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# Encapsulated Cell Biodelivery of Nerve Growth Factor to the Basal Forebrain in Patients with Alzheimer's Disease

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**Key Words**

Nerve growth factor · Alzheimer's disease · Basal forebrain · Neurosurgery · Encapsulated cell biodelivery

**Abstract**

***Background/Aims:*** Degeneration of cholinergic neurons in the basal forebrain correlates with cognitive decline in patients with Alzheimer's disease (AD). Targeted delivery of exogenous nerve growth factor (NGF) has emerged as a potential AD therapy due to its regenerative effects on the basal forebrain cholinergic neurons in AD animal models. Here we report the results of a first-in-man study of encapsulated cell (EC) biodelivery of NGF to the basal forebrain of AD patients with the primary objective to explore safety and tolerability. ***Methods:*** This was an open-label, 12-month study in 6 AD patients. Patients were implanted stereotactically with EC-NGF biodelivery devices targeting the basal forebrain. Patients were monitored with respect to safety, tolerability, disease progression and implant functionality. ***Results:*** All patients were implanted successfully with bilateral single or double implants without complications or signs of toxicity. No adverse events were related to NGF or the device. All patients completed the study, including removal of implants at 12 months. Positive findings in cognition, EEG and nicotinic receptor binding in 2 of 6 patients were detected. ***Conclusions:*** This study demonstrates that surgical implantation and removal of EC-NGF biodelivery to the basal forebrain in AD patients is safe, well tolerated and feasible.



## Introduction

Alzheimer's disease (AD) is a progressive disorder with a successive decline in cognitive functions. One early symptom is episodic memory loss, where episodic memory tests can be useful early in the disease process [1]. The progressive memory loss in AD patients is associated with neurodegeneration and dysfunction of the central cholinergic neurons located in the basal forebrain, wherefrom the cholinergic neurons project to the cerebral cortex and hippocampus [2, 3]. There are several reasons why nerve growth factor (NGF) is being explored as

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a symptomatic and disease-modifying therapeutic agent attenuating the rate of degeneration of basal forebrain neurons in AD. NGF has demonstrated regenerative and neuroprotective effects on basal forebrain cholinergic neurons in animals [4–6]. Endogenous NGF is synthesized by post-synaptic cortical and hippocampal neurons, and signals through the TrkA and p75 receptors located on the cholinergic terminals. The activated receptor complex is then retrogradely transported from the target areas, i.e. the cortex and hippocampus, to the cholinergic nuclei in the basal forebrain [7]. Studies have reported decreased levels of NGF and NGF receptors in the basal forebrain in patients with AD [8, 9], while NGF levels in the hippocampus and cortex are unchanged or increased, supporting the hypothesis that the normal NGF signalling and/or transport in AD is impaired [10]. It has been suggested that a loss of trophic support to cholinergic neurons precedes the loss of the cholinergic phenotype seen in AD [10]. Moreover, studies show that basal forebrain cholinergic neurons are viable, albeit dysfunctional, for an extended period, while these cells die in a late stage of the disease. Thus, these degenerating cells may be amenable to pharmacotherapeutic interventions in early and intermediate stages of the disease [11].

The molecular mechanisms of NGF signalling are not fully understood, but an interesting picture is emerging. The NGF precursor, pro-NGF, is increased in AD brains [12]. In AD, a diminished conversion of the NGF precursor molecule to its mature form as well as an augmented degradation of mature NGF has been shown [13]. Pro-NGF has been shown to bind to the p75 receptor resulting in pro-apoptotic signalling, while NGF binding to the TrkA receptor enhances survival signalling. In AD, there may be an imbalance in the ratio of pro-NGF and NGF suggestive of a shift towards pro-apoptotic signalling. It is also interesting to note that TrkA reduces and p75 activates β-secretase cleavage of the amyloid precursor protein to Aβ, further emphasizing the link to AD [11, 13, 14].

There is no cure for AD and the current drug treatment available today, i.e. cholinesterase inhibitors [15] and memantine [16], is symptomatic. Thus, there is a need for disease-modifying therapies and NGF has emerged as a potential therapeutic candidate. We have previously explored intracerebroventricular infusion of NGF in 3 AD patients and found normalization of the EEG pattern, upregulation of nicotinic receptors and increased glucose metabolism in the brain [17, 18]. However, these patients developed side effects, where spinal pain was the most prominent, making this route of administration non-tolerated and unsafe [18]. Subsequently, we demonstrated that when NGF was infused directly into the brain parenchyma of rats, no pain-related effects were observed while intracerebroventricular administration resulted in pain [19]. Other delivery routes of NGF to the brain, such as nasal administration, have been shown to be well tolerated in mice [20], and development of a recombinant NGF molecule without algogenic effect is in progress [21].

In order to avoid side effects, NGF needs to be delivered locally to targets in the cholinergic basal forebrain. In man, this poses a clinical and technical challenge. Age-related cholinergic decline in primates can be reversed by lentiviral gene delivery of NGF to the basal forebrain [22]. Recently, it was shown in AD patients that local delivery of NGF by implantation of genetically modified, autologous fibroblasts to the basal forebrain is safe, and may even slow the disease progression [23].

However, the in vivo gene therapy approach using viral vectors also suffers from certain limitations and safety concerns, particularly the permanent genetic modification of the patients' brain cells and inability to control or stop the release of bioactive substance. In order to circumvent these problems, we have applied an encapsulated cell technology (EC biodelivery), developed by NsGene A/S, Denmark. The implant is a catheter-like device containing a genetically engineered NGF-secreting human cell line housed behind a semi-permeable hollow fibre membrane at its tip. This encapsulation is immuno-isolatory and protects the transplanted allogeneic cell line from host immune system rejection while nutrients, oxygen and therapeutic products can diffuse freely across the wall of the capsule, keeping the cells functioning in animals for at least 12 months [24–26]. A great advantage over traditional ex vivo or in vivo gene therapy approaches is that the NGF delivery can be stopped by removing the device with the genetically modified NGF-producing cells from the brain. Here we report the results of the first clinical trial in AD patients using encapsulated NGF-producing cells with safety and tolerability as primary outcome measures.

## Patients and Methods

This was a 12-month, open-label, single-centre, dose-escalation phase Ib study of EC biodelivery of NGF to the cholinergic basal forebrain of patients with mild to moderate AD.

*Subjects*

Subjects were recruited from patients referred to the memory clinic at a university hospital. The inclusion criteria were as follows: patients had a probable diagnosis of mild to moderate AD according to the NINCDS-ADRDA criteria [27], they were aged

50–80 years, had a Mini-Mental State Examination (MMSE) [28] score of 15–24, were living in their own homes with a caregiver, had been treated with a stable dose of acetylcholinesterase inhibitors (AChEI) for at least 3 months before enrolment and were to remain on stable AChEI for the study period. Exclusion criteria were ongoing medical and/or psychiatric conditions, antipsychotic drug treatment and smoking (not to interfere with nicotine binding, see below). Stable doses of other baseline medications were maintained.

Prior to the study, patients underwent medical examination according to the clinical routine at the memory clinic, including a medical history and somatic assessment. Computerized brain tomography (CT) or magnetic resonance imaging (MRI) of the brain, psychometric testing of cognition and routine blood sampling were also performed. Based on this information, a diagnosis of AD was established by the clinician. The AD diagnosis was then reaffirmed by a member of the study team (M.E.-J.).

The study was approved by the Swedish Medical Products Agency for 6 patients. Ethical approval was obtained from the regional human ethics committee of Stockholm. Both patient and caregiver gave written informed consent prior to study entry.

*The NGF Biodelivery Device*

The NGF biodelivery device, NsG0202, is a gene therapy medicinal product under the European regulatory classification and a combination product according to the FDA. It consists of NGC0295 cells expressing human NGF (but no pro-NGF [26]) housed in an implantable device. The NGC0295 cells are derived from the ARPE-19 human retinal pigment epithelial cell line (ATCC, USA), transfected and stably modified with the human NGF gene under control of a modified cytomegalovirus promoter containing an intron. The tip of the device (active part) consists of a polyethersulphone hollow fibre membrane (Akzo Membrana, Wuppertal, Germany). This active part is 11 mm long and contains the NGC0295 cells attached to polyvinyl alcohol foam scaffolding. The active part is attached to an inert 1-mm-wide polyurethane tether. The entire catheter-like device is compatible with stereotactic neurosurgical implantation. At implantation, the active part is placed in the cholinergic basal forebrain targets and the inert tether is cut to length and secured at the burr hole level with a titanium plate (for further details, see Wahlberg et al. [29]).

*Implantation and Explantation Procedure*

The device implantation and explantation procedures were performed by the neurosurgical team (B.L., G.L.). The surgical procedure is described in detail elsewhere [29]. Briefly, following baseline assessments, the patients were implanted stereotactically under general anaesthesia. Bilateral single implants were positioned with the active part targeting the nucleus basalis of Meynert (Ch4) of the lateral basal forebrain in the first 3 patients (patients 1–3, first dose cohort). Three months into the study, the collected safety data from these patients were assessed by an independent safety data review board. Based on their assessment, patients 4–6 were approved for inclusion and implantation of bilateral double implants (second dose cohort). Patients 4–6 were implanted with bilateral NsG0202 devices in the Ch4 as well as with bilateral single implants in the vertical limb of the diagonal band (Ch2).

The definition of the anatomical targets was performed based on brain atlas coordinates and modified according to each cranial MRI to fit the individual brain anatomy. The surgery was preceded by a stereotactic cranial MRI examination to localize surgical targets and to define trajectories for implantation. A cranial CT scan was performed immediately after the surgical procedure for safety assessment, and the position of implants was documented by image fusion technique (BrainLAB AG, Feldkirchen, Germany). All patients received postoperative care for 2–3 days at the neurosurgical ward followed by in-patient care for 3–6 days at a geriatric ward, before discharged to go home. At the end of the study, all patients underwent surgical removal of the implants under general anaesthesia.

*Histopathology*

A cortical brain biopsy was obtained at the entry point of the implant (at a gyrus of the frontal lobe, 20 mm anterior to the coronary suture and 30–50 mm lateral to midline) at the start of the procedure. Tissue was immediately fixed in 4% paraformaldehyde solution for 72 h followed by cryostat sectioning. To visualize Aβ and tau histopathology, tissue sections were stained with (a) polyclonal antibodies against Aβ40 and Aβ42 (1:1,000) and (b) antibodies against hyperphosphorylated tau (Innogenetics Br-3, clone AT-8, dilution 1:500), respectively. The reaction product was visualized using the Zymed Lab-SA detection system (Zymed, San Francisco, Calif., USA).

*Monitoring*

All patients were monitored for 12 months and followed up with a total of 13 visits during the study period (in addition to daily in-patient assessments in adjunction with surgery). A clinical evaluation was also performed at 3 and 7 months after the implants were removed.

*Outcome Measures*

The primary objective was to explore the safety and tolerability of NsG0202 in AD patients. Outcome measures included vital signs, concomitant medications, adverse events (AEs), pain (assessed with a visual analogue scale), BMI and haematology/clinical chemistry. An immediate postoperative CT scan and MRI scans at 3 and 12 months after implantation were performed to verify the positions of the implants and to screen for signs of infection, bleeding, oedema or necrosis. Cerebrospinal fluid (CSF) samples, obtained at baseline, and at 3 and 12 months after implantation, were analysed for signs of infection, inflammation and haemorrhage. Assays of NGF (ELISA kit, minimal detection level 31 pg/ml, R&D systems, Minneapolis, Minn., USA) and an ELISA detection of anti-NGF antibodies in serum and CSF were developed and validated by Vecura AB, Stockholm, Sweden according to GLP.

Secondary outcome measures included cognition assessed by clinical rating scales, and EEG, MRI and positron emission tomography (PET) at 3 and 12 months compared with baseline recordings. Cognition was assessed using the MMSE and Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-Cog) [30]. PET imaging was performed at the Uppsala PET Centre. Measurements of regional cerebral blood flow and cerebral nicotine receptor binding were performed using a dual tracer protocol including $^{15}$O-water and (S)(–)-(N-$^{11}$C-methyl) nicotine [31]. A flow-compensated parameter $k_2$* was calculated from the rate constant $k_2$ for nicotine divided by cerebral blood flow [32, 33]. A low $k_2$* value indicates high $^{11}$C-nicotine binding. The glucose metabolism was measured with $^{18}$F-fluoro-2-deoxy-D-glucose.

**Table 1.** Demographic data on included patients

| | Patients | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| Age, years | 63 | 55 | 65 | 57 | 60 | 73 |
| Gender | F | F | M | M | F | F |
| Duration of memory symptoms, years | 6 | 3 | 6 | 1 | 4 | 4 |
| AD diagnosis, years | 2 | 2 | 1 | 1 | 2 | 1 |
| MMSE score at inclusion (0–30) | 23 | 19 | 24 | 21 | 23 | 23 |
| ADAS-Cog score at inclusion (0–85) | 22 | 27 | 27 | 24 | 28 | 34 |
| Duration of AChEI at inclusion, months | 12 | 21 | 8 | 11 | 26 | 12 |

Parametric maps of cerebral glucose metabolism (Patlak images) were created by the Patlak technique using the time course of $^{18}$F-fluoro-2-deoxy-D-glucose in plasma as the input function and a lumped constant of 0.418. EEG activity was recorded in a resting awake condition with eyes closed and under strict vigilance control. Data were acquired on a computer-based system (Nervus, version 5.3.0, Cephalon, Denmark). Data sampling and analysis are described in detail by Jelic et al. [34]. The EEG variables chosen were absolute power in alpha, beta, delta and theta frequency bands. A composite variable in form of a ratio of fast and slow EEG frequencies was chosen to represent overall change in EEG activity, higher values indicating a favourable increase in frequency of dominant posterior EEG activity. CSF samples were aliquoted in polypropylene tubes and stored at –80°C until analysis. Analyses of CSF Aβ1–42, total tau and phosphorylated tau 181 were performed with xMAP technology using the Inno-Bia AlzBio 3 kit (Innogenetics, Gent, Belgium) as described previously [35]. CSF levels of neurofilament light (NFL) and glial fibrillary acidic protein (GFAP) were analysed using previously described ELISA methods [36, 37].

*Statistical Analyses*

Data are presented for each individual since the number of patients limits the use of group statistics. Each patient is his own control. The Statistica® 7.0 software package (Statsoft, Tulsa, Okla., USA) was used for the statistical calculations. Non-parametric statistics (median for basic descriptions) and comparisons using the Wilcoxon matched-pairs test were used comparing baseline data with 3- and 12-month postoperative time points when appropriate. Spearman's rank correlations were used in correlation analyses. A p value of $<0.05$ was deemed significant.

## Results

Six patients, 2 men and 4 women, mean age 62 ± 6 years with a median MMSE score of 23, were enrolled and completed the study. All patients had been treated with AChEI as concomitant medication for a mean duration of 15 ± 7 months at study start (table 1), and were on stable AChEI throughout the study. Three of these patients were on laevothyroxine due to a previous hypothyroid condition and had been euthyroid for several years. One patient was on treatment for a mild asthmatic condition, which was stable throughout the study. The patients were closely monitored for 12 months after implantation, and then followed according to clinical routine. The glucose metabolism pattern of the brain as assessed with PET imaging at baseline was consistent with an AD diagnosis in all 6 patients. The diagnosis of AD was histopathologically confirmed on tissue from cortical biopsies at the implantation site in 5 of the 6 patients. Numerous amyloid-positive plaques and tau-positive tangles were found in all tissue specimens (fig. 1). In 1 patient, the biopsy could not be analysed for technical reasons.

*Safety and Tolerability*

NGF-producing EC biodelivery devices were safely and accurately implanted bilaterally to the basal forebrain of all 6 patients. The mean level of NGF released from each device just prior to implantation was 1.7 ± 0.2 ng NGF/24 h. There were no intra-operative complications. Immediate postoperative cranial CT scans showed the implants in the intended anatomical target positions without adverse radiographic findings. Overall, the postoperative course was uneventful. Five of the 6 patients experienced transient mild headache. One patient developed mild confusion that resolved over the course of 3 days. This patient also developed atrial fibrillation postoperatively which was successfully treated. The surgical scalp incisions healed without complications in all patients.

All patients were carefully monitored for 12 months after implantation. No findings gave rise to specific safety concerns regarding vital signs, ECG, laboratory values or AE profiles. There were no significant decreases in BMI during the trial nor were there any pain responses attributed to the NGF treatment.

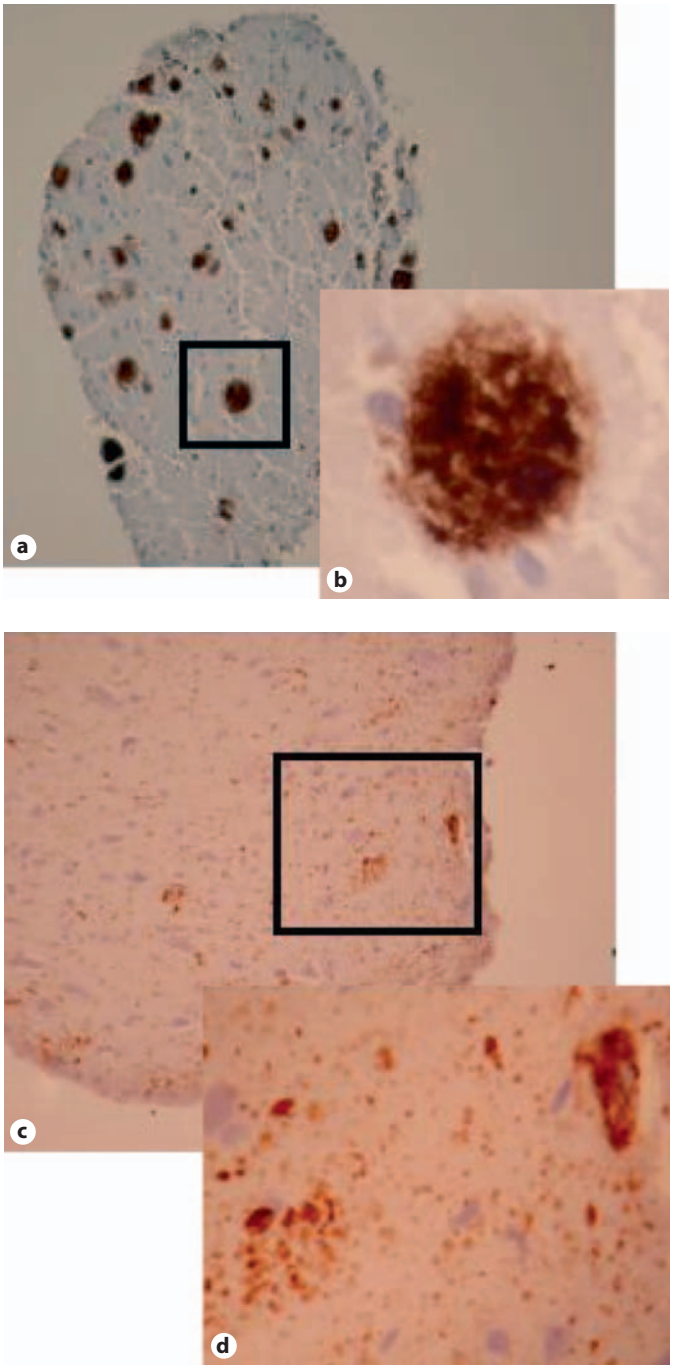


**Fig. 1.** Brain cortical biopsies from 2 patients in the study, obtained at the entry point of the implant at a gyrus of the frontal lobe at the start of the implantation procedure. Several Aβ42-positive plaques are seen in the cortical biopsy (×10) in **a** and enlarged (×40) in **b**. In **c** (×10) and in the enlarged region (×20) in **d**, tau-positive immunoreactivity is found.

Two serious AEs and a total of 57 AEs were reported for the entire study. The 2 serious AEs consisted of 2 hospitalizations of 1 patient with postoperative delirium after elective orthopaedic surgery. The most common AEs were mild headache (n = 11), nausea (n = 7) and nasopharyngitis (n = 6) (table 2). There were 3 AEs of medical importance, a left subacute subdural haematoma in 1 patient, a mild CSF pleocytosis in another patient, and 1 case of subcortical white matter changes on cranial MRI. The left subacute subdural haematoma was incidentally discovered at the 3-month MRI follow-up in 1 patient. The haematoma had a temporo-parieto-occipital distribution with a local mass effect, but without midline shift and did not extend frontally to the 2 implants on the left side. There were no neurological symptoms. The haematoma was conservatively managed and fully resolved within 3 months with the implants in the correct positions (fig. 2). In 1 patient, the analysis of CSF prior to explantation of devices at 12 months showed a CSF pleocytosis of mononuclear cells with negative microbiology. A repeat CSF analysis a week later showed reduced pleocytosis. The results of further analyses suggested that the patient suffered from subclinical borreliosis (Lyme disease), an infectious disease endemic to the area of the patient's residence. The patient remained asymptomatic but received standard antibiotic treatment. Another patient was diagnosed with multiple discrete signal changes in the subcortical white matter including some associated with the tethers of all 4 implants at 12 months. No changes were seen in the brain tissue surrounding the active part of the implants. Review of previous MRI images revealed scattered discrete subcortical white matter changes already present prior to implantation. A follow-up cranial MRI at 3 months after removal of the implants showed partial resolution of these white matter changes along the implant tracts. These findings were similar to asymptomatic white matter changes previously reported with implants for deep brain stimulation [38].

At 12 months, all patients underwent surgical retrieval of the EC biodelivery devices. Devices were easily removed and recovered for analysis. The surgical procedure was safe and without any complications. All patients were discharged the day after surgery. No side effects related to the removal procedure were reported. All 18 implants were retrieved intact with no tissue adherence or haemorrhage. The analysis of NGF release by the 6 EC biodelivery devices in the first dose cohort revealed that the levels of NGF were reduced to 10% of the levels at implantation. Areas of both healthy-appearing nucleated cells and degenerating cells were detected. In the second

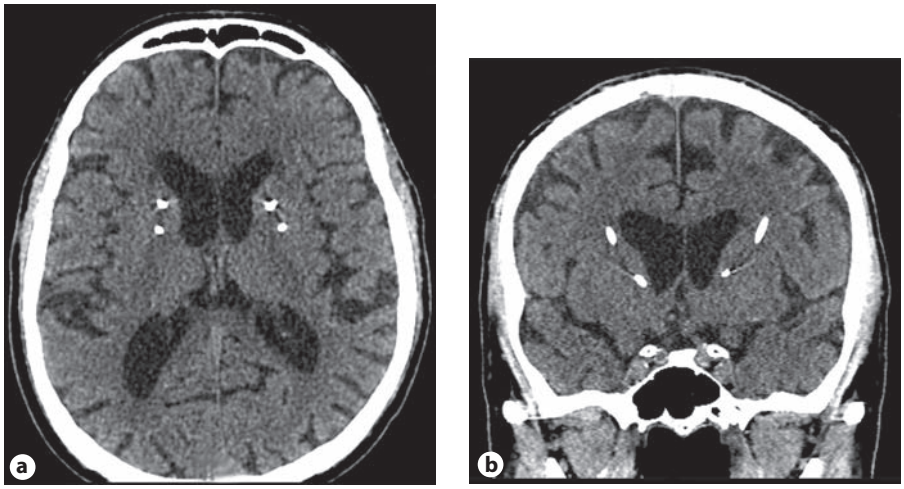

**Fig. 2.** The CT images showing the position and trajectories of the implants on axial (**a**) and coronal (**b**) sections at 6 months after implantation. The barium-impregnated tethers are seen as white dots. The tip of the tethers with the EC is not barium-impregnated and thus not visible on CT scan.

cohort at explantation, detectable levels of NGF in 2 of 12 devices were found and only degenerating cells were seen on histology.

*Cognition, PET and EEG*

Median MMSE scores decreased and median ADAS-Cog scores increased at 3 and 12 months as compared with baseline but did not reach statistical significance. Two patients (patient 1 in cohort 1 and patient 6 in cohort 2) showed improved MMSE and ADAS-Cog scores following implantation (table 3; fig. 3a). After 2 years of progressive cognitive decline prior to implantation, patient 1 showed an increased MMSE score at 3 months after implantation, which remained stable during the implant duration, and returned to the preoperative MMSE score at 3 months after removal of implants (fig. 4). Patients 1 and 6 also showed increased cortical $^{11}$C-nicotine binding as assessed by PET imaging at 3 and 12 months compared to baseline (table 3; fig. 3b). A significant negative correlation between change in ADAS-Cog scores (but not with MMSE scores) and change in $^{11}$C-nicotine binding at 12 months compared with baseline was seen ($r = -0.84$, $p < 0.05$). In patient 1, unchanged cerebral glucose metabolism, and fast to slow ratio in EEG activity at 12 months compared to baseline were found (table 3).

*CSF Analyses*

No anti-NGF antibodies were detected in serum or in CSF in any of the patients at 3 or 12 months. The NGF levels in CSF were below detection limit of the assay at baseline, 3 and 12 months.

Compared with baseline levels, NFL in CSF increased significantly at 3 months ($p < 0.03$) in all patients but re-

**Table 2.** Number of reported AEs during the study

| Adverse events | Cohort | | Total number |
|---|---|---|---|
| | 1 | 2 | |
| Abnormal clinical chemistry findings[a] | 3 | 1 | 4 |
| Confusion | 1 | 1 | 2 |
| Contusion chest | | 1 | 1 |
| Dizziness | | 3 | 3 |
| Glaucoma, reduced visual acuity | 2 | | 2 |
| Headache | 5 | 6 | 11 |
| Heart failure, chest pain, AV-block I | | 3 | 3 |
| Haematuria | 1 | | 1 |
| Hypertension | 2 | | 2 |
| Migraine | 1 | | 1 |
| Nasopharyngitis | 1 | 5 | 6 |
| Nausea | 2 | 5 | 7 |
| Pain back | 1 | 1 | 2 |
| Pain hip | | 1 | 1 |
| Paraesthesia | | 1 | 1 |
| Pneumonia | 1 | | 1 |
| Pruritus, hair loss | 2 | | 2 |
| Stomatitis | | 1 | 1 |
| Subdural haematoma | | 1 | 1 |
| Urinary tract infection | 1 | | 1 |
| Urticaria | 3 | | 3 |
| White matter changes on MRI | | 1 | 1 |
| Total | 26 | 31 | 57 |

[a] Hypokalaemia, hyperglycaemia, CSF pleocytosis, pathological liver enzymes.

**Table 3.** Positive (+), negative (–) or no (0) changes in cognitive performance measured with the MMSE and ADAS-Cog, EEG ratio of fast and slow activity and percentage change in mean cortical glucose metabolism (rCMRglc) and nicotine binding ($^{11}$C-Nic) assessed by PET during NGF treatment after 3 and 12 months as compared to baseline (0 months)

| Patient | Change from baseline | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MMSE | | | ADAS-Cog | | | EEG | | PET rCMRglc | | PET $^{11}$C-Nic | |
| | 3 m | 6 m | 12 m | 3 m | 6 m | 12 m | 3 m | 12 m | 3 m | 12 m | 3 m | 12 m |
| 1 | + | + | + | 0 | – | + | + | 0 | 0 | 0 | + | + |
| 2 | – | – | – | – | – | – | + | + | 0 | 0 | – | 0 |
| 3 | – | – | – | + | – | – | – | – | 0 | – | + | – |
| 4 | – | – | – | – | – | – | – | – | – | – | 0 | – |
| 5 | – | – | – | + | – | – | – | – | – | – | 0 | 0 |
| 6 | 0 | + | – | + | + | + | 0 | – | – | – | + | + |

An increase/decrease was defined as ≥10% and ≤10% compared with baseline for the PET and EEG data while a change of 1 point or more in the MMSE and ADAS-Cog scores defined increases/decreases of these assessments. m = Months.

turned to baseline levels at 12 months. No parallel increases in GFAP levels were found. The Aβ42 and total tau proteins did not change during the study, while a significant ($p < 0.02$) decrease in phosphorylated tau was seen at 3 months which was sustained at 12 months.

## Discussion

We have demonstrated that deep brain implantation of encapsulated, genetically modified NGF-producing cells is feasible, safe and well tolerated by patients with AD. In contrast to a previous pilot study exploring intracerebroventricular infusion of NGF [18], no NGF-related AEs, neither pain nor weight loss, were detected. In the present study, the intraparenchymal delivery of NGF did not result in increased NGF levels in CSF, supporting the hypothesis that NGF side effects of pain and weight loss are related to elevated levels of NGF in CSF and the concomitant activation of NGF receptors located on dorsal root ganglia and the hypothalamus, respectively. Furthermore, no antibodies to NGF could be detected in CSF or in serum throughout the duration of the study, consistent with little or no systemic exposure of recombinant NGF. Moreover, pro-NGF, which has been suggested to stimulate apoptosis [11, 13, 14], was not significantly secreted by the EC [26].

The basal forebrain cholinergic neurons project widely to the cerebral cortex and to the hippocampus, and depend on retrograde NGF signalling from the cortical and hippocampal target areas to the cell bodies in the basal forebrain [7]. In AD, the endogenous NGF signalling to these neurons is insufficient, and based on a large body of animal experiments, the clinical delivery of exogenous NGF has been attempted for therapeutic purposes. In one clinical study, autologous fibroblasts, genetically modified to produce NGF, were implanted in the lateral part of the basal forebrain, targeting the basal nucleus of Meynert (Ch4) [23]. Similarly, the patients in the first dose cohort of our study received bilateral single implants to Ch4, whereas patients in the second dose cohort also received implants in the vertical limb of the diagonal band (Ch2), since neurons located in this nucleus project to the hippocampus and degenerate in AD. In fact, this is the first time ever that implantation to the Ch2 region has been successfully performed in man [29].

All patients were on stable AChEI treatment (range 8–26 months) before study start, and continued on the same dose during the study. In the longest reported (12 months) placebo-controlled study of AChEI treatment in AD patients, both groups declined in cognition and activities of daily living function over time, although the AChEI-treated group showed less decline than the placebo group [39]. In our trial, all patients except 1 had been receiving AChEI for 11 months or more. This 1 patient did not improve cognitively during the trial, and the 2 who did improve were anticipated to decline cognitively as expected with long-term AChEI treatment. The cognitive improvements observed in these 2 patients are therefore not likely to be attributed to the AChEI treatment.

In the previous two studies of NGF delivery to AD patients, improved MMSE score and cerebral glucose me-

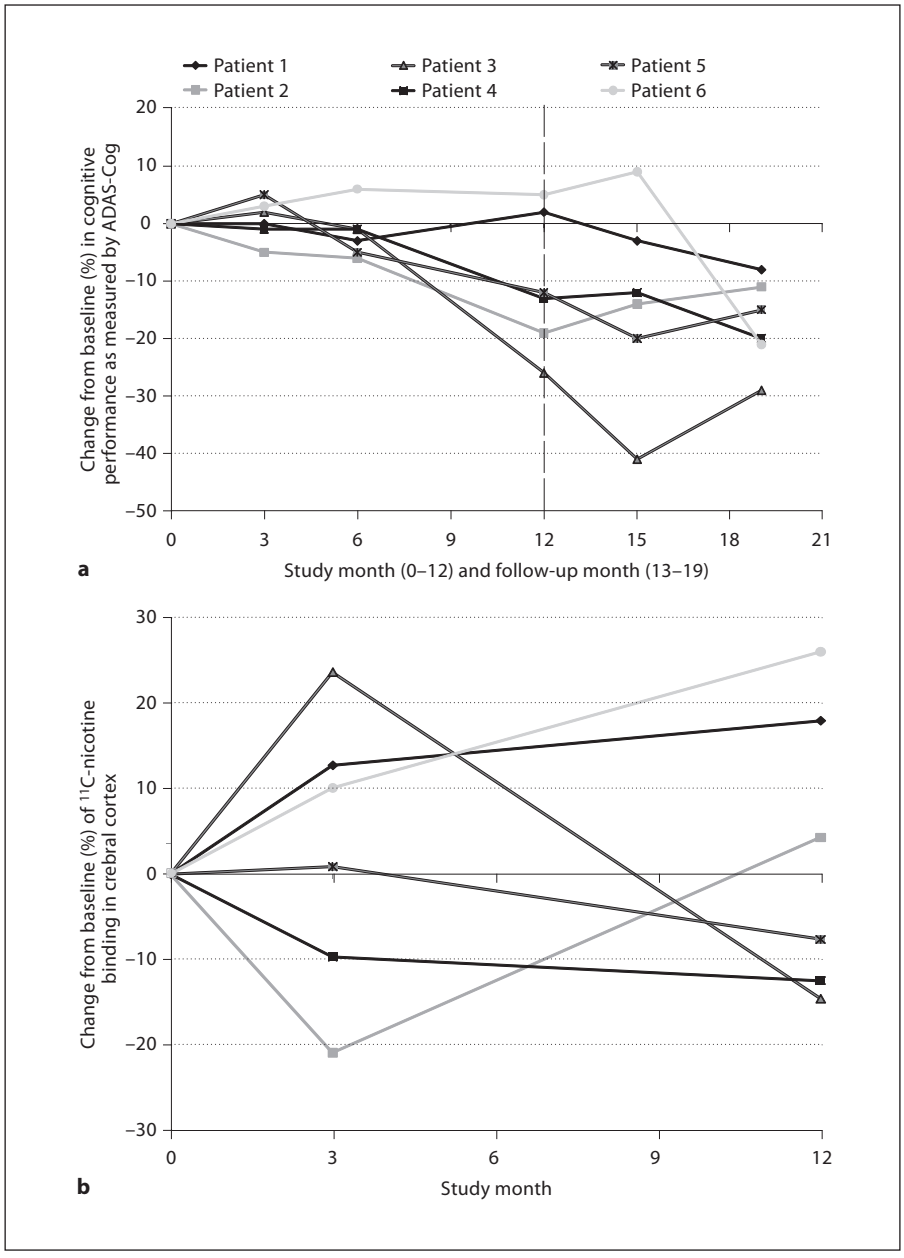


**Fig. 3.** Change from baseline (%) in cognitive performance as measured by ADAS-Cog (**a**) and change from baseline (%) in $^{11}$C-nicotine binding in the cerebral cortex assessed by PET imaging (**b**). Please note that the cognitive performance improved during treatment at 12 months in patients 1 and 6 (**a**). The $^{11}$C-nicotine binding in mean cortical areas increased in both patients 1 and 6 at 3 and 12 months compared to baseline, at 12 months with 18 and 26%, respectively (**b**). In patient 3, the $^{11}$C-nicotine binding increased at 3 months but then markedly decreased to which concomitant illness may have contributed. $^{11}$C-nicotine binding was expressed as percentage increase from baseline value by using the following formula: $100 - \%k_2^* = 100 - (k_{2(f)}^*/k_{2(b)}^* \times 100)$ where f and b indicate the time of follow-up (3 and 12 months) and baseline (0 months), respectively.

tabolism [23] as well as increased nicotine binding, blood flow and an improved EEG pattern [17, 18] were found in a subset of patients. Similarly, 2 out of 6 patients in the present study showed improved cognition with corresponding improvement of PET and EEG biomarkers. A negative correlation between ADAS-Cog scores and $^{11}$C-nicotine binding (indicating an improved cognitive performance with increased nicotine binding) was found in our study. Positive correlations of cortical $^{11}$C-nicotine binding and performance in cognitive tests have been shown in AD patients after 12 months of treatment with AChEI [40]. One patient in the first cohort in our study had been followed clinically for 2 years prior to enrolment and had been treated with AChEI during that time. This patient showed improved cognition at 3 and 12 months after implantation, as measured by the MMSE, and compared with the anticipated rate of decline in AD patients receiving AChEI treatment as assessed with the MMSE [41]. The fact that the cognitive function of this patient returned to the preoperative baseline level at 3 and 7

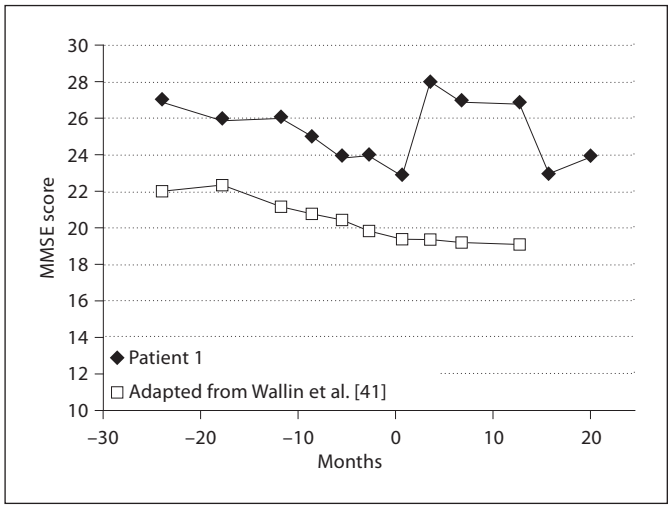


**Fig. 4.** MMSE scores in patient 1 are shown. This patient had been assessed with the MMSE during treatment with AChEI for 2 years prior to enrolment in the study. The trajectory of the cognitive function assessed with mean MMSE (SD range 4.6–7.3) in AD patients on AChEI treatment adapted from the study of Wallin et al. [41] is added in the figure to give an appreciation of the rate of decline in AD patients on long-term AChEI treatment. Please note the improved MMSE score in patient 1 during the NGF treatment, clearly shown at 3, 6 and 12 months (just prior to explantation) with a marked decline at 15 months, i.e. 3 months after explantation.

months after device explantation also suggests that the NGF implants may have slowed the rate of cognitive decline. However, one should bear in mind that these findings could also be due to chance or rater bias. Since this was a non-placebo-controlled trial, the effects found in a subset of the patients could be due to a placebo effect only. However, positive effects were found not only on cognitive tests but also on EEG activity and nicotine binding assessed by PET, with the latter investigations less prone to rater bias.

The dose of NGF delivered in this study was about 1,000-fold lower than the dose delivered intracerebroventricularly in our previous study [17, 18]. The initial mean dose of 1.7 ng/24 h NGF may be at the lower limit needed for a therapeutic effect. The rate of decline of the NGF release from the implants is also unknown. Since there were no marked side effects in man with the initial dose of 1.7 ng/day of NGF, further dose escalation can be justified. Animal studies suggest an intraparenchymal dose of 100 ng/24 h to be desirable for a biological effect [42, 43]. The devices have been tested in the rat striatum and shown to positively affect the surrounding normal cholinergic neurons with NGF immunoreactivity up to 2 mm from the implant edge [44]. Similarly, diffusion of NGF from the devices implanted in the Göttingen minipig brain suggests a spread of at least 2 mm [26] which should be sufficient to reach the cholinergic neurons in the Ch2 and Ch4 regions from a close implant. Moreover, in contrast to the majority of the devices in the patients in this study, devices implanted in the minipig brain showed that NGF was delivered for at least 12 months [26]. The reasons for this discrepancy are discussed below.

One important aspect of this new technology is the ability to remove the genetically modified NGF-producing cells from the host brain, thus combining the safety of a retrievable device with the efficacy of gene therapy. A minimally invasive surgical procedure was required to remove the implants, which were easily and safely retrieved intact at the end of the study [29]. All devices were analysed for NGF release and cell morphology. Consistent with the finding of surviving cells, NGF release could be detected and quantified from all implants retrieved from patients in the first dose cohort at 12 months, at about 10% of the pre-implantation level, whereas in the second cohort, degenerating cells were found in the implants and detectable levels of NGF only in 2 out of 12 devices. Viable, NGF-releasing cells and detectable NGF levels in the first cohort at the end of the study indicate delivery of NGF throughout the study period. The presence of degenerating cells in the devices in the second cohort suggests that NGF had been delivered over an extended time period also to these patients. The survival and function of the implants were, however, less than expected, as results from a 12-month minipig toxicology study have shown essentially intact cell viability and function of similar implants during a 12-month period [26]. The discrepancy between the minipig and patient studies may be related to the difference in implantation instrumentation used between the species or differences in the diffusion of nutrients and/or waste products through the semi-permeable capsule wall in the AD versus the minipig brain. Immunological host-versus-graft reaction is a less likely explanation of poor cell survival in the AD study, as the NGF-secreting human retinal pigment epithelial cell line was well protected from immune rejection in the xenogeneic milieu of the minipig brain.

The CSF levels of Aβ and tau did not change during the trial. However, NFL levels in CSF increased significantly in all patients at 3 months but returned to baseline values at 12 months. This is an intriguing and highly

consistent finding which needs to be further explored. The increase could be due to the surgical intervention, as repeated brain trauma after amateur boxing has shown increased NFL and GFAP in CSF [45]. However, in that study, levels returned to normal after 3 months' rest from boxing, while the elevated NFL levels in our study were present at 3 months after the surgery and were not paralleled by an increase in GFAP, making it inconsistent with trauma. Alternatively, the elevated NFL levels could be indicative of regeneration, as an increased NFL activity around regenerating neurons has been shown [46].

In conclusion, our study shows that EC biodelivery of NGF delivery is safe and well tolerated by AD patients. There were no complications with the implantation or explantation procedures. We here demonstrate the first step in a new possible therapeutic strategy for AD based on local delivery of a bioactive molecule to cholinergic neurons in the basal forebrain. With optimization of dose and long-term function of new cell lines, aided by device improvement and a reloading technique, it is anticipated that EC biodelivery of NGF has the potential to become a new treatment strategy for AD with both symptomatic and disease-modifying effects. We are well aware that this strategy is not suitable for all AD patients, but may become a treatment option for younger AD patients in otherwise relatively good medical condition, and eligible for neurosurgical intervention.


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## Disclosure Statement

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