Human neural stem cells in patients with chronic ischaemic stroke (PISCES): a phase 1, first-in-man study

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Summary

Background **CTX0E03 is an immortalised human neural stem-cell line from which a drug product (CTX-DP) was developed for allogeneic therapy. Dose-dependent improvement in sensorimotor function in rats implanted with CTX-DP 4 weeks after middle cerebral artery occlusion stroke prompted investigation of the safety and tolerability of this treatment in stroke patients.**

Methods **We did an open-label, single-site, dose-escalation study. Men aged 60 years or older with stable disability (National Institutes of Health Stroke Scale [NIHSS] score ≥6 and modified Rankin Scale score 2–4) 6–60 months after ischaemic stroke were implanted with single doses of 2 million, 5 million, 10 million, or 20 million cells by stereotactic ipsilateral putamen injection. Clinical and brain imaging data were collected over 2 years. The primary endpoint was safety (adverse events and neurological change). This trial is registered with ClinicalTrials.gov, number NCT01151124.**

Findings **13 men were recruited between September, 2010, and January, 2013, of whom 11 (mean age 69 years, range 60–82) received CTX-DP. Median NIHSS score before implantation was 7 (IQR 6–8) and the mean time from stroke was 29 (SD 14) months. Three men had subcortical infarcts only and seven had right-hemisphere infarcts. No immunological or cell-related adverse events were seen. Other adverse events were related to the procedure or comorbidities. Hyperintensity around the injection tracts on T2-weighted fluid-attenuation inversion recovery MRI was seen in five patients. At 2 years, improvement in NIHSS score ranged from 0 to 5 (median 2) points.**

Interpretation **Single intracerebral doses of CTX-DP up to 20 million cells induced no adverse events and were associated with improved neurological function. Our observations support further investigation of CTX-DP in stroke patients.**

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Introduction

Stroke is the most common cause of adult neurological disability worldwide, with the annual incidence being around 795 000 per year in the USA and 152 000 in the UK. Incidence, prevalence, and disability-adjusted life-years lost are predicted to rise as the age of the general population increases.[1] Stroke has profound effects on patients and carers, and places an enormous economic burden on society. In the UK, stroke care accounts for 5% of total health-care costs, with a total spend on direct and indirect costs of £8·9 billion per year.[2] Among survivors, dependence in activities of daily living 3 months after the event varies from 16·2%[3] to 19·2%.[4] Rehabilitative approaches aid functional recovery and brain reorganisation,[5] but the effects decrease over time[6] and plateau several weeks to months after stroke, indicating limited endogenous capacity for recovery.

At a tissue level, the capacity of the brain for neurogenesis and angiogenesis suggests that it might be possible to improve endogenous recovery processes.[7] Pharmacological attempts to stimulate repair have so far not improved clinical outcomes, although several agents remain under investigation.[8] Cell-based therapies offer greater potential to increase brain repair and offer a more dynamic biological response to a diverse and changing environment than do drug therapies.[9] Studies of cell therapies in animal models of disease have identified effects on cell differentiation, immunomodulation, inflammation, and stimulation of endogenous repair processes, such as angiogenesis and neurogenesis. Functional improvements after treatment with human neural stem cells (hNSCs) in animal studies of stroke support the potential of this therapeutic strategy.[10,11] Intracerebral delivery of neural stem cells, the preferred route in animals, helps to control dosing and leads to superior cell delivery and survival over intravenous or intra-arterial routes, which are preferred for mesenchymal stromal or related tissue-derived cell populations.[12]

In rats with induced middle cerebral artery obstruction, CTX0E03 cells injected after 4 weeks were associated with improvements in behavioural outcome measures dependent on dose[10] and implantation site.[11] Histological evidence of increased host striatal angiogenesis[13] and neurogenesis[14] was also seen. Preclinical evidence supported long-term safety. These data along with those on pharmacodynamic interactions, pharmacokinetic biodistribution, and toxicology formed the basis for a first-in-human trial. Here we report the results of the Pilot Investigation of Stem Cells in Stroke (PISCES) phase 1 dose-escalation trial, in which we investigated the safety

Research in context

Evidence before this study

We searched PubMed from inception to March 16, 2016, for articles published in any language, with the search terms "neural stem cells", "ischaemic stroke", and "clinical trial or study". We excluded articles concerning mesenchymal stem cells, bone-marrow-derived cells, animal studies, and non-ischaemic stroke. We found no studies that had investigated intracranial delivery of neural stem cells alone. One study compared and reported intracisternal delivery of human fetal neural stem progenitor cells of unspecified origin and mesenchymal stem cells, compared with intravenous mesenchymal stem cells alone in six patients between 1 week and 2 years after stroke. Intracranial delivery of autologous cells in stroke has been reported for teratocarcinoma-derived cells. Several published and ongoing studies are investigating intravenous autologous mesenchymal stem cells, which have several differences from neural stem progenitor cells, including timing, mechanism of action, and delivery. Although a few studies have provided proof of concept, the cell lines have not been developed further for various reasons, including safety.

Added value of this study

Our results add to the available data because of the novel potential for treatment of stroke. Functional improvements seen are promising despite little expectation for patients with chronic stroke. Some microstructural improvement in brain white matter is in line with experimental stroke evidence seen in laboratory studies.

Implications of all the available evidence

This study of neural stem-cell therapy in human beings, together with experimental and proof-of-concept studies, show potential for the treatment of stroke. Further research in carefully selected patients and larger trials is needed before this therapy can be considered for use in practice.

and feasibility of intracerebral stereotactic implantation of the CTX0E03 cell line drug product (CTX-DP) in patients with chronic stable ischaemic stroke.

Methods

Patients

Eligible patients were men aged 60 years or older who had had a first ischaemic stroke 6–60 months previously, with stable neurological deficits, defined as National Institutes of Health Stroke Scale (NIHSS)[15] scores of 6 or higher, and moderate to severe disability, defined as modified Rankin Scale[16] scores of 2–4. Patients were identified through referral from rehabilitation services or we allowed self-referral triggered by media awareness. Other inclusion criteria were surgical fitness under anaesthesia and minimum infarct diameter of 1 cm on MRI. We excluded men who had any unstable disorder with expected survival shorter than 12 months, had received an unlicensed pharmaceutical product as part of a clinical trial in the previous 3 months, had had major surgery in the previous 30 days or had such surgery planned, had a history of epilepsy or a blood coagulation disorder, had undergone allogeneic stem-cell, tissue, organ, or bone-marrow transplantation, and who had any contraindication to MRI. We also excluded those taking tamoxifen or similar analogues and excluded any women to minimise the chance of exposure to tamoxifen, a minor metabolite of which is the ligand for the *c-mycER TAM* transgene that governs CTX0E03 replication. All patients gave fully informed consent.

Trial design

PISCES was a phase 1, open-label, single-centre, dose-escalation trial of intracerebral stereotactic implantation of CTX-DP. The study was approved by the UK Medicines and Healthcare Products Regulatory Agency (MHRA) and National Research Ethics Service (previously the Gene Therapy Advisory Committee). We adhered to European Union and MHRA guidelines on advanced therapy investigational medical products.[17]

Patients were recruited into cohorts to which sequentially increasing doses of 2 million, 5 million, 10 million, or 20 million CTX0E03 hNSCs would be given in 40, 100, 200, or 400 µL volumes. The cohort size for each of the first three doses was three patients, and for the highest dose was two patients. The final sample size was decided after cell manufacture was interrupted due to changes in ownership of a contracted manufacturing site and on the basis of MHRA consultation and assessment of safety data by the independent data and safety monitoring committee (DSMC). Consistent fulfilment of inclusion criteria and clinical stability were confirmed at three visits in the 2 months before stereotactic implantation. Regular follow-up over 2 years included collection of clinical and imaging data on days 1, 2, and 7, and at 1, 3, 6, 12, and 24 months after surgery. Additionally, patients were contacted by telephone on days 14 and 21 and at 2, 9, and 18 months after surgery. Adverse events were documented and reviewed by the study team.

Study oversight and independent review

The DSMC included stroke, imaging, and neurosurgical experts who reviewed the clinical and imaging data. The data for the first patient in each dose cohort were assessed 1 month after implantation and before further patients in that cohort received treatment. Data for the last patient in each cohort were assessed 3 months after implantation and before dose escalation was recommended.

Clinical assessments

We assessed neurological impairment,[15] disability,[16] spasticity (modified Ashworth scale),[18] activities of daily

living (Barthel Index),[19] and health-related quality of life (EuroQoL Five Dimensions questionnaire).[20] A general physical examination was done and vital signs were recorded at each visit. Blood analyses included alloantibodies, blood count, infective markers, and renal and liver functions.

CTX0E03 cell manufacture and delivery

CTX0E03 hNSCs are clonally derived from human fetal cortical neuroepithelial cells following retroviral insertion of a conditional immortalisation transgene, *c-mycER TAM*.[21] The transgene generates a MYC–ER fusion protein that acts as a growth promoter in the cells under the control of 4-hydroxy tamoxifen, and confers phenotypic and genotypic stability through long-term expansion culture. *MYC*-dependent cell replication is curtailed by the removal of 4-hydroxy tamoxifen from cultures. The hNSCs are obtained by early expansion of a single isolation from 12-week fetal cortical neuroepithelium. The CTX0E03 cell line has undergone cell expansion and banking and long-term storage in liquid nitrogen in accordance with good manufacturing practice. CTX-DP is manufactured from cryopreserved CTX0E03 cells as an advanced therapy investigational medicinal product intended for allogeneic treatment.[22]

The CTX-DP is aseptically manufactured as a colourless, opaque, slightly viscous suspension composed of CTX0E03 cells at a concentration of 5×10^4 cells per µL. The diluent, HypoThermasol-FRS (Biolife Solutions, Bothell, WA, USA), is made up of ions, buffers, impermeants, colloid, metabolites, and an antioxidant. The final formulation is devoid of 4-hydroxy tamoxifen and growth factors, which restores the cells' capability to differentiate. For each patient in our study, CTX-DP was manufactured in a commercial good manufacturing practice facility on the day of the surgery, transported to the hospital pharmacy under strict temperature control (2–8°C), and implanted intracerebrally within 3 h of reaching room temperature in the operating theatre. Cell implantation was targeted to the putamen, ipsilateral to the infarct, because this was equivalent to the site used in rats, and previous clinical experience with similar volumes of cells supported the safety of this approach.

Surgical procedure

Patients were reviewed by the study neurosurgeon before admission. They were admitted to hospital 1 day before surgery for clinical assessments and anaesthetic review and to give surgical consent. On the day of surgery, after the quality of the CTX-DP was approved by a qualified person from the manufacturer, patients under general anaesthesia were fitted with a Leksell stereotactic frame (Elekta Instruments, Stockholm, Sweden) and underwent head CT. The operating surgeon identified suitable targets and trajectories within the basal ganglia of the affected side on a T1-weighted three-dimensional MRI scan acquired before surgery. The surgeon then used the MRI and CT images together, viewed with BrainLab iStereotaxy software (version 3.0), to generate coordinates for the targets and entry points.

One 15 mm burr hole situated according to the coordinates was fashioned with a craniotome. CTX-DP doses in the first two cohorts were delivered via a single injection tract, those in the third cohort via two tracts, and those in the fourth cohort via four tracts. A maximum of 100 µL was delivered per tract, at the rate of 5 µL/min in 20 µL boluses at each of five points separated by 1 mm along the tract. Implantation cannulae were made of sterile stainless steel with inner diameter 0·35 mm, outer diameter 0·9 mm, and length 235 mm (ReNeuron, Guildford, Surrey, UK; CE marked as a class III medical device), based on a design described by Kondziolka and colleagues.[23] We used a luer needle hub mounted within a Backlund injection needle (Elekta) and attached to a 250 µL Hamilton syringe (ReNeuron; CE marked as a sterile, class I medical device) for delivery. Patients were observed in the recovery ward until they were fully awake and physiologically stable, after which they were returned to a neurosurgical ward.

Brain imaging

We obtained brain images with a 3-Tesla GE-Signa-Excite-HDxt (General Electric, Milwaukee, WI, USA) MRI scanner. The protocol for structural brain imaging used a T1-weighted sagittal fluid-attenuation inversion recovery (FLAIR) sequence (time to echo [TE] 8·5 ms, time to repetition [TR] 2·5 s, inversion time [TI] 920 ms), a T1-weighted three-dimensional inversion flip-angle gradient echo train sequence (TE 1·5 ms, TR 7·2 ms, TI 500 ms), a T2-weight propeller fast spin echo sequence (TR 5 s, TE 109·2 ms), a T2* gradient echo sequence (TE 22 ms, TR 670 ms, flip angle 10°), and a T2-weighted

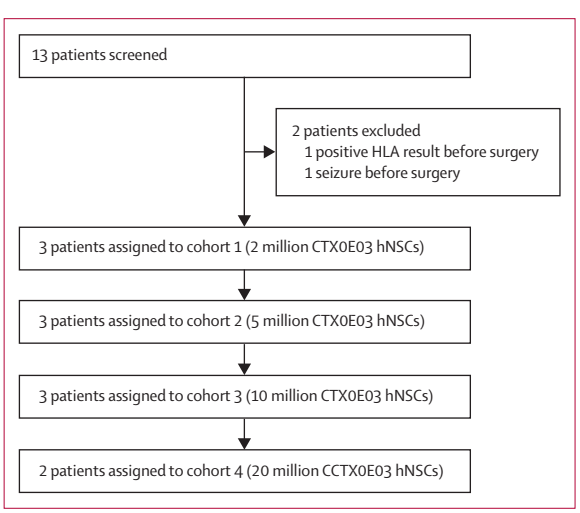

***Figure 1*: Trial profile**
hNSCs=human neural stem cells. Safety was assessed by the data safety monitoring committee 1 month after implantation in the first patient of each cohort and 3 months after implantation in the last patient of each cohort and before dose escalation.

	Age (years)	Time since stroke (months)	Infarct hemisphere and vascular territory	Risk factors	NIHSS score	Modified Rankin Scale score	Barthel Index score
2 million cells							
P1	68	14	Left cortical, MCA	Smoking, high cholesterol	8	4	12
P2	82	21	Right subcortical, MCA	Smoking, hypertension, family history of stroke and diabetes	9	4	10
P3	78	51	Left subcortical, MCA	Smoking, family history of diabetes	6	4	11
5 million cells							
P4	75	32	Right cortical, PCA	Smoking, hypertension, history of myocardial infarction	6	3	14
P5	69	33	Right cortical, MCA and ACA	Smoking, hypertension, high cholesterol, diabetes	10	4	9
P6	61	12	Right cortical, MCA	Smoking, high cholesterol, family history of stroke and diabetes	8	4	12
10 million cells							
P7	64	14	Left cortical, MCA	Smoking, high cholesterol, atrial fibrillation	7	2	16
P8	68	46	Right subcortical, MCA	Hypertension, family history of stroke	8	3	14
P9	60	18	Left cortical, MCA	Smoking, hypertension, diabetes	7	3	13
20 million cells							
P10	61	36	Right cortical, MCA	Smoking, peripheral vascular disease, alcohol excess	6	3	15
P11	71	44	Right cortical, MCA	Smoking, angina, atrial fibrillation	7	3	12
Overall							
Median (IQR)	68 (61–75)	32 (14–44)	..	..	7 (6–8)	3 (3–4)	12 (11–14)

MCA=middle cerebral artery. ACA=anterior cerebral artery. NIHSS=National Institutes of Health Stroke Scale.

***Table 1*: Baseline demographic data**

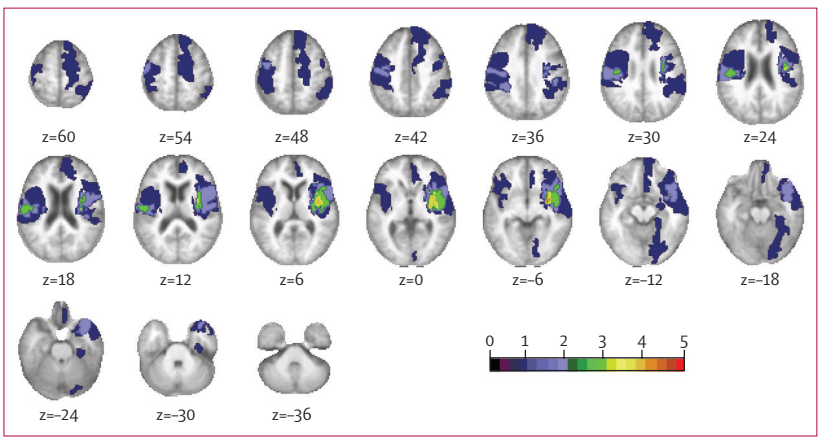

***Figure 2*: Overlapped positioning of infarcts in all 11 patients**
In all images, left side of image=left side of brain.

See Online for appendix

FLAIR sequence (TE 140 ms, TR 10 s, TI 2250 ms, slice thickness 5 mm, slice gap 1·5 mm). MRI was done 56 and 21 days before surgery and at 1, 3, 12, and 24 months after surgery. After a scanner upgrade in January, 2014, we added a T1-weighted three-dimensional sequence with gadolinium contrast and a T2-weighted three-dimensional FLAIR sequence (TE 128·3 ms, TR 6000 ms, and TI1 857 ms) to the imaging protocol. An experienced neuroradiologist (CS) reviewed all images.

Diffusion tensor imaging was acquired 21 days before surgery and 1 and 12 months after surgery to measure longitudinal change in fractional anisotropy, which is a surrogate marker of white-matter integrity, around the needle tracts. One acquisition of diffusion tensor images (TR 11 s, TE 87·1 ms, matrix 128×128, field of view 240 mm, 1·8×1·8×5·0 mm voxels, 34 directions with b values 0 and 1000 s/mm) was collected at each timepoint. Methods for image preprocessing and region-of-interest analyses are provided in the appendix.

Immunological monitoring

Patients did not receive any immunosuppressive therapy. Venous blood was obtained for analysis of HLA class I and II antibodies against CTX0E03 before treatment and at 1, 3, 6, 12, and 24 months after surgery. Patients positive for alloantibodies before implantation were excluded.

Endpoints

The primary endpoint was safety, including adverse events, neurological deterioration, and mortality. Secondary endpoints were functional change on days 1, 2, and 7, and at months 1, 3, 6, 12, and 24 after implantation.

Statistical analysis

Functional outcome data are reported as median (IQR) or mean (SD). All statistical analyses were done with SAS version 9.3, Microsoft Excel (2010 version), and Minitab

version 16. Change in fractional anisotropy on diffusion tensor imaging is reported as Cohen's *d* effect size. This study is registered with ClinicalTrials.gov, number NCT01151124.

Role of funding source

The funders of the study contributed to study design but had no role in data collection, data analysis, or data interpretation. They reviewed the trial report before submission for publication. All authors had full access to all the data in the study and the corresponding author and the DSMC chair had final responsibility for the decision to submit for publication.

Results

13 men were recruited between September, 2010, and January, 2013, of whom two were excluded before implantation, one because of a seizure and the other because of a possible positive alloantibody result. 11 men received CTX-DP (figure 1, table 1). Median follow-up after implantation was 44 months (range 33–60), with the last recruited patient having been followed up for 33 months. The positions of lesions overlapped substantially (figure 2, appendix). Operative times (first incision to last stitch) ranged from 50 to 140 min.

All patients were discharged home on day 2 after surgery. All serious adverse events were related to the neurosurgical procedure or to incidental or known medical conditions (table 2) and non-serious adverse events were mostly mild (appendix). One new ischaemic stroke—an occipital infarct not present on brain imaging on days 56 and 21 before surgery—was noticed retrospectively on the presurgical CT scan after new visual symptoms were described by the patient some weeks after surgery. A superficial malignant melanoma occurred in one patient with a history of chronic sun exposure. No event was judged to be attributable to CTX-DP. All patients who received treatment were HLA negative before and after intervention.

Preoperative neurological deficits and spasticity were stable in all patients before surgery (figure 3, appendix). After CTX-DP implantation, improvements were seen over time in NIHSS, summed arm and leg Ashworth scale, and Barthel Index scores. Disability at 12 months was unchanged in seven of 11 patients and improved by one grade in four (modified Rankin Scale). At 24 months, disability was unchanged in seven patients, worsened by two grades in one, and improved by one grade in three. Patient-reported overall health status had improved by a median of 18 points (IQR –5 to 30) at 12 months compared with baseline.

Qualitative brain imaging showed hyperintensity around the needle injection tract on T2-weighted FLAIR MRI in five patients (figure 4). Hyperintensity was first seen at 1 month and persisted to 24 months. Two further patients had slight increases in pre-existing peri-infarct white matter hyperintensity between months 1 and 12 (figure 4). No changes were seen in the other patients. The MRI changes were not associated with clinical changes. The DSMC's qualitative safety review of all scans concluded that the increased hyperintensity was not significant over time.

	Cohort	Time after surgery (months)	Attributed cause	SUSAR
Within 1 month after surgery				
Extradural haematoma (asymptomatic)	1	1	Procedure	Yes
Subdural haematoma (asymptomatic)	1	1	Procedure and anticoagulant use	Yes
Right occipital infarct	3	0	Withholding antiplatelet therapy before surgery	No
1–6 months after surgery				
Cystoscopy (elective surveillance)	1	6	Hospital admission	No
Minor bleed at the burr hole on MRI*	1 and 2	1	Procedure	No
Malignant melanoma (left ear pinna)	3	6	Pre-stroke high risk	No
>6 months after surgery				
Diverticulitis (flare up)	1	7	Pre-stroke risk	No
Haematemesis	1	8	Pre-stroke risk	No
Perforated sigmoid diverticulum	1	16	Pre-stroke risk	No
Altered bowel†	2	8	Pre-stroke risk	No
Seizure	3	10	Alcohol withdrawal	No
Alcohol withdrawal syndrome	3	12	Regular alcohol use	No
Collapse‡	3	18	Acute-on-chronic hyponatraemia	No
Gastroenteritis	3	23	Infection	No
Community-acquired pneumonia	4	11	General infection risk	No

SUSAR=sudden unexpected serious adverse reaction.*Seen in two patients. †Required colonoscopy. ‡Due to low sodium concentration.

***Table 2*: Serious adverse events**

Quantitative brain imaging, done in five patients, showed that mean fractional anisotropy on an axial region of interest was reduced at 1 month after implantation compared with baseline because voxels within the injection tract contributed zero values. At 12 months, compared with 1 month, fractional anisotropy was reduced in 17 of 28 sampled slices from four patients and was increased in the other nine slices (figure 5). Of the nine slices showing increase fractional anisotropy four were closer to the putamen and five were closer to the cortex. In one patient, who received the lowest dose, all slices showed reduced fractional anisotropy.

Discussion

This first-in-man study offers preliminary data on the feasibility, tolerability, and cell-related safety of stereotactic intracerebral injection of genetically modified CTX0E03 hNSCs in patients with chronic ischaemic stroke. We noted serious adverse events related to the procedure in four of 11 patients, none of which was symptomatic, which is consistent with general safety data for brain stereotactic procedures.[24] Unlike previous trials in which terato-carcinoma-derived neuronal cells[25,26] and fetal porcine cells[27] have been used to treat stroke, we saw no seizures

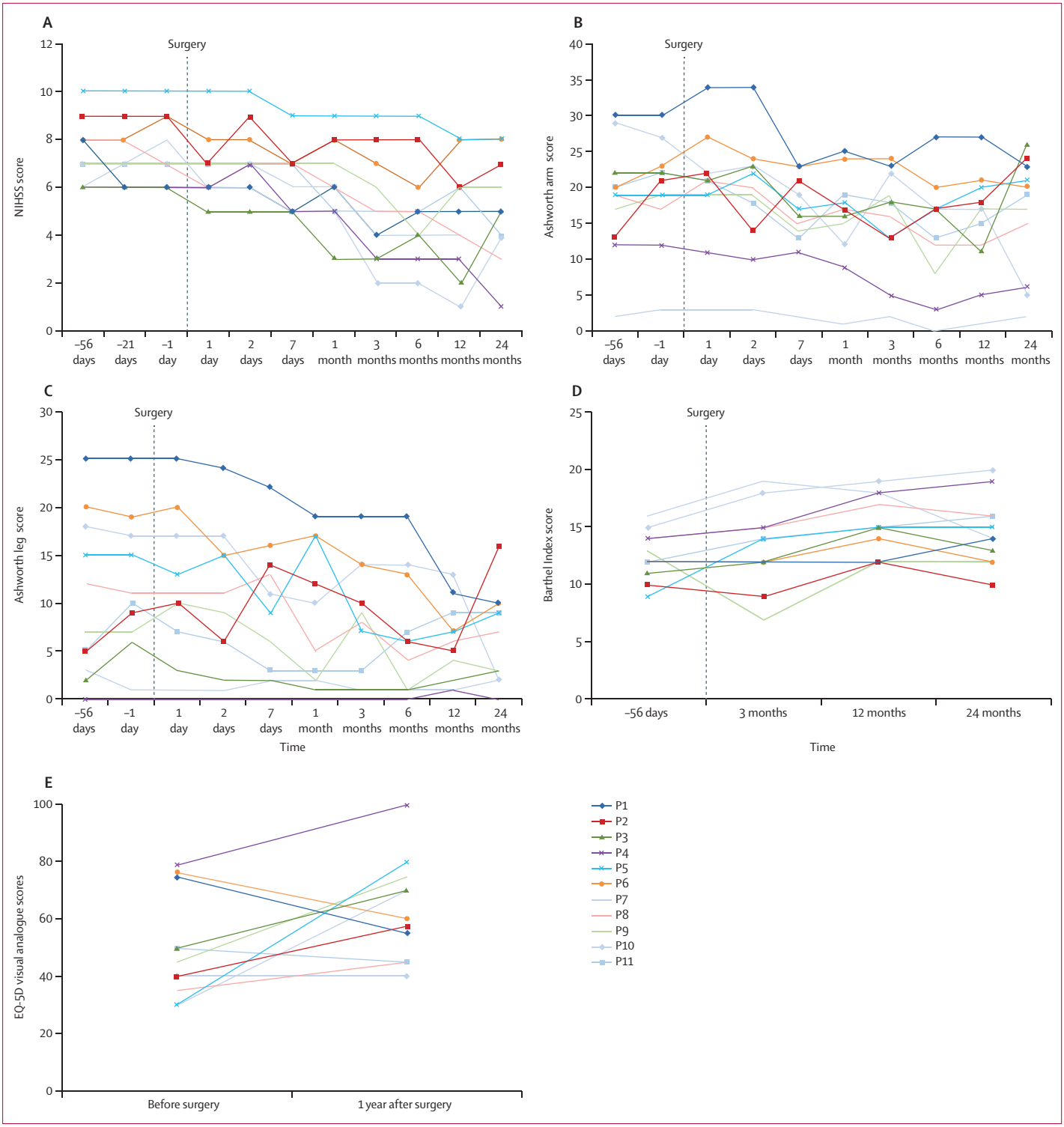

***Figure 3*: Changes from baseline in functional outcome measures**
(A) Neurological defects. (B) Arm spasticity. (C) Leg spasticity. (D) Activities of daily living. (E) Overall health. NIHSS=National Institutes of Health Stroke Scale. EQ-5D=EuroQoL Five Dimensions questionnaire. P=patient.

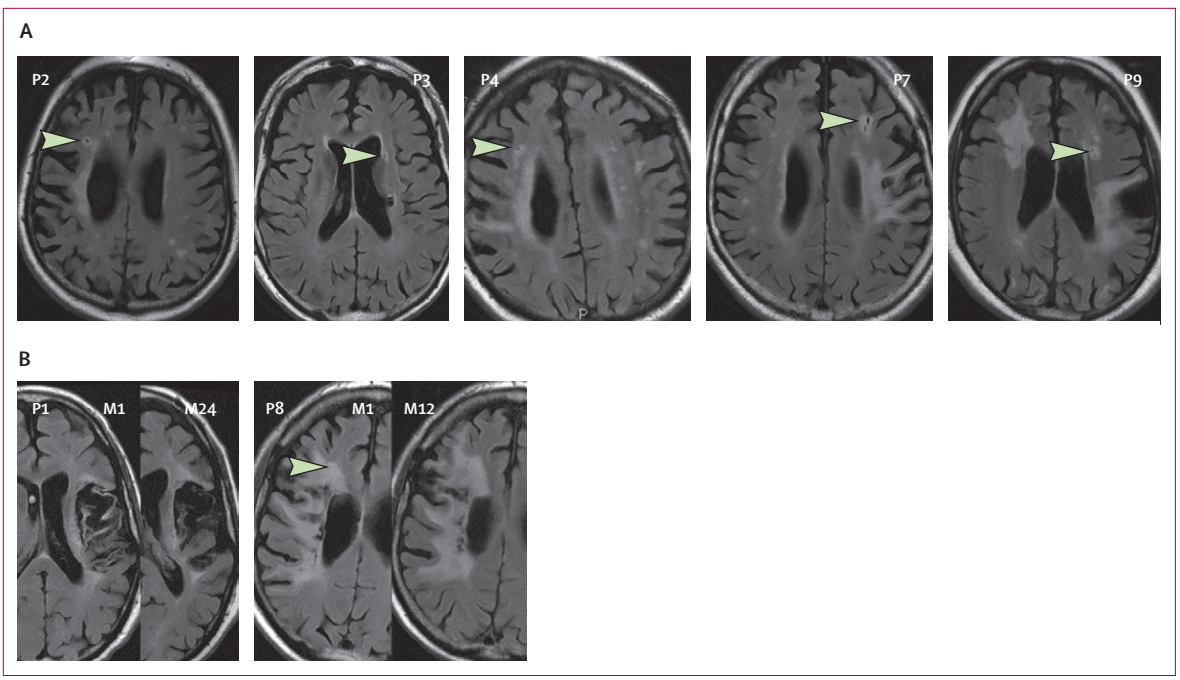

***Figure 4*: T2-weighted fluid-attenuation inversion recovery MRI**
In all images, left side of image=right side of brain. (A) Hyperintensity around injection tract was seen in five patients distinct from the lesion or pre-existing gliosis in an axial region of interest (arrows). (B) Increased white-matter hyperintensity around the infarct was seen at 24 months for P1 and 12 months for P8, compared with at 1 month. P=patient. M=month.

after surgery. A seizure event in one patient 10 months after implantation was thought to be precipitated by alcohol withdrawal. Superficial melanoma was diagnosed histologically (pT1a N0 M0[28]) in one patient 6 months after elective excision of a painful mole that had been present in a sun-exposed region for longer than 10 years. This patient had previously been prescribed antimetabolite skin creams for sun-related skin injury. Most other adverse events were due to systemic comorbidities, falls, or elective procedures that required hospital admissions, which is in line with the expected profile in disabled stroke survivors with multiple comorbidities.[29]

Hyperintensity on T2-weighted FLAIR MRI was seen around the needle tract in five patients at some point during the follow-up period. Hyperintensity could have various causes, including localised inflammation, graft–host reaction, gliosis, or dysmyelinosis. Few longitudinal imaging data are available for patients who have had stereotactic procedures for functional reasons and, therefore, whether this imaging feature is related specifically to cell injection is unclear. Increased fractional anisotropy after cell implantation, as was observed in several axial slices along the tract, has been related to increased myelination in some conditions,[30,31] which suggests improvement in microstructural white matter. Planned post-mortem pathological studies might offer additional data to characterise this finding.

In animal studies, stem cells of various kinds have been associated with improved neurological outcomes after focal brain ischaemia. hNSCs have the potential to differentiate into neural cells as well as having paracrine effects. Neural stem-cell therapies have most commonly been developed as allogeneic therapy, giving the potential flexibility of implantation in acute or subacute periods without dependence on successful cell harvest, the need for extracorporeal cell expansion in a laboratory, which can take days to weeks, and the uncertain dosing that is inherent in autologous cell therapies. Stereotactic intracranial injection in humans ensures that the intended cell dose is delivered adjacent to the ischaemic damage, replicating the conditions of animal studies, which is more likely than less invasive routes to yield proof of concept. Intravenous or intra-arterial administration might be safer, but animal data indicate that these routes result in negligible cell engraftment in the brain,[10] making the treatment reliant on diffuse paracrine or even peripherally mediated therapeutic effects.[32]

Our secondary endpoints were exploratory indices of efficacy. Given the small number of patients, the heterogeneous population, and the open-label, single-arm study design, we cannot draw reliable conclusions about the effects of cell implantation on neurological or functional change. Of note, however, despite selection of chronic, stable patients at late stages after stroke, most showed some improvement across several indices of function, including in four individuals who moved across a modified Rankin Scale threshold at a median of

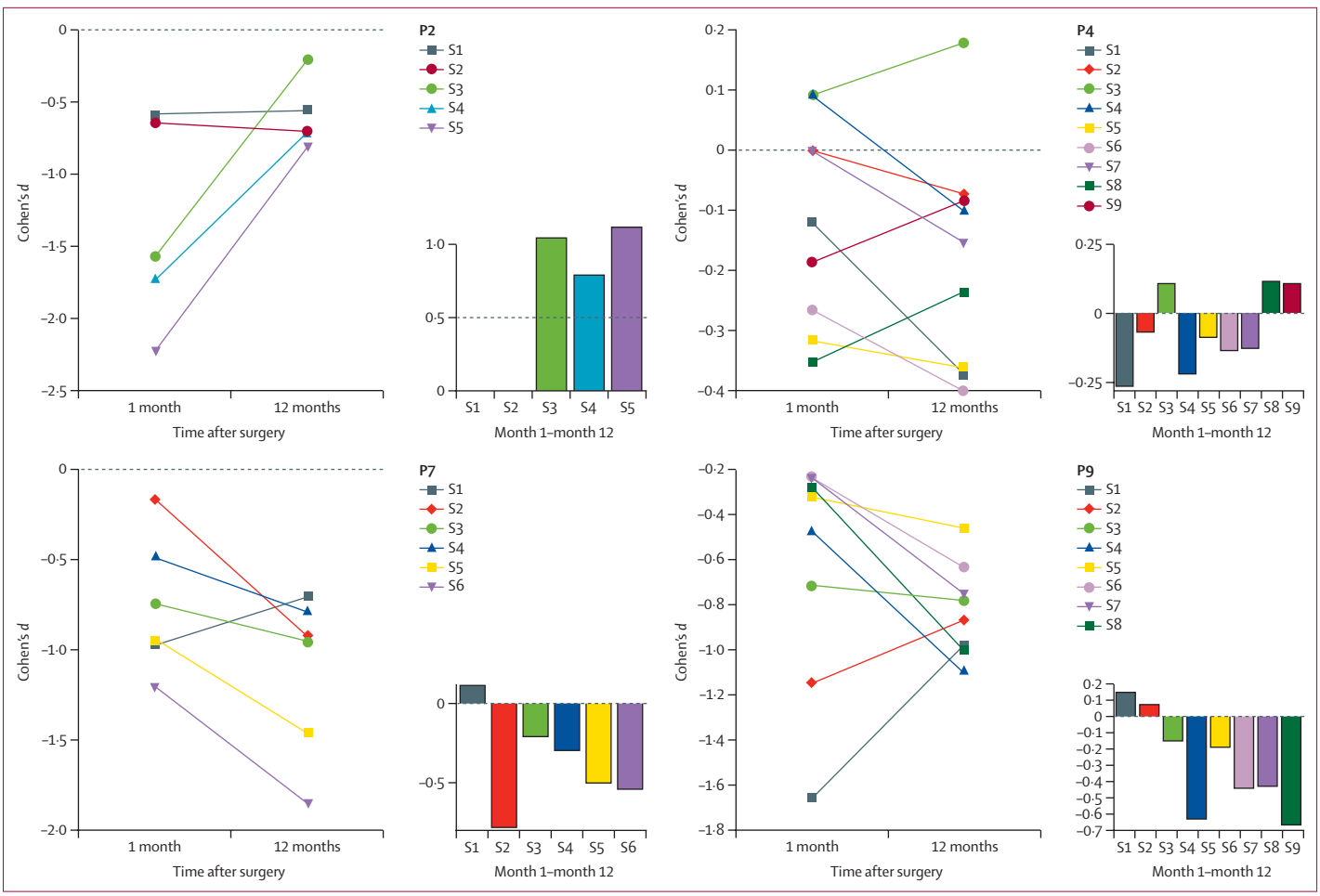

***Figure 5*: Changes from baseline in fractional anisotropy after cell implantation in four patients**
Change in Cohen's *d* values from baseline. Bar charts show relative changes between month 1 and month 12 after intervention. P=patient. S=brain slice.

32·5 months after stroke (range 21–51). Whether attributable to cell implantation or to other factors, such as engagement with trial assessments and increased generic medical input, change in this population suggests that trials of interventions are worthwhile late after stroke, when recovery is not generally believed to be attainable. Anecdotal accounts described reduced spasticity, minor return of finger movement at phalangeal joints, and improved visual perception and better bed-to-chair transfers. These findings are supported by the changes we saw in spasticity, health-related quality of life, activities of daily living, and neurological impairment.

The NIHSS score was selected as an objective tool for identifying deterioration after implantation. Other indices of neurological function are likely to offer better sensitivity to changes in future trials. Given the early nature of stem-cell research with no reproductive toxicology evidence available for stem cells of other origin or CTX-neural stem cells in particular, which have used a tamoxifen analogue receptor for in-vitro control of cell number replication,[33] we only assessed men in this early trial. Future studies, however, will not be limited to men as we saw no preclinical evidence of in-vivo cell-cycle switching and the safety profile was good.

Patients were not given immunosuppressive therapy because non-clinical studies of CTX0E03 hNSCs showed no evidence that cell survival and efficacy required immunosuppression. Additionally, in-vitro studies for MHC class II (DR) and MHC class I (A, B, and C) showed low protein expression for CTX0E03 hNSCs. Further immunosuppressive therapy might lead to infection after stroke, which is independently associated with poor outcomes.

The putamen was chosen for implantation on the basis of preclinical data as the closest intact subcortical neuronal cluster, and was preferred to white-matter injections, which can cause further axonal injury related to increased pressure. We selected CTX-DP doses by scaling up from those that were efficacious in rats, and the ascending dose design allowed cautious incremental increases after safety review. Inclusion of appropriate concurrent controls and measures to ensure blinding will be essential for future

investigations assessing efficacy. The usefulness of including control groups in early phase clinical investigations involving invasive procedures in small numbers of severely disabled participants is debated. We considered including a non-operated control group, but felt it was unlikely to provide valid control data as stroke lesions are heterogeneous and the number of patients would have been small. A placebo surgery control group raises ethical concerns because of surgical and anaesthesia risks, and might be unacceptable to patients.[34]

The study has several limitations. The small sample size limited the number of patients exposed to each dose level, particularly the highest dose, which was given to only two patients because of cell production issues. Any adverse events of low incidence, therefore, might not have been identified. Safety was assessed over a 2-year period, but longer-term safety issues could occur. Thus, we are providing lifelong surveillance. The open-label design and lack of a control group mean that exploratory efficacy data should be regarded with extreme caution. It is not possible to exclude the possibility that any neurological change over time might result from stereotactic injection rather than cell implantation, although such effects have not been observed in animals after injection with placebo.

Intracerebral implantation of CTX-DP led to no adverse events after treating 11 chronic stroke patients. The longitudinal clinical findings suggest that this novel cell therapy for ischaemic stroke is feasible, safe, and warrants a larger, phase 2 trial.

Contributors

DK recruited patients, collected and analysed data, wrote the first draft, and revised later versions with input and key revisions from all authors. JS and KP developed the stem-cell product. CH and AM managed the trial statistics. WS coordinated patients' visits. JM and CS managed the acquisition of imaging data and safety reporting. PMB chaired the data and safety monitoring committee and helped to design the study. LD did all surgeries. KWM was chief investigator and designed and managed the study. All authors reviewed and approved the final report.

Data and safety monitoring committee

Philip W Bath (chair), University of Nottingham, Nottingham, UK, Joanna Wardlaw (neuroradiologist), Ian Whittle (neurosurgeon), and Christopher Weir (biostatistician), University of Edinburgh, Edinburgh, UK.

Declaration of interests

DK has received travel grants from Guarantors of Brain, Jim Gatheral, and Mac Robertson scholarship. JS and KP are employees and stock holders of ReNeuron. JS has a patent for cell lines and a patent for neural transplantation issued to ReNeuron, and KP holds US patent 7,416,888 B2. CH and AM's university employer has received funding from ReNeuron. PMB has received honoraria from ReNeuron. KWM has received trial funding from ReNeuron for PISCES and for an ongoing phase 2 stroke stem-cell trial. The other authors declare no competing interests.

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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Kalladka D, Sinden J, Pollock K, et al. Human neural stem cells in patients with chronic ischaemic stroke (PISCES): a phase 1, first-in-man study. *Lancet* 2016; published online Aug 3. http://dx.doi.org/10.1016/S0140-6736(16)30513-X.

WEB-APPENDIX

Pilot Investigation of Human Neural Stem Cells in Chronic Ischaemic Stroke Patients (PISCES): A Phase 1, First-in-Man Study

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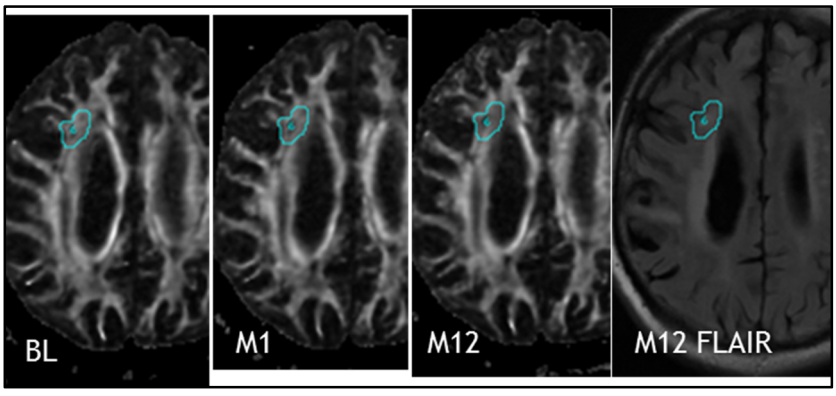
Supplementary Information

DTI Processing: The detailed review of pre-processing has been previously published.[1,2] Briefly, the dataset was motion corrected by iterative steps that included aligning each volume to the geometric mean volume, re-computing the geometric mean; align each group's geometric mean to first acquired I_0 image, transformation using the 9 parameter affine rigid body method. Multi-modal image registration between anatomical (T2W FSE and 3D T1W IR-FSPGR) using the intensity correlation maximization. The first (I_0, b=0) volume of each DTI dataset was then registered to the T2W FSE image, which was further registered with the 3D T1W IR-FSPGR image and subsequently registered to an atlas representative target that conformed to the Talairach system.[3] The diffusion tensor and its 3 eigenvalues were computed using log-linear regression in each voxel and the 3 eigenvalues were used to calculate radial diffusivity, axial diffusivity and mean diffusivity. Anisotropic diffusion was expressed as FA, which was normalized to assume a range from 0 to 1.[4]

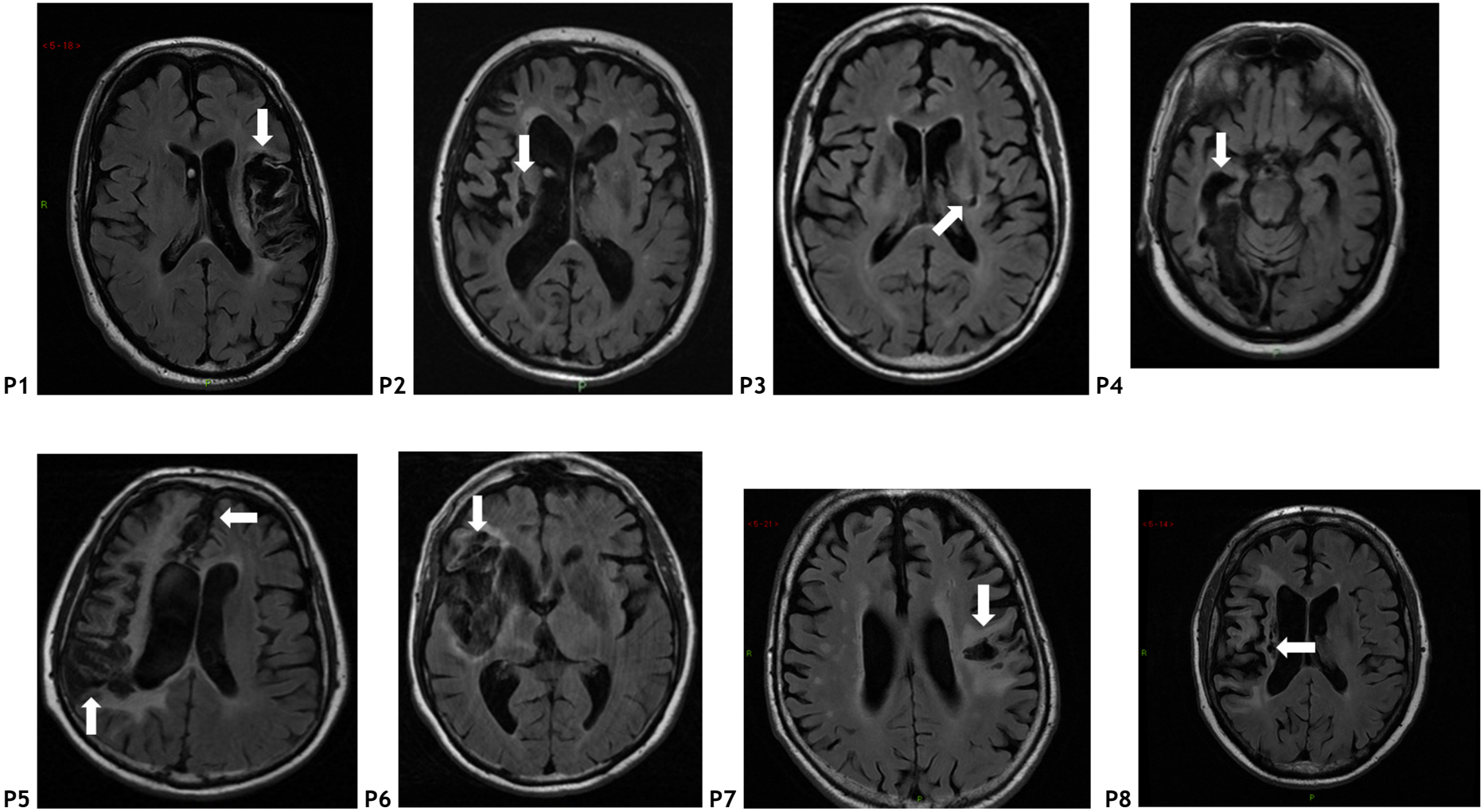
Region-of-Interest (ROI) analysis: The ROIs on which the FA was measured and sampled were defined on the atlas transformed 12 month T2W FLAIR images using ANALYZE 10.0 (Mayo Clinic, Rochester, USA). ROIs around the tract including the hyper-intense areas were manually drawn on an axial plane. Sequential ROIs (4 to 9 counts) were drawn along the tract starting near the putamen and working upwards towards the cortex, for a given subject. These ROIs were then used on the atlas transformed FA images to record the average (mean & SD) FA value of all the voxels in a given ROI (figure 8). The Cohen's d effect size of the change in FA between the 3 time points was calculated.[5]

Figure 1:

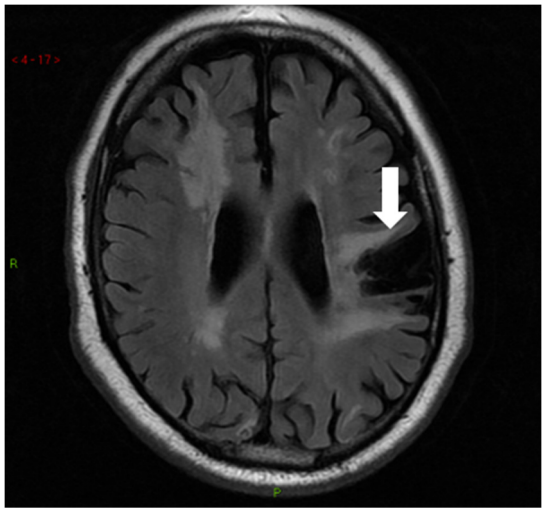


Atlas transformed Fractional Anisotropy DTI images from BL, M1 and M12 showing one of the ROIs used for measuring mean FA. The M12 T2W FLAIR image that was used to create the ROI around the injection needle tract is shown on the right.

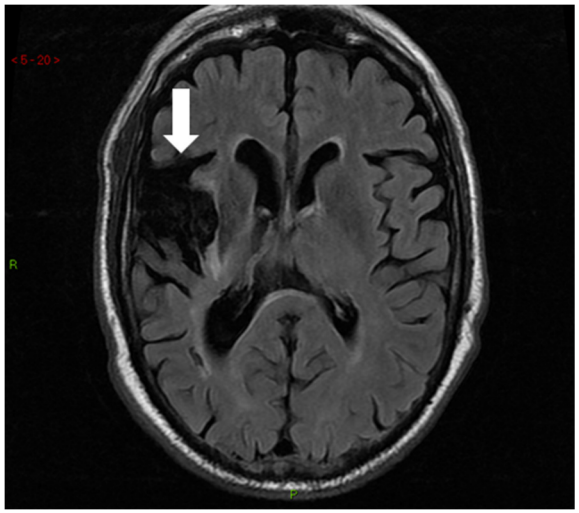
Figure 2: Representative axial MR images of all 11 patients (P1 to P11) to show infarct site and size.



P9



P10



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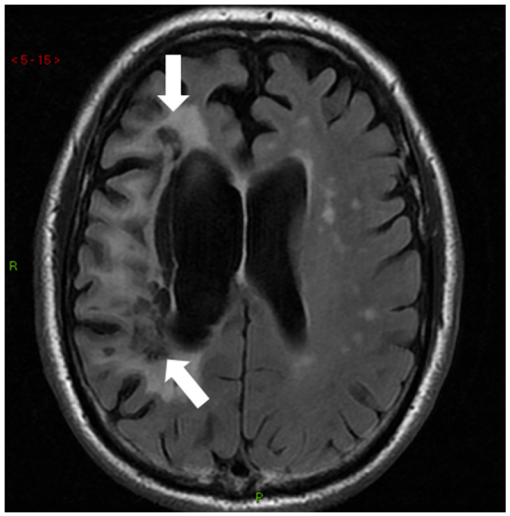
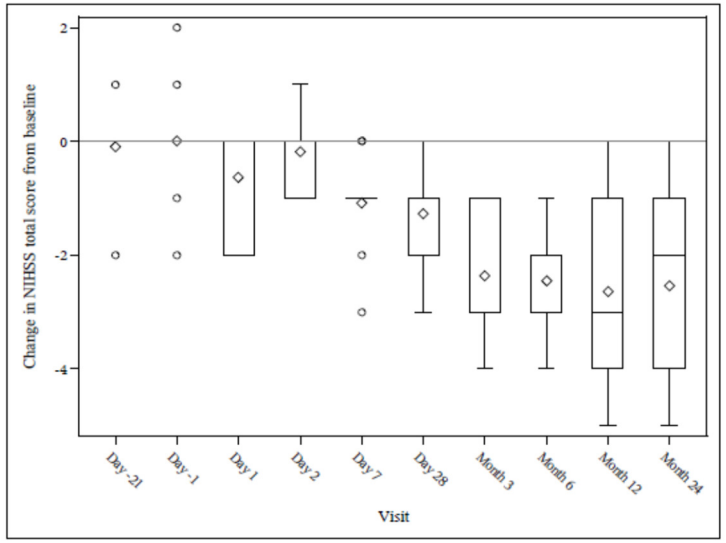


Table 1: Adverse Events

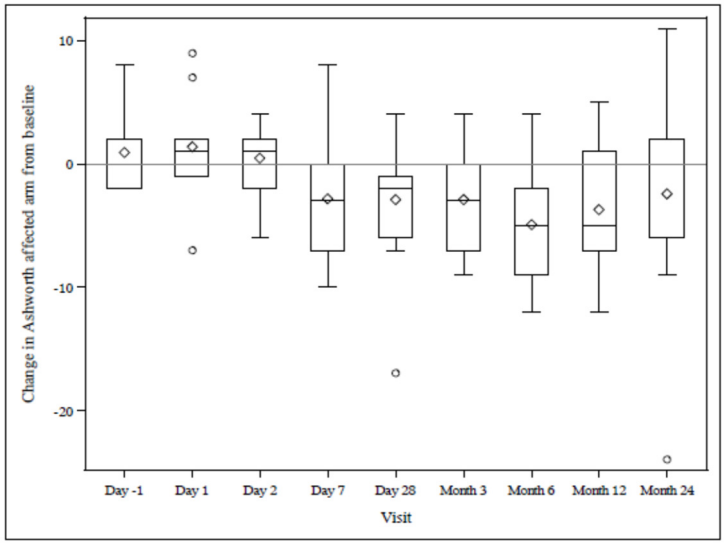
Event	Number of events				Number of patients
	Mild	Moderate	Severe	Total	
TOTAL	106	39	2	147	11
Surgery/Anaesthesia related					
Hypotension	6	3	0	9	9
Superficial skin laceration	2	0	0	2	2
Pain					
Neck and shoulder	0	1	0	1	1
Low back	0	1	0	1	1
Wound site	3	0	0	3	3
Abdomen	1	0	0	1	1
Non-specific pain	2	3	0	5	3
Neurological					
Headache	8	1	0	9	8
Seizure - Alcohol withdrawal	0	2	0	2	1
Muscle fasciculation	2	0	0	2	1
Tingling sensation	1	0	0	1	1
Difficulty to initiate walk	1	1	0	2	1
Increased spasticity in right hand	0	1	0	1	1
Twitch in left arm on waking up	1	0	0	1	1
Abnormal movement of left arm	0	1	0	1	1
Depression	0	1	0	1	1
Systemic					
Generally unwell / pyrexia	2	2	0	4	4
Respiratory tract Infection	4	2	0	6	5
Chest pain- non-cardiac	0	0	1	1	1
Cardiac arrhythmia -Atrial fibrillation	0	1	0	1	1
Fatigue	0	1	0	1	1
Urinary tract infection	8	0	0	8	3
Microscopic haematuria	1	0	0	1	1
Proteinuria	1	0	0	1	1
Prostrate hypertrophy	3	0	0	3	3
Choking	1	0	0	1	1
Gastroenteritis	0	0	1	1	1
Hematemesis	0	1	0	1	1
Change in bowel habit	0	1	0	1	1
Gastro-oesophageal reflux / Vomiting	4	0	0	4	3
Diarrhoea	4	1	0	5	4
Constipation	3	1	0	4	4
Diverticular disease	0	1	0	1	1
Rectal bleeding	1	0	0	1	1
Gout	0	1	0	1	1
Cataract	0	2	0	2	1
Diabetic retinopathy worsening	1	0	0	1	1
Dental – Abscess / bleed / extraction	2	2	0	4	4

Osteoporosis	0	1	0	1	1
Reduced mobility	1	0	0	1	1
Impaired Fasting glycaemia	1	0	0	1	1
Potassium depletion	1	0	0	1	1
Ankle swelling	1	0	0	1	1
Skin					
Erythematous rash / itch	7	0	0	7	3
Blister	1	1	0	2	2
Cellulitis	2	0	0	2	1
Fungal Infection- Groin	1	0	0	1	1
Transient Acantholytic dermatosis (Grover's)	1	0	0	1	1
Biopsy right cheek	1	0	0	1	1
Skin biopsy buttock	1	0	0	1	1
Investigations					
MRI hyper-intensity around surgical tract	6	0	0	6	6
Abnormal Blood results	3	3	0	6	6
Abnormal ECG	1	0	0	1	1
MRI-intracranial hemosiderin around meninges	4	0	0	4	2
Procedures					
Cystoscopy- inflamed bladder	0	1	0	1	1
Cataract day surgery	2	0	0	2	1
Others					
Mechanical Fall / difficulty to walk	9	2	0	11	7
Swelling/pain Leg from splint	1	0	0	1	1

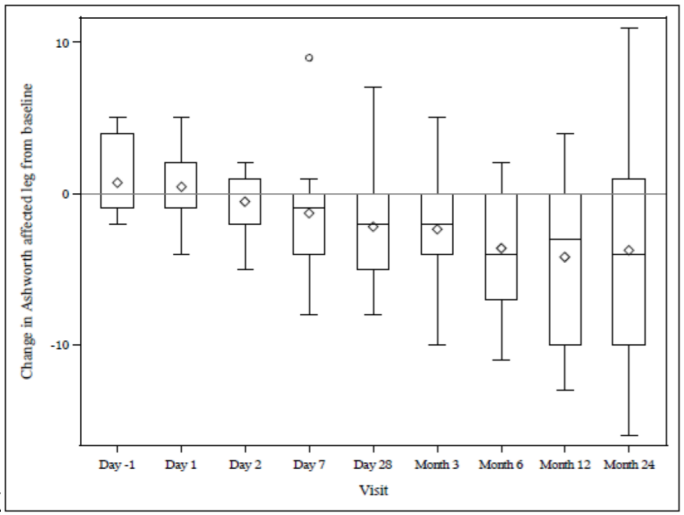
Figure 3: Functional Outcome Measures change from baseline for all patients.



2a



2b



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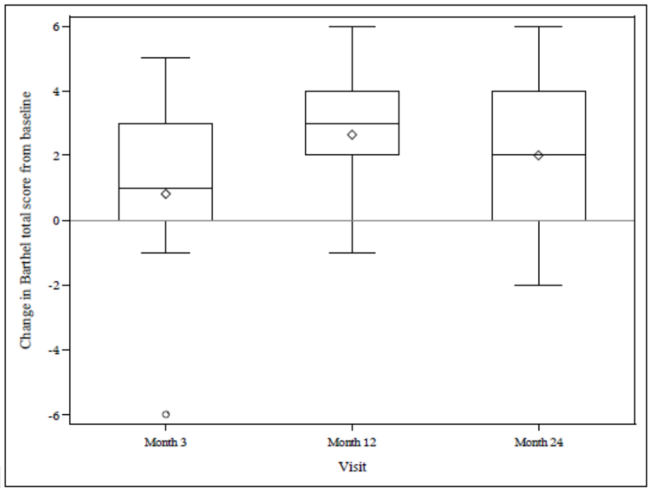
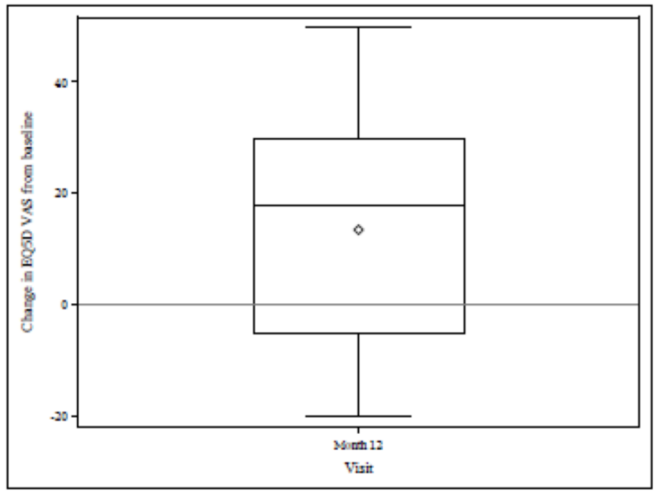
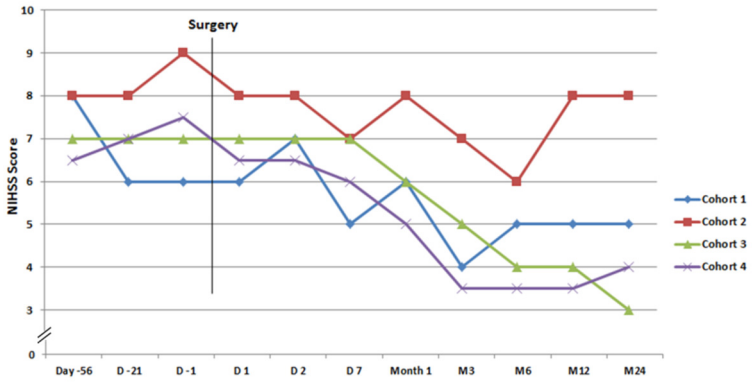
2d 

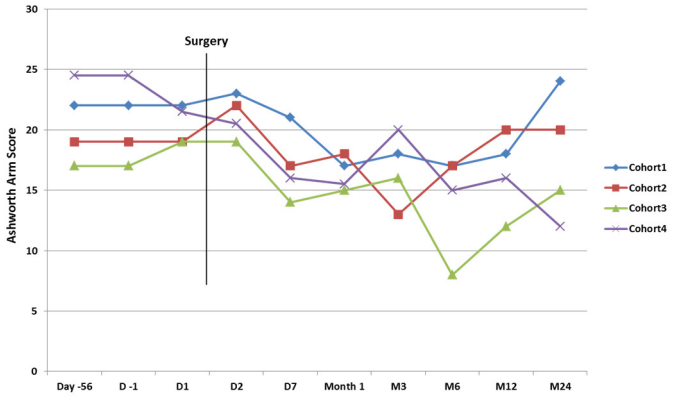
2e 

Box and whiskers plots with median, IQR and range at D-21 (left) and M12 or M24 (right) for each figure is shown. 2a. NIHSS measures neurologic deficits. 2b. Arm spasticity measured using Ashworth scale. 2c. Leg spasticity measured using Ashworth scale. 2d. Barthel Index measures activities of daily living. 2e. EQ-5D Visual Analogue Scores measures the patient reported overall health state.

Figure 4: Functional outcome measures of the four dose cohorts.



3a



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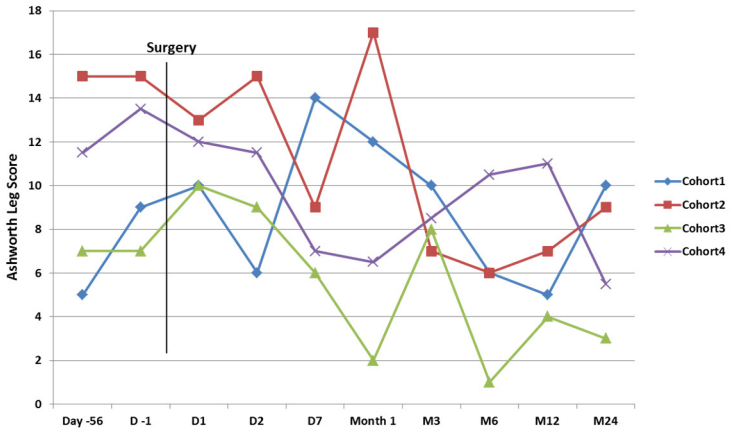

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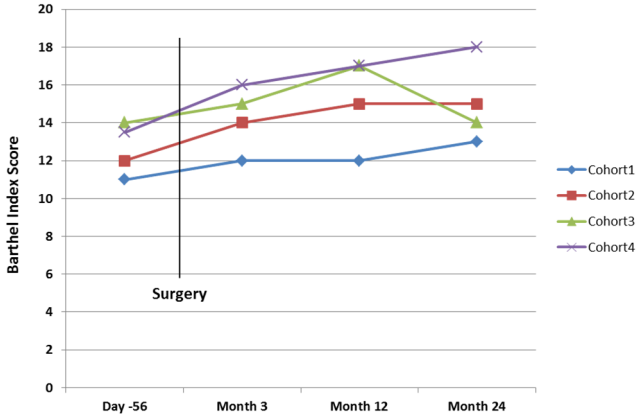
3d



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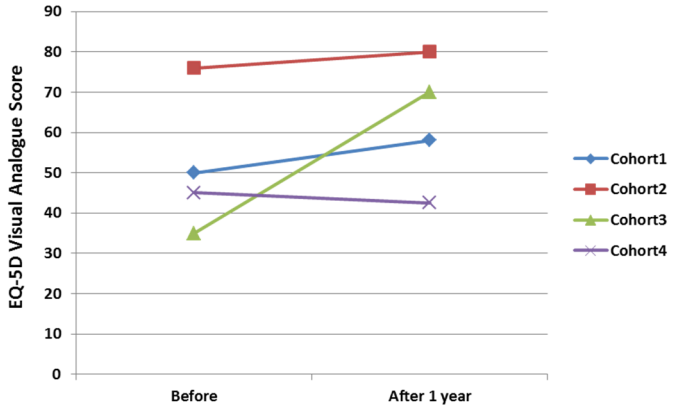

3a. Median NIHSS measuring neurologic deficits. 3b. Median Ashworth arm scores measuring arm spasticity. 3c. Median Ashworth leg scores measuring leg spasticity. 3d. Median Barthel Index trend measures activities of daily living. 3e. EQ-5D Visual Analogue Score trend measures the patient reported overall health state. Cohort1= 2million cells, cohort2= 5million cells, cohort3= 10million cells and cohort4= 20million cells.

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