting and regrowth into the area of high GDNF expression (indicated by green color in C), which in turn is accompanied by a gradual functional recovery (as illustrated in Fig. 4). Injection of the vector into the substantia nigra (D), by contrast, protects the nigral cell bodies but is unable to preserve the axonal projection to the striatum. Instead, there is extensive local sprouting of TH-positive fibers in regions close to the rescued cell bodies, i.e. into the area of high GDNF expression (green color in D). (Based on data from Ref [34].)
Fig. 7. The ability of GDNF to preserve a functional nigrostriatal projection in the rat PD model depends both on the site of administration and the timing relative to the toxin injection, as revealed in experiments using injections or infusion of the recombinant protein. GDNF is most effective when given prior to or shortly after the toxic insult. (Based on data from Refs. [33,60] and Kirik et al., unpublished data.)
Fig. 6. Immunohistochemical visualization of the GDNF protein in an animal receiving injections of the LV-GDNF vector (3×1 μl) in the striatum. 10 weeks survived. Similar to the results obtained with the AAV4-GDNF vector (Fig. 3), the transduced GDNF is widely distributed throughout the striatum (STR, A–C) and along the axons of the striatal projection neurons to the globus pallidus (GP, D–F), substantia nigra pars reticulata (SN, J–L). The level of GDNF, as measured by ELISA, was 4–6 ng/mg tissue in the striatum and 1–2 ng/mg in the substantia nigra (see Table 1), showing that the factor is efficiently distributed throughout the nigrostriatal system (Giorgiopolski et al., unpublished data).
Ex vivo gene therapy:

GDNF in Parkinson’s Disease
Early transplantation of an encapsulated glial cell line–derived neurotrophic factor–producing cell demonstrating strong neuroprotective effects in a rat model of Parkinson disease

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Department of Neurological Surgery, Okayama University Graduate School of Medicine and Dentistry, Okayama, Japan
Fig. 5. Bar graphs. Upper: Analysis of TH-positive fibers in the striatum performed using computerized image analysis system. Data is shown relative to BHK-GDNF cell line and control.
Ex vivo gene therapy:

NGF in Alzheimer’s Disease
Although the precise pathogenesis of AD is unknown, certain pathological features accompany the disease. These pathological features include the abnormal accumulation of extracellular amyloid, the formation of intraneuronal neurofibrillary tangles, synapse loss, and cellular degeneration. Cellular degeneration occurs in several neuronal populations in the central nervous system. Among the neuronal populations that degenerate in AD, loss of basal forebrain cholinergic neurons is particularly severe. Loss of cholinergic neurons in AD correlates best with severity of dementia, the density of amyloid plaques in the brain, and the amount of synapse.
To date, the only FDA-approved therapies for Alzheimer's Disease focus on augmenting the function of degenerating cholinergic neurons. The present trial will move beyond compensating for cholinergic neuronal degeneration by attempting to 1) protect cholinergic neurons from degeneration, and 2) augment the function of remaining cholinergic neurons by directly elevating choline acetyltransferase (ChAT) function in neurons. These two therapeutic interventions will be brought about by the delivery of human NGF to the brain. NGF has been shown to prevent both lesion-induced and spontaneous, age-related degeneration of basal forebrain cholinergic neurons. Further, NGF infusions reversed both lesion-induced memory loss and spontaneous, age-related memory loss in rodents. Based on these findings, NGF administration offers significant potential as a neuroprotective strategy in Alzheimer's disease. Grafts of primary fibroblasts transduced to express human nerve growth factor have been shown to sustain NGF in vivo gene expression for at least eighteen months in the rodent central nervous system. In addition, these grafts sustain NGF messenger RNA production for at least 14 months in vivo. In primate systems, ex vivo NGF gene therapy has been demonstrated to sustain NGF protein production in the brain in the rhesus monkey for at least one year. Thus, the available data suggests that ex vivo NGF gene therapy is an effective means of preventing loss of basal forebrain cholinergic neurons and of augmenting cholinergic function in the primate brain. In animals, this procedure is safe and well tolerated.
Background

• Based on these data, clinical trials of ex vivo NGF gene therapy in Alzheimer's disease has begun. This is an 18 month, open label, prospective Phase I clinical trial of Ex Vivo Gene Therapy for Alzheimer's disease in 8 patients with a mild degree of cognitive impairment. Patients will be screened for the diagnosis of Probable Alzheimer's disease of mild severity. After obtaining informed consent, three skin biopsies will be obtained to generate cultures of primary, autologous fibroblasts. These cells will be cultured, then genetically modified to produce and secrete the human nerve growth factor (NGF) molecule. If fibroblasts are deemed acceptable based on NGF production rates and standard cell culture sterility tests, then patients will receive intracerebral injections of their own primary fibroblasts into the region of basal forebrain cholinergic neurons in the brain, where neurons are undergoing atrophy as a result of Alzheimer's disease.
Reading #4:
A Phase I Study of Ex Vivo Nerve Growth Factor Gene Therapy for Alzheimer's Disease

Condition:
Alzheimer Disease

Treatment or Intervention:
Gene Transfer: Human Nerve Growth Factor (Phase I)

Purpose
This Phase I clinical trial is the first step in testing gene therapy. This study is called a "Safety/Toxicity" study by the Food and Drug Administration, and primarily aims to determine whether the experimental protocol is safe for humans. It will determine whether the study procedure causes side effects in humans, and may also give us a preliminary sense of whether this will be effective in combating Alzheimer's disease in humans.
A Phase I Study of Ex Vivo Nerve Growth Factor Gene Therapy for Alzheimer's Disease

Principal Investigator: Marc Tuszynski, PhD, UCLA
Sponsors and Collaborators: The Shiley Family Trust Institute for the Study of Aging University of California, San Diego
Information provided by: National Institute on Aging (NIA)
Study Type: Interventional
Study Design: Treatment, Open Label, Uncontrolled, Safety Study
Expected Total Enrollment: 8
Study completion: November 2003

Ages Eligible for Study: 50 Years and above
Genders Eligible for Study: Both
Criteria
- Neurologist certified diagnosis of probable Alzheimer's disease
- Early stage of Alzheimer's disease (generally within three years of onset)
- Normal speaking ability and normal ability to understand
- Ability to understand the potential risks of participation in this study
- Willing to visit the San Diego area and be available for many visits in the first year
- Willing to discontinue use of drugs Cognex, Aricept, Exelon, or Reminyl for the first 18 months of the trial
Eligibility Criteria

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Further Study Details

- Although the precise pathogenesis of AD is unknown, certain pathological features accompany the disease. These pathological features include the abnormal accumulation of extracellular amyloid, the formation of intraneuronal neurofibrillary tangles, synapse loss, and cellular degeneration. Cellular degeneration occurs in several neuronal populations in the central nervous system. Among the neuronal populations that degenerate in AD, loss of basal forebrain cholinergic neurons is particularly severe. Loss of cholinergic neurons in AD correlates best with severity of dementia, the density of amyloid plaques in the brain, and the amount of synapse. To date, the only FDA-approved therapies for Alzheimer's Disease focus on augmenting the function of degenerating cholinergic neurons. The present trial will move beyond compensating for cholinergic neuronal degeneration by attempting to 1) protect cholinergic neurons from degeneration, and 2) augment the function of remaining cholinergic neurons by directly elevating choline acetyltransferase (ChAT) function in neurons. These two therapeutic interventions will be brought about by the delivery of human NGF to the brain. NGF has been shown to prevent both lesion-induced and spontaneous, age-related degeneration of basal forebrain cholinergic neurons. Further, NGF infusions reversed both lesion-induced memory loss and spontaneous, age-related memory loss in rodents. Based on these findings, NGF administration offers significant potential as a neuroprotective strategy in Alzheimer's disease. Grafts of primary fibroblasts transduced to express human nerve growth factor have been shown to sustain NGF in vivo gene expression for at least eighteen months in the rodent central nervous system. In addition, these grafts sustain NGF messenger RNA production for at least 14 months in vivo. In primate systems, ex vivo NGF gene therapy has been demonstrated to sustain NGF protein production in the brain in the rhesus money for at least one year. Thus, the available data suggests that ex vivo NGF gene therapy is an effective means of preventing loss of basal forebrain cholinergic neurons and of augmenting cholinergic function in the primate brain. In animals, this procedure is safe and well tolerated. Based on these data, clinical trials of ex vivo NGF gene therapy in Alzheimer's disease has begun. This is an 18 month, open label, prospective Phase I clinical trial of Ex Vivo Gene Therapy for Alzheimer's disease in 8 patients with a mild degree of cognitive impairment. Patients will be screened for the diagnosis of Probable Alzheimer's disease of mild severity. After obtaining informed consent, three skin biopsies will be obtained to generate cultures of primary, autologous fibroblasts. These cells will be cultured, then genetically modified to produce and secrete the human nerve growth factor (NGF) molecule. If fibroblasts are deemed acceptable based on NGF production rates and standard cell culture sterility tests, then patients will receive intracerebral injections of their own primary fibroblasts into the region of basal forebrain cholinergic neurons in the brain, where neurons are undergoing atrophy as a result of Alzheimer's disease.
Stem cells in PD
The promise of stem cells in Parkinson disease

J. William Langston

Parkinson's Institute, Sunnyvale, California, USA.
Dopaminergic neurons generated from monkey embryonic stem cells function in a Parkinson primate model

Yasushi Takagi,1,2 Jun Takahashi,1 Hidemoto Saiki,3 Asuka Morizane,1 Takuya Hayashi,4 Yo Kishi,1 Hitoshi Fukuda,1 Yo Okamoto,1 Masaomi Koyanagi,1 Makoto Ileguchi,1 Hideki Hayashi,1 Takayuki Imazato,1 Hiroshi Kawasaki,5 Hirofumi Suemori,6 Shigeki Omachi,7 Hidehiko Iida,8 Nobuyuki Itoh,7 Norio Nakatsuji,6 Yoshiki Sasai,2,6 and Nobuo Hashimoto1

Key points:
Figure 1
Neural progenitors induced from primate ES cells. (A) Time course of neural progenitor marker expression in monkey ES cells cultured on PA6 cells. (B) Detached ES cell colonies formed spheres similar to those of neural progenitor cells. (C–E) Spheres were immunoreactive for NCAM (C, green), Musashi-1 (D, red), and Nestin (E, green). Scale bar: 100 μm.
Figure 2
Expression of differentiated neural and neuronal subtype markers. Differentiated spheres were stained with antibodies against TuJ1 (A and B, green; E–H, blue), GFAP (B, red), Map2ab (C and D, green), GalC (D, red), GABA (E, red), glutamate (Glu; F, green), serotonin (Ser; G, red), and ChAT (H, green). Scale bar: 100 µm. The proportions of cells expressing differentiated neural (I) and neurotransmitter-related (J) markers are expressed as the mean ± SD of 3 independent cultures.
Figure 3
DA neurons differentiated from ES cell-derived neurospheres. (A–D) Differentiated spheres treated with FGF2 and FGF20 were stained with antibodies against TH (red) and TuJ1 (green). Scale bars: 50 μm. (E) The proportion of TH-positive cells to TuJ1-positive cells is expressed as the mean ± SD of 3 to 5 independent cultures. *P < 0.05. (F) RT-PCR for mesen-
Table 1  
Neurological scores of MPTP-treated monkeys

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alertness</td>
<td>Normal, 0; reduced, 1; absent, 2</td>
</tr>
<tr>
<td>Head-checking movement</td>
<td>Present, 0; reduced, 1; absent, 2</td>
</tr>
<tr>
<td>Eyes</td>
<td>Normal, 0; reduced blinking, 1; eyes closed, 2</td>
</tr>
<tr>
<td>Posture</td>
<td>Normal, 0; mildly abnormal, 1; abnormal, 2; grossly abnormal, 3</td>
</tr>
<tr>
<td>Balance</td>
<td>Normal, 0; impaired, 1; frequent falling, 2; no movement, 3</td>
</tr>
<tr>
<td>Motility, at rest</td>
<td>Normal, 0; mild bradykinesia, 1; bradykinesia, 2; akinesia, 3</td>
</tr>
<tr>
<td>Motility, reaction to external stimuli</td>
<td>Normal, 0; mildly reduced, 1; reduced, 2; absent, 3</td>
</tr>
<tr>
<td>Walking</td>
<td>Normal, 0; mildly reduced, 1; reduced, 2; no walking, 3</td>
</tr>
<tr>
<td>Tremor</td>
<td>Absent, 0; mild/not always, 1; moderate, 2; severe, 3</td>
</tr>
</tbody>
</table>

**Figure 4**  
Function of ES cell–derived neurospheres in MPTP-treated monkeys. Behavioral scores (A) and PET study (B and C) of ES cell–transplanted (n = 6) and sham-operated animals (n = 4). (B) Mean Ki values from entire putamen. (C) Increased 18F-fluorodopa uptake in the putamen of ES cell–transplanted animals. All values are mean ± SD. *P < 0.05.
Figure 5
Survival of ES cell–derived DA neurons in the striatum. (A–C) Grafted cells (BrdU-labeled, green) survived and differentiated into DA neurons (TH-positive, red) along the needle tract (merged image C). Scale bar: 500 μm. (D–I) Colocalization (arrows in F and I) of BrdU (D, F, G, and I, green) and TH (E and F, red) or DAT (H and I, red) shows that graft-derived cells have dopaminergic character. Scale bar: 50 μm.
Ex vivo gene therapy: BDNF in spinal cord injury
BDNF-expressing marrow stromal cells support extensive axonal growth at sites of spinal cord injury

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\textsuperscript{b}Veterans Administration Medical Center, San Diego, CA 92165, USA
Fig. 10. Functional outcomes. No significant functional recovery is observed following grafting of MSC-GFP and MSC-BDNF-GFP cells into cervical dorsal column lesion sites on (A) a rope-walking test and (B) a tape-removal test.