Long-term safety and tolerability of ProSavin, a lentiviral vector-based gene therapy for Parkinson's disease: a dose escalation, open-label, phase 1/2 trial

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Summary

Background Parkinson's disease is typically treated with oral dopamine replacement therapies; however, long-term treatment leads to motor complications and, occasionally, impulse control disorders caused by intermittent stimulation of dopamine receptors and off-target effects, respectively. We aimed to assess the safety, tolerability, and efficacy of bilateral, intrastriatal delivery of ProSavin, a lentiviral vector-based gene therapy aimed at restoring local and continuous dopamine production in patients with advanced Parkinson's disease.

Methods We undertook a phase 1/2 open-label trial with 12-month follow-up at two study sites (France and UK) to assess the safety and efficacy of ProSavin after bilateral injection into the putamen of patients with Parkinson's disease. All patients were then enrolled in a separate open-label follow-up study of long-term safety. Three doses were assessed in separate cohorts: low dose ($1·9\times10^7$ transducing units [TU]); mid dose ($4·0\times10^7$ TU); and high dose (1×10^8 TU). Inclusion criteria were age 48–65 years, disease duration 5 years or longer, motor fluctuations, and 50% or higher motor response to oral dopaminergic therapy. The primary endpoints of the phase 1/2 study were the number and severity of adverse events associated with ProSavin and motor responses as assessed with Unified Parkinson's Disease Rating Scale (UPDRS) part III (off medication) scores, at 6 months after vector administration. Both trials are registered at ClinicalTrials.gov, NCT00627588 and NCT01856439.

Findings 15 patients received ProSavin and were followed up (three at low dose, six mid dose, six high dose). During the first 12 months of follow-up, 54 drug-related adverse events were reported (51 mild, three moderate). Most common were increased on-medication dyskinesias (20 events, 11 patients) and on–off phenomena (12 events, nine patients). No serious adverse events related to the study drug or surgical procedure were reported. A significant improvement in mean UPDRS part III motor scores off medication was recorded in all patients at 6 months (mean score 38 [SD 9] *vs* 26 [8], n=15, p=0·0001) and 12 months (38 *vs* 27 [8]; n=15, p=0·0001) compared with baseline.

Interpretation ProSavin was safe and well tolerated in patients with advanced Parkinson's disease. Improvement in motor behaviour was observed in all patients.

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Introduction

Parkinson's disease is a common neurodegenerative disorder mainly characterised by motor dysfunction resulting in bradykinesia, rigidity, tremor, gait impairment, and postural instability. The disease has a prevalence of around 1% in people aged 60 years, affecting around 5 million people worldwide.[1] Several risk factors are associated with Parkinson's disease including inheritance (5–10% of patients)[2] and exposure to chemicals such as pesticides.[3] A crucial pathological component is the progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta that project axons to the striatum, where dopamine is released. The rate of dopamine biosynthesis is limited by three enzymes that are expressed in nigral neurons: tyrosine hydroxylase and cyclohydrolase 1, which facilitate the conversion of tyrosine to levodopa, and aminoacid decarboxylase (AADC), which converts levodopa to dopamine.[4–6]

Current therapies for Parkinson's disease, which are mainly based on the oral dopamine precursor levodopa, provide excellent control of motor symptoms in the initial stages of the disease.[7] However, as the disease progresses, levodopa therapy becomes less effective as side-effects emerge such as on–off phenomena (when the patient has improved mobility [on] due to levodopa and which is then followed by sudden unpredictable impaired mobility [off]) and dyskinesias.[8] Previous studies have shown that the intermittent nature of oral levodopa administration and subsequent irregular stimulation of postsynaptic dopamine receptors is at least partly responsible for these motor

complications.[9] Inappropriate dopaminergic stimulation of the mesolimbic area by systemic intake of dopaminergic drugs can also induce dopamine dysregulation syndrome and impulse control disorders.[10] Thus, a therapeutic approach that provides continuous and stable dopamine replacement, restricted to the dopamine-depleted striatum, might provide an effective long-term treatment without the onset of behavioural complications.

Gene transfer technology can provide long-term expression of therapeutic proteins in vivo. Three therapeutic approaches using adeno-associated virus (AAV) vectors have been evaluated in clinical trials of Parkinson's disease. These strategies were aimed at neuroprotection using the neurotrophic factor neurturin; enhanced conversion of levodopa to dopamine by gene transfer of AADC; and modulation of basal ganglia activity with glutamic acid decarboxylase.[11–15] All these AAV-based gene therapies were shown to be safe and well tolerated, and showed some benefit in clinical motor assessments.[12,16,17] Gene therapy approaches using lentiviral vectors have not previously been evaluated in clinical trials of CNS disorders. However, these vectors have been repeatedly shown to have low immunogenicity and an ability to transduce neuronal cells with high efficiency and carry a larger therapeutic cargo than AAV vectors.[18–21]

We have generated a tricistronic lentiviral vector (ProSavin, Oxford BioMedica, Oxford, UK) that is based on the equine infectious anaemia virus encoding the rate-limiting dopamine biosynthetic enzymes tyrosine hydroxylase, AADC, and cyclohydrolase 1. Previous studies have shown that expression of these three enzymes in nondopaminergic cells, such as striatal neurons, is sufficient to enable these cells to manufacture dopamine.[22] Thus, the therapeutic rationale for ProSavin is to deliver the vector to the motor region of the striatum (putamen) and convert striatal cells into so-called "dopamine factories", thereby replacing the constant source of dopamine that is lost in Parkinson's disease.

Integrating vectors have the potential for insertional mutagenesis, as observed in clinical trials using gammaretroviral vectors to modify haematopoietic stem cells.[23] Several risk factors for insertional mutagenesis were identified in these clinical studies. These factors included the use of gammaretroviral vectors with active promoter regions (and their preference for insertion at transcription start sites), the nature of the transgenes expressed, the proliferative nature of the target cell type, and the immunocompetence of the patients treated.[23] The risk of insertional mutagenesis has been mitigated in the design of the lentiviral vector by removal of the viral control signals from the duplicated long terminal repeat by using the self-inactivating vector configuration, thereby reducing the potential for gene activation. ProSavin has also been shown to target postmitotic neurons and this factor is also likely to minimise the risk of oncogenesis. Additionally the preference of lentiviral vectors to integrate into active genes suggests that insertion near a proliferation or oncogene is unlikely. The genes delivered by ProSavin do not impart a proliferative advantage and the patients in this study are immunocompetent. We believe, therefore, that the weight of evidence suggests that the risk of oncogenesis is very low. No evidence for insertional mutagenesis was observed in supporting non-clinical studies.

In preclinical studies, surgical administration of ProSavin to the striatum was well tolerated and led to local dopamine production and significant behavioural improvement in rat and non-human primate models of Parkinson's disease.[24] We aimed to assess the safety, tolerability, and efficacy of bilateral, intrastriatal delivery of ProSavin in patients with advanced Parkinson's disease.

Methods

Study design and setting

We undertook an open-label, dose-escalation phase 1/2 study with 12-month follow-up at two study sites (France and UK). All patients were subsequently enrolled into a separate open-label follow-up study to provide long-term (up to 9 years) safety data. After that point annual survival is assessed by follow-up phone calls for life. The study protocols (NCT00627588 and NCT01856439) were approved by the institutional review board of each participating institution and complied with the Declaration of Helsinki, current Good Clinical Practice guidelines, and local laws and regulations. An independent data monitoring committee consisting of a virologist and neurologists ensured the integrity of the trial and safety of participants. All participants provided written informed consent before enrolment. Appropriate ethics and regulatory approvals were received before study initiation.

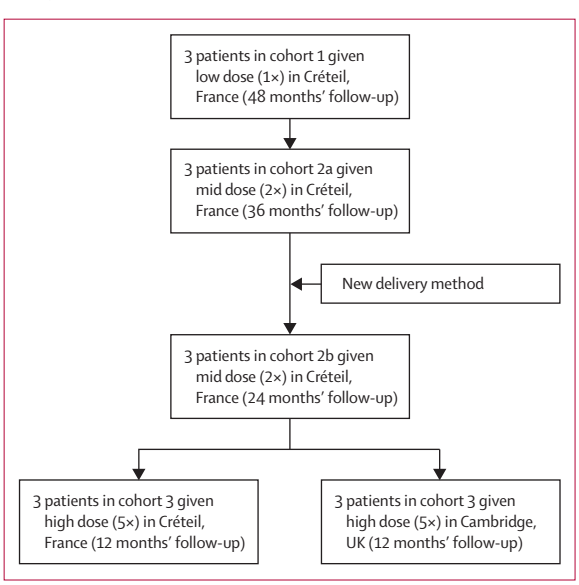

Figure 1: Trial design

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See **Online** for appendix

	Cohort	Age (years)	Disease duration (years)	UPDRS motor score		Total UPDRS score		Levodopa equivalent daily dose (mg)
				Off	On	Off	On	
L1	1	62	8	23	6	49	19	2547
L2	1	57	8	30	8	61	24	1329
L3	1	58	16	28	11	70	35	1998
M4	2a	57	17	29	8	63	20	2164
M5	2a	56	12	30	14	58	24	1572
M6	2a	49	9	34	7	74	23	2523
M7	2b	64	9	49	19	83	31	1772
M8	2b	59	13	38	15	67	31	1088
M9	2b	57	15	46	9	68	20	1775
H10	3	48	22	37	8	59	21	1535
H11	3	58	10	35	10	71	27	1844
H12	3	61	26	52	13	91	25	1180
H13	3	63	16	49	23	90	52	1690
H14	3	57	19	52	23	94	47	699
H15	3	55	9	44	18	71	30	1593
Mean (SD)	..	57·4 (4·3)	13·9 (5·3)	38 (9·3)	13 (5·5)	71 (12·7)	29 (9·4)	1687 (487·7)

Patients are listed in the order in which they received treatment. Off=off-medication state. On=on-medication state. L=low dose ($1·9 \times 10^7$ transducing units [TU]). M=mid dose ($4·0 \times 10^7$ TU). H=high dose (1×10^8 TU).

Table 1: **Baseline demographic data, by patient number**

	Number of events				Number of patients
	Mild	Moderate	Severe	Total	
Events in the first 12 months					
Total	51	3	0	54	14
Nervous system disorders	41	2	0	43	14
On and off phenomenon	12	0	0	12	9
Dyskinesia	19	1	0	20	11
Headache	4	0	0	4	4
Akinesia	3	0	0	3	3
Balance disorder	1	0	0	1	1
Tremor	0	1	0	1	1
Brain oedema*	1	0	0	1	1
Speech disorder	1	0	0	1	1
Injury, poisoning, and procedural complications	1	0	0	1	1
Subdural haematoma†	1	0	0	1	1
Investigations	5	0	0	5	4
Nuclear MRI brain abnormality	2	0	0	2	2
Nuclear MRI abnormality	1	0	0	1	1
Weight decreased	1	0	0	1	1
Weight increased	1	0	0	1	1
Psychiatric disorders	3	1	0	4	4
Anxiety	1	1	0	2	2
Abnormal dreams	1	0	0	1	1
Hallucinations	1	0	0	1	1
Musculoskeletal and connective tissue disorders	1	0	0	1	1
Musculoskeletal pain	1	0	0	1	1

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Participants

Patients with bilateral idiopathic Parkinson's disease, as defined by the diagnostic criteria from the 1999 Core Assessment Program for Surgical Interventional Therapies,[25] were enrolled in the study. Entry criteria included: age 48–65 years; disease duration of at least 5 years; Hoehn and Yahr stage 3 or 4 in the off medication state; Unified Parkinson's Disease Rating Scale (UPDRS) part III (off medication) score between 20 and 60; motor complications associated with levodopa therapy; stable treatment regimen for at least 6 weeks before surgery; and 50% or more improvement in UPDRS part III score between the off and on medication states. Full criteria are listed in the study protocol.

Procedures

The ProSavin vector was produced by a triple transient transfection of HEK293T cells as previously described.[18] The vector was purified and concentrated by anion exchange chromatography and hollow fibre ultrafiltration. A schematic of the ProSavin genome is shown in the appendix.

Three dose levels of ProSavin were assessed in four patient cohorts: dose level one (low dose, $1·9 \times 10^7$ transducing units [TU]; cohort 1); dose level two (mid dose, $4·0 \times 10^7$ TU; cohorts 2a and 2b); and dose level three (high dose, 1×10^8 TU; cohort 3). A modified delivery method was introduced for cohorts 2b and 3 to increase the rate of delivery and enhance the distribution of the vector (methods in the appendix). All patients were given ProSavin bilaterally into the striatum under general anaesthesia. The vector was administered vertically from the dorsal surface of the brain and targeted to the sensorimotor part of the striatum. The putaminal targets were identified and localised with the Leksell stereotactic frame (G frame, Elekta, Stockholm, Sweden) and MRI guidance (T1 3D MRpage, 1·5 T, Siemens, München, Germany) using neuronavigation technology (Stealth station, Medtronic, Minneapolis, MN, USA).

The primary endpoints of the study were the number and severity of adverse events associated with ProSavin administration and motor responses as assessed with UPDRS part III (off medication) scores, at 6 months after vector administration. Patients were clinically evaluated at screening, weekly during the first month after treatment, and then at 1, 2, 3, 6, 9, and 12 months. Safety data are shown up to February, 2013. Thereafter assessments will be done twice a year for 3 years, then annually for 7 years, and after that point annual survival is assessed by follow-up phone calls for life. Adverse events were assessed at every visit; all events were recorded, including those reported spontaneously on general questioning and those observed directly by the investigators. Efficacy assessment including UPDRS parts I, II, and III (in the off and on medication states), UPDRS part IV, Rush dyskinesia rating scale (RDRS),[26] and quality of life (using the Parkinson's

Disease Questionnaire, PDQ-39) were assessed at baseline and at 1, 3, 6, and 12 months (and yearly thereafter in the open-label follow-up). UPDRS off-medication assessments were done in the practically defined off state after overnight drug withdrawal. UPDRS on-medication assessments were done 1 h after a dose of levodopa that was tailored for each patient at baseline and the same dose used at each assessment. Neuropsychological tests were completed before surgery and at 6 and 12 months. Individual doses of dopaminergic drug were unchanged throughout the study unless alterations were needed in response to adverse events. Doses were assessed at every visit and expressed as levodopa equivalent daily dose (LEDD).

Each patient had two PET scans on the same day, in the defined off-medication state, using the radioligands ^{18}F-levodopa and ^{11}C-raclopride. PET imaging was done in all patients (except for three patients in cohort 3, because of the unavailability of appropriate PET imaging at that clinical site), before surgery and 6 months after ProSavin administration. All PET scans were done with a high-resolution tomograph (ECAT EXACT HR+, CTI-Siemens, Knoxville, TN, USA). Cortical excitability and reflex recordings were studied before ProSavin administration and at 6 and 12 months after administration. All recordings were done while the patient was in the off-medication state.

Serum samples were prepared from blood samples from each patient before surgery and 2 weeks, 4 weeks, and 3, 6, and 12 months after treatment. Antibody responses against ProSavin components were measured with semiquantitative ELISA. Biodistribution of ProSavin to blood cells was assessed by analysis of DNA extracted from buffy coat samples with a real-time PCR-based (quantitative PCR) assay. Vector shedding was assessed by analysis of RNA extracted from urine.

Statistical analysis

Data management and statistics were done by Quanticate (Hitchin, UK) and serious adverse events were registered and reported by Parexel International (Harrow, UK). Adverse events were tabulated and rated for severity (mild, moderate, and serious) and their relation to the study intervention. UPDRS scores were analysed by Wilcoxon paired test at 6 and 12 months. Changes in ^{11}C-raclopride binding potential and ^{18}F-levodopa uptake between baseline and 6 months were analysed by Friedman ANOVA. Dose effects in PET were analysed by Kruskal Wallis.

Both trials are registered at ClinicalTrials.gov, NCT00627588 and NCT01856439 (EudraCT numbers 2007-001109-26 and 2009-017253-35).

Role of the funding source

The study sponsor had responsibility for the trial design, manufacture and supply of drug, data collection, and clinical monitoring. The data were analysed by Quanticate (Hitchin, UK) as described. The formal clinical study report was written by Quanticate (Hitchin, UK). Authors from the sponsor were involved in drafting this Article on the basis of the data and analysis from Quanticate. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

	Number of events				Number of patients
	Mild	Moderate	Severe	Total	
(Continued from previous page)					
Events in the first 12–48 months					
Total	22	0	0	22	5
Nervous system disorders	17	0	0	17	5
On and off phenomenon	8	0	0	8	5
Dyskinesia	8	0	0	8	5
Dysarthria	1	0	0	1	1
Psychiatric disorders	4	0	0	4	3
Delusional perception	2	0	0	2	2
Confusional state	1	0	0	1	1
Hallucinations, visual	1	0	0	1	1
Musculoskeletal and connective tissue disorders	1	0	0	1	1
Myalgia	1	0	0	1	1

*Along the injection site only. †Bleeding under burr hole.

Table 2: **Drug-related adverse events**

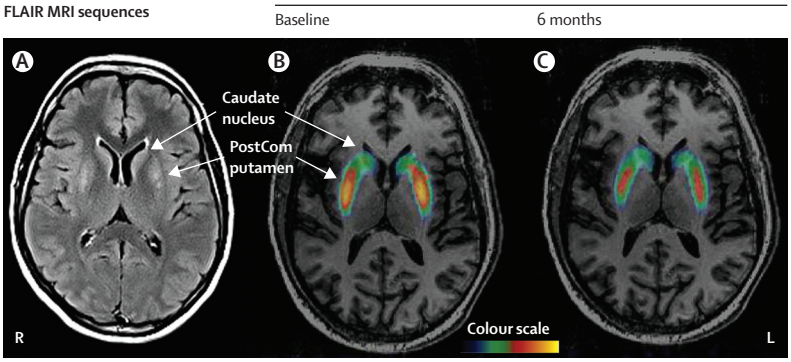

Figure 2: **PET and MRI studies**
(A) Two-dimensional FLAIR MRI sequences done 1 month after surgery, showing increased signal within the postcommissural (PostCom) putamen corresponding to motor putamen injection sites (lower arrow), and no signal change in the non-injected caudate nucleus (upper arrow). (B, C) Binding potential parametric maps from ^{11}C-raclopride PET scans of patient 11 (cohort 3) done at baseline (B) and 6 months after ProSavin injection (C) within the putamen. Note a decrease in a binding potential index only in the left and right postcommissural putamen area (B, lower arrow), compared with no binding potential change on either side of the non-injected caudate nucleus (B, upper arrow).

Results

Between Jan 14, 2008, and Aug 8, 2011, 17 patients were enrolled in the study; two patients (both men) withdrew before receiving treatment (both screening failures; the first due to an intercurrent illness and the second due to a failure to meet the inclusion criteria of 50%

improvement in UPDRS part III). The remaining 15 patients received ProSavin and were followed up according to the protocol (figure 1). Three patients were included at dose level one, six patients at dose level two, and six patients at dose level three. Table 1 shows baseline patient characteristics. Data are presented for all 15 patients beyond the primary study endpoint of 6 months: three patients at 48 months, six at 36 months, nine at 24 months, and 15 at 12 months of follow up.

All patients were ambulatory within 24 h of surgery and were discharged from hospital after a further 7–9 days. Adverse events were reported in all patients, with most (170 of 246 events in 48 months) judged to be unrelated to ProSavin (appendix). Eight serious adverse events (right inguinal hernia, large vessel vasculitis, disabling on-medication dyskinesia, fall due to dyskinesia, aspiration pneumonia, uncontrolled diabetes, deep brain stimulation surgery for two patients) occurred, but all were unrelated to the study drug. During the first 12 months of follow-up 54 drug-related adverse events were reported (table 2), and of these 51 were mild and three moderate. The most common drug-related adverse events were increased on-medication dyskinesias (20 events, 11 patients) and on–off phenomena (12 events, nine patients). Increased dyskinesias resolved with a reduction in the patients' oral dopaminergic medication. The safety profile across all dose cohorts was similar with the exception that early increases in on-medication dyskinesias were more consistently observed in the highest dose group. In this group, all six patients had an increase in on-medication dyskinesias by 6 weeks after surgery, whereas only one of three patients from the low dose group and three of six patients from the mid dose group had a similar change. During long-term follow-up (12–48 months) of the nine patients from cohorts 1, 2a, and 2b, we noted 22 adverse events that were related to ProSavin (table 2), of which the most common were on–off phenomena and dyskinesias (eight events, five patients, for both events). No deaths or new neurological deficits were reported in any patients during either the planned 1 year course of the study or in the extended follow-up to date.

Neuropsychological measurements showed no significant changes compared with baseline after 12 months in any of the modalities tested (data not shown). MRI scans done at 4 weeks and 3, 6, and 12 months after surgery showed no evidence of abnormalities, such as haemorrhage or oedema, at the injection sites. Expected signal changes around the entry point and along the needle trajectory were observed and confirmed the correct placement of the vector in the striatum (figure 2).

Immunological analyses showed no detectable antibody responses against any of the ProSavin transgene products in any patients. Low-level antibody responses against the VSV-G envelope protein were detected in four of the six patients from cohort 3, at 3 or 6 months after treatment; antibodies to p26 protein (part of the gag protein that makes up the viral particle) were observed in three of these four patients at the same timepoints. MRI showed no corresponding inflammatory responses. ProSavin RNA (vector particles) and DNA sequences (cell-associated vector) were not detected in most blood and urine samples and there was no indication of consistent vector RNA or DNA presence (appendix).

UPDRS part III (off medication) motor scores were significantly reduced compared with baseline at 6 months (mean score 38 *vs* 26, n=15, p=0·0001) and 12 months (38 *vs* 27; n=15, p=0·0001) in all 15 patients. No significant difference was seen between the different dose cohorts (all p>0·05; table 3, figure 3). Long-term follow-up

	Baseline	6 months	12 months	24 months	36 months	48 months
Off state						
L1	23	12 (–48%)	13 (–43%)	16 (–30%)	20 (–13%)	19 (–17%)
L2	30	26 (–13%)	27 (–10%)	30 (0%)	35 (17%)	32 (7%)
L3	28	20 (–29%)	19 (–32%)	20 (–29%)	21 (–25%)	22 (–21%)
M4	29	21 (–28%)	24 (–17%)	30 (3%)	26 (–10%)	NA
M5	30	24 (–20%)	26 (–13%)	19 (–37%)	24 (–20%)	NA
M6	34	16 (–53%)	15 (–56%)	18 (–47%)	16 (–53%)	NA
M7	49	34 (–31%)	39 (–20%)	44 (–10%)	NA	NA
M8	38	24 (–37%)	32 (–16%)	39 (3%)	NA	NA
M9	46	18 (–61%)	24 (–48%)	22 (–52%)	NA	NA
H10	37	26 (–30%)	17 (–54%)	NA	NA	NA
H11	35	24 (–31%)	27 (–23%)	NA	NA	NA
H12	52	46 (–12%)	39 (–25%)	NA	NA	NA
H13	49	29 (–41%)	33 (–33%)	NA	NA	NA
H14	52	33 (–37%)	38 (–27%)	NA	NA	NA
H15	44	32 (–27%)	26 (–41%)	NA	NA	NA
Mean	38·4	25·7 (–33%)	26·6 (–31%)	NA	NA	NA
SD	9·28	8·12	8·13	NA	NA	NA
On state						
L1	6	7 (17%)	6 (0%)	7 (17%)	14 (133%)	10 (67%)
L2	8	10 (25%)	11 (38%)	11 (38%)	19 (138%)	19 (138%)
L3	11	7 (–36%)	7 (–36%)	10 (–9%)	15 (36%)	12 (9%)
M4	8	7 (–13%)	8 (0%)	10 (25%)	10 (25%)	NA
M5	14	14 (0%)	14 (0%)	13 (–7%)	16 (14%)	NA
M6	7	7 (0%)	6 (–14%)	9 (29%)	7 (0%)	NA
M7	19	15 (–21%)	15 (–21%)	17 (–11%)	NA	NA
M8	15	13 (–13%)	15 (0%)	12 (–20%)	NA	NA
M9	9	5 (–44%)	5 (–44%)	8 (–11%)	NA	NA
H10	8	8 (0%)	8 (0%)	NA	NA	NA
H11	10	8 (–20%)	9 (–10%)	NA	NA	NA
H12	13	11 (–15%)	9 (–31%)	NA	NA	NA
H13	23	16 (–30%)	21 (–9%)	NA	NA	NA
H14	23	24 (4%)	33 (43%)	NA	NA	NA
H15	18	20 (11%)	15 (–17%)	NA	NA	NA
Mean	12·8	11·5 (–9%)	12·1 (–7%)	NA	NA	NA
SD	5·50	5·29	7·07	NA	NA	NA

Data are scores (change, as a percentage). UPDRS=Unified Parkinson's Disease Rating Scale. NA=data not available.

Table 3: **UPDRS part III scores in on and off states for each patient, by timepoint**

showed continued improvement in UPDRS part III (off medication) motor scores compared with baseline in six of nine patients at 24 months, five of six patients at 36 months, and two of three patients at 48 months (table 3). No significant improvement in UPDRS part III (on medication) motor scores were observed at any time in any group (table 3). Two patients (M7 and M8) received deep brain stimulation at 27 and 28 months after ProSavin administration, respectively. As such, efficacy analysis was not done on these patients after the 24 month assessment.

Mean total UPDRS (off medication) motor scores were significantly reduced at 6 months versus baseline (52 *vs* 71; n=15, p=0·0001) and at 12 months versus baseline (54 *vs* 71; n=15, p=0·0001) in the total patient population (table 4). Significant improvements were also observed in total UPDRS (on medication) scores at 6 months versus baseline (22 *vs* 29; n=15, p=0·0006) and at 12 months versus baseline (24 *vs* 29; n=15, p=0·002; table 4).

Analysis of the UPDRS part I scores showed a significant decrease in mean on-medication scores between baseline and 6 months in the total patient population (1·6 *vs* 0·3; n=15, p=0·02; appendix). A significant decrease in mean scores at baseline and 6 months was also noted in the UPDRS part II assessments (off state: 21 *vs* 17; n=15, p=0·02; on state: 4·2 *vs* 2·2; n=15, p=0·02; appendix). UPDRS part IV scores were improved relative to baseline in ten of 15 patients at 6 months and 11 of 15 patients at 12 months (appendix) with a significant reduction in mean score between baseline and 12 months (10 *vs* 8; n=15, p=0·03; appendix). No differences in mean RDRS scores were recorded between baseline and 6 or 12 months (data not shown). Analysis of PDQ-39 scores showed a significant improvement in mean scores between baseline and 6 months (33·3 *vs* 27·9; n=14, p=0·04; appendix), but not at 12 months (p=0·9). Patient diary data suggested a shift in the mean time spent in the off-medication (complete or partial) to on-medication state (without dyskinesias or with non-troublesome dyskinesias) in 13 of 15 patients. There was no overall difference between the different dose cohorts with respect to this finding (appendix).

11 of 15 patients at 6 and 12 months needed a reduction in LEDD compared with baseline (table 5). Of the four patients at 12 months who did not need a decrease in LEDD, three showed no change and one had a small increase in LEDD compared with baseline. The need for LEDD reduction was most evident in the highest dose group (cohort 3), in which all six patients had an increase in on-medication dyskinesias at 6 weeks after ProSavin administration, whereas in the low or mid dose cohorts, four of nine patients had an increase in on-medication dyskinesias by the same timepoint.

PET imaging results showed that ^{18}F-levodopa Ki values at baseline were similar across all 12 patients analysed. There was no significant overall difference between baseline and 6 months in ^{18}F-levodopa Ki values

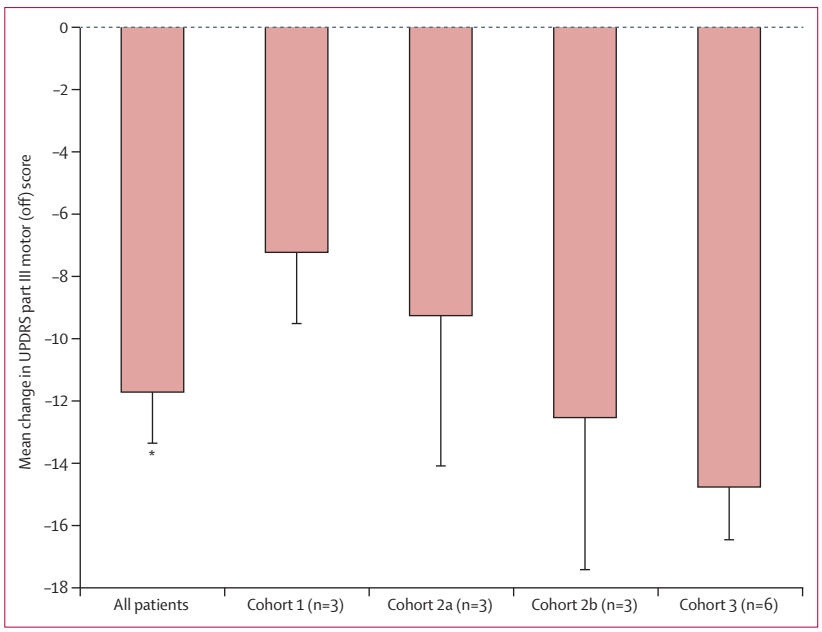

***Figure 3*: Mean change in UPDRS III (off medication) motor score relative to baseline at 12 months**
Bars show SE. UPDRS=Unified Parkinson's Disease Rating Scale. *Significant decrease at 12 months compared with baseline (p=0·0001).

for the patient population (appendix). Baseline ^{11}C-raclopride binding potentials were similar across all patients analysed. A significant dose effect (p=0·02) of putaminal binding potential change between baseline and 6 months was observed, with mean changes of 5·33% in the low dose cohort, –9·57% in the mid dose cohort, and –10·07% in the high dose cohort. In the total population, the change in binding potential at 6 months relative to baseline was significantly higher in the target putamen region compared with the control uninjected caudate nucleus region (p=0·03; figure 2). Patients receiving the highest ProSavin dose (cohort 3) had a significant change in binding potential relative to baseline in the putaminal subregions (anterior putamen, p=0·046; and posterior putamen, p=0·027; appendix).

Neurophysiological investigation showed restoration of intracortical motor inhibitory control at rest and increased motor cortical output during voluntary movement (p=0·0062 and p=0·0137, respectively; appendix). These results support an improvement of motor control after ProSavin, likely to reduce motor disturbances at rest and assist movement execution.

Discussion

We report the results of a clinical trial describing the first-in-man use of a lentiviral-based gene therapy vector for a chronic neurodegenerative disorder of the CNS (panel). In view of the novelty of this approach the safety findings were of paramount importance and the favourable safety profile observed is highly encouraging. In terms of efficacy, a significant improvement in motor function was shown up to 12 months in all patients. Long-term

	Baseline	6 months	12 months	24 months	36 months	48 months
Off state						
L1	49	30	30	37	44	43
L2	61	45	59	62	73	65
L3	70	41	43	38	42	49
M4	63	50	52	69	59	NA
M5	58	54	53	30	47	NA
M6	74	54	44	58	53	NA
M7	83	67	69	84	NA	NA
M8	67	46	58	67	NA	NA
M9	68	44	54	51	NA	NA
H10	59	54	49	NA	NA	NA
H11	71	58	65	NA	NA	NA
H12	91	84	73	NA	NA	NA
H13	90	47	53	NA	NA	NA
H14	94	63	64	NA	NA	NA
H15	71	44	39	NA	NA	NA
Mean (SD)	71·3 (12·73)	52·1 (12·29)	53·7 (11·25)	NA	NA	NA
On state						
L1	19	13	14	15	23	22
L2	24	19	28	26	38	42
L3	35	15	18	17	22	27
M4	20	17	17	28	21	NA
M5	24	24	22	19	28	NA
M6	23	25	22	26	25	NA
M7	31	29	26	39	NA	NA
M8	31	27	29	22	NA	NA
M9	20	18	16	20	NA	NA
H10	21	18	21	NA	NA	NA
H11	27	22	25	NA	NA	NA
H12	25	24	21	NA	NA	NA
H13	52	27	34	NA	NA	NA
H14	47	33	41	NA	NA	NA
H15	30	25	22	NA	NA	NA
Mean (SD)	28·6 (9·41)	22·4 (5·41)	23·7 (6·89)	NA	NA	NA

UPDRS=Unified Parkinson's Disease Rating Scale. NA=data not available.

Table 4: **Total UPDRS score in on and off states for each patient, by timepoint**

follow-up data showed long-term tolerability and evidence of clinical benefit for up to 4 years after treatment. Long-term motor efficacy is consistent with the results of preclinical efficacy studies[24] and the fact that lentiviral vectors mediate sustained transgene expression through integration of the vector genome into the host cell.[24] These data are encouraging in view of the expected disease progression of a 3–4 point increase in UPDRS part III (off medication) motor score per year.[27,28] Although the efficacy findings show promise, the magnitude of effects are within the placebo range reported in other clinical trials for Parkinson's disease using surgical techniques,[12,13] and must be interpreted with caution.

Although dose effects are difficult to assess in small sample populations, there are indications that the highest dose evaluated in this study provided the greatest level of dopaminergic activity. Specifically, patients in the highest dose cohort had a consistent requirement for a reduction in dopaminergic medication, the highest mean improvement in UPDRS part III (off medication) motor scores relative to baseline, and a significant change in ^{11}C-raclopride binding potential. Interestingly, although the PET data are based on a small sample set, the magnitude of effects, in terms of change in ^{11}C-raclopride binding potential, is similar to that previously reported in patients with Parkinson's disease before and after levodopa challenge.[29]

The antibody responses observed in four patients at the highest dose were consistent with preclinical studies in which anti-VSV-G responses were seen in rats and non-human primates (unpublished data). The timing of the antibody responses (3 and 6 months after ProSavin administration) suggests that transgene expression is unlikely to be affected since vector integration and initiation of expression is thought to be at a maximum by 1 month after administration.[30] Similar to preclinical studies vector antibody responses did not give rise to any inflammatory response in patients as assessed by sequential brain MRI or any deterioration in their clinical state.

Most adverse events observed in this study were associated with stereotactic brain surgery or Parkinson's disease, and none were thought to be clinically significant or unexpected. In previous studies investigating dopaminergic cell transplants, off-medication graft-induced dyskinesias (a form of disabling dyskinesia that persists after levodopa is stopped) were observed in some patients.[31,32] In the present study, no patients developed off-medication dyskinesias, whereas on-medication dyskinesias were common and were reversed by a reduction in the oral dopaminergic intake. This pattern is as expected for delivery of an efficacious dopaminergic therapy.[33] The increased occurrence of on-medication dyskinesias in the highest dose group might correlate with the fact that this group has the highest dopaminergic delivery to the striatum from ProSavin, as noted in non-human primate models of Parkinson's disease.[24]

Although the mechanism of action of ProSavin has not been fully elucidated, one hypothesis is that dopamine manufactured by ProSavin in the striatal neurons diffuses into the extracellular environment and stimulates postsynaptic dopamine receptors in an autocrine or paracrine fashion, thus restoring the normal dopaminergic signalling pathway. This mechanistic model is supported by preclinical non-human primate studies using ProSavin that show a restoration of the extracellular dopamine tone in the striatum and normalisation of signalling within the basal ganglia networks.[24] Similarly, indirect measurement of dopamine release in this study using ^{11}C-raclopride PET scanning suggests a partial restoration of striatal dopamine tone in a dose-related fashion. The lack of an effect in ^{18}F-levodopa uptake after ProSavin administration is

probably a consequence of the unavailability of the cellular machinery in the transduced striatal neurons to store and accumulate radiolabelled dopamine.

Several clinical trials of AAV-based vectors and different approaches have been done in patients with Parkinson's disease. These studies support the safety of a gene-based therapy approach for the treatment of neurological disorders. Promising efficacy results obtained in these phase 1 clinical trials[13–15] were disappointing in the subsequent randomised phase 2 studies, in which fairly mild or no benefit over placebo was recorded.[16,17] For this reason, the delivery of ProSavin must be optimised before the necessary phase 2 studies. Further clinical optimisation studies will also provide additional long-term safety data before phase 2 evaluation.

In summary, the data from these early phase clinical trials provide preliminary evidence for the safety and potential clinical benefit of ProSavin as a long-term treatment for Parkinson's disease. Additionally, we have adopted an iterative process in this trial to try to optimise delivery and use of this vector in patients with Parkinson's disease, and only when we have an optimum mode and dose of delivery will we proceed to a more definitive double-blind placebo controlled trial.

	Baseline	6 months	12 months	24 months	36 months	48 months
L1	2547	2257	2382	2507	2507	3102
L2	1329	1319	1103	1019	1582	1582
L3	1998	1448	750	1149	1732	2499
M4	2164	2164	1025	1229	1487	NA
M5	1572	1572	1572	1238	1238	NA
M6	2523	2257	2257	1548	1615	NA
M7	1772	1923	2023	2305	NA	NA
M8	1088	1088	1088	910	NA	NA
M9	1775	1525	1400	1400	NA	NA
H10	1535	1260	1260	NA	NA	NA
H11	1844	1549	1615	NA	NA	NA
H12	1180	1030	1130	NA	NA	NA
H13	1690	1391	1391	NA	NA	NA
H14	699	633	699	NA	NA	NA
H15	1593	1530	1468	NA	NA	NA
Mean (SD)	1687·2 (487·74)	1529·6 (447·99)	1410·8 (482·94)	NA	NA	NA

NA=data not available.

Table 5: **Levodopa equivalent daily dose (mg) for each patient, by timepoint**

Panel: Research in context

Systematic review

We searched PubMed for articles without date or language restrictions up to Sept 17, 2013. We used the full-text search terms "lentiviral vector" AND "Parkinson's disease" AND "clinical trials". We also searched PubMed for reports including the search terms "brain delivery", "tyrosine hydroxylase", "aromatic amino acid decarboxylase", and "cyclohydrolase 1". We found no reports of clinical trials using lentiviral vectors for the treatment of Parkinson's disease other than that described here.

Interpretation

This Article is the first report of the use of lentiviral vectors in clinical trials for Parkinson's disease. Furthermore, this is the first report of a clinical trial using in-vivo administration of lentiviral vectors in man in any disease indication.

Contributors

SP was investigator coordinator and principal investigator for the French site. RB was principal investigator for UK sites. SP, HL, GSR, NDM, SMK, PR, SN, OR, and KAM designed the study. Preparation and characterisation of viral vectors were supervised by PAR, JM, and KAM. Analysis of patient samples for shedding, biodistribution, and antibodies were supervised by JM, MK, and RH. SP supervised all surgical procedures in France, CW and SP in the UK. RH did statistical analysis. SD and CL contributed to trial coordination. SP, JMG, GF, DPB, SM, and NVG did patient screening. UPDRS ratings, other clinical measurements, and PET studies, before surgery and at all subsequent timepoints, were undertaken by SP, JMG, GSR, HL, SL, PB, CW, HI, CT, GF, IG, KA, PCB, XD, NT, AK, BG, PL, PD, DPB, SM, NVG, RB, and PR. Neurophysiological investigation was designed and undertaken by JPL. SP, JMG, GSR, SN, CL, and KAM primarily did data interpretation and writing of the Article, with contributions from all authors including PLC, NDM, OR, PH, and PC.

Conflicts of interest

GSR, SD, JM, PAR, MK, NDM, RH, SN, SMK, and KAM are employees or former employees of Oxford BioMedica, which funded this study. They and their families have ownership interests in the company. SP, OR, PR, RAB, PB, and CW are consultants or former consultants for Oxford BioMedica. The remaining authors, including those responsible for the assessment of study eligibility, and for the clinical measurements and statistical analyses, have no involvement in Oxford BioMedica and declare that they have no conflicts of interest.

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