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

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Key Words: Stroke, neural stem cells, clinical trial, cerebrovascular disease.

Abstract:

Background: CTX0E03 is an immortalised human neural stem cell line, developed for allogeneic therapy (CTX-DP). Dose-dependent improvement in sensorimotor function in rats implanted with CTX-DP four weeks after middle cerebral artery occlusion stroke prompted investigation of the safety and tolerability of intra-cerebral implantation of CTX-DP in stroke patients.

Methods: In an open label, single site, ascending dose study (ClinicalTrials.gov, NCT01151124),male patients (aged≥60years) with stable disability (National Institutes of Health Stroke Scale [NIHSS] ≥6 and modified Rankin Scale [mRS] 2-4) after ischaemic stroke 6-60 months previously were implanted with single doses of 2, 5, 10 or 20 million cells by stereotaxic ipsilateral putamen injection. Clinical and brain imaging data were collected over 2 years. The primary endpoint was safety (adverse events and neurological change).

Findings: Eleven male patients (mean age 69 years; range 60-82) received CTX-DP. Median (IQR) pre-implantation NIHSS was 7 (6, 8) and mean (±SD) time from stroke 29±14 months. Three had sub-cortical-only and 7 had right hemisphere infarcts. Up to 2 years after implantation, no immunological or cell-related adverse events were observed. Other adverse events were related to the procedure or comorbidities. Hyperintensity around injection tracts on magnetic resonance imaging T2W-FLAIR was observed in 5 patients. At 2 years, range of improvement (median) in NIHSS was 0 to 5 (2) points.

Interpretation: In single intracerebral doses of up to 20 million cells, no cell-related adverse events were observed in over 24 months. Neurological function was improved at 24 months. Observations support further investigation of CTX-DP in stroke.

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Introduction:

Stroke is the most common cause of adult neurologic disability worldwide, with an incidence of approximately 795,000 and 152,000 people per year in the USA and UK, respectively. Incidence, prevalence and disability-adjusted life-years lost are predicted to rise further with population ageing.[1] Stroke has profound effects on patients and their carers alike, with an enormous economic burden to society. In the UK stroke care accounts for 5% of total healthcare costs, approximately £8.9 billion per year in direct and indirect costs.[2] Among survivors, dependence in activities of daily living 3 months after onset varies from 16.2%[3] to 19.2%[4] . Stroke rehabilitative approaches aid functional recovery and brain reorganisation[5] but the effects of rehabilitation decrease with time after the event[6] and a "plateau" of recovery from stroke is observed with the first weeks to months, indicating limited endogenous recovery capacity.

At a tissue level, the capacity of the brain for neurogenesis and angiogenesis suggests that it may be possible to enhance endogenous recovery processes.[7] Pharmacological attempts to stimulate repair have to date not improved clinical outcomes, although several agents remain under investigation.[8] Cell-based therapies offer the potential to enhance brain repair, offering a more dynamic biological response to a diverse and changing environment in the injured brain than can be achieved with drug therapy.[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 in experimental stroke animal models treated with human neural stem cells (hNSCs) support the potential of this therapeutic strategy. Intracerebral delivery of stem cells, the preferred route in animal stroke studies of neural stem cells, has the advantages of controlled dosing, and improved cell delivery and survival over intravenous (IV) or intra-arterial (IA) routes that have been preferred in studies of mesenchymal stromal or related tissue-derived cell populations.[10]

In rat middle cerebral artery obstruction (MCAo) models, CTX0E03 cells injected 4 weeks after MCAo, showed a dose[11] and implantation site[12] dependent improvement in behavioural outcome measures along with histological evidence of increased host striatal angiogenesis[13] and neurogenesis.[14] Together with preclinical evidence

supporting long-term safety, pharmacodynamic interactions, pharmacokinetic bio-distribution and toxicology data formed the basis for a first-in-human clinical trial.

We report the results of Pilot Investigation of Stem Cells in Stroke (PISCES), a phase-1 dose escalation trial undertaken to investigate the safety and feasibility of intra-cerebral stereotactic implantation of CTX-DP in patients with chronic stable ischaemic stroke.

Methods:

Patients

Patients with stable neurological deficits and moderate to severe disability (defined by National Institutes of Health Stroke Scale[15] (NIHSS) ≥6 and modified Rankin Scale[16] (mRS) of 2-4) resulting from a first ischaemic stroke 6 months to 5 years previously were recruited. All patients gave fully informed consent. Patients were identified through referral from rehabilitation services or self-referral triggered by media awareness. Male patients only were recruited in order to minimise any chance of exposure to Tamoxifen, a minor metabolite of which is the ligand for the modified c-myc growth factor gene (c-mycER^{TAM}) governing replication of CTX0E03 cells (detailed under "CTX0E03 Human neural stem cells") and the "first-in-man" stage of novel investigation. Full inclusion and exclusion criteria are listed in Table 3 in supplementary information.

Trial Design

PISCES was a phase-1, open-label, single centre, dose-escalation trial of intra-cerebral stereotactic implantation of CTX0E03 hNSCs. The study was approved by the United Kingdom Medicines and Healthcare Products Regulatory Agency (MHRA), and National Research Ethics Service (NRES) [previously Gene Therapy Advisory Committee (GTAC)]. The study was registered with ClinicalTrials.gov, number NCT01151124. European Union and MHRA guidelines pertaining to Advanced Therapy Investigational Medical Products (ATIMP) were adhered to.[17] Eligible patients were recruited and in a sequential ascending dose design, 3 cohorts of 3 patients each received a single implantation of 2, 5 and 10 million CTX0E03 hNSC (40, 100 and 200 µL volume respectively) with a final cohort of 2 patients receiving 20 million cells (400 µL). The final sample size of 11 subjects was decided after interruption of cell manufacture to

changes in ownership of a contracted manufacturing site, following MHRA consultation and presentation of safety data. Consistent fulfilment of inclusion criteria and clinical stability were confirmed at three visits from two months before stereotactic implantation of CTX0E03 hNSC under general anaesthesia. Regular follow-up over 2 years included clinical and imaging data acquired at days 1 (D1), 2 (D2), 7 (D7) and months 1 (M1), 3 (M3), 6 (M6), 12 (M12), 24 (M24) along with interspersed telephone visits at days 14 (D14), 21 (D21) and months 2 (M2), 9 (M9) and 18 (M18). Adverse events were documented and reviewed. The primary endpoint was safety including adverse events, neurological deterioration or mortality. Secondary endpoints included functional change at D1, D2, D7 and M1, M3, M6, M12, M24, post implantation.

Study Oversight and Independent Review

An independent data and safety monitoring committee (DSMC) comprising of stroke, imaging and neurosurgical experts reviewed clinical and imaging data. The DSMC reviewed the M1 data for the first subject at each dose level before proceeding to subsequent subjects and M3 data after the last subject of each cohort before recommending escalation of the cell dose.

Clinical Assessments

Assessments covered neurological impairment (NIHSS)[15], disability (mRS)[16], spasticity (modified Ashworth scale)[18], activities of daily living (Barthel Index, BI)[19] and health-related quality of life (EuroQoL, EQ-5D)[20]. General physical examination and vital signs were recorded at each visit. Blood analyses included allo-antibodies, blood count, infective markers, renal and liver function.

CTX0E03 hNSC manufacture and delivery

The human Neural Stem Cell line CTX0E03[21] was clonally derived from human foetal cortical neuro-epithelial cells following retroviral insertion of a conditional immortalisation transgene, c-mycER^{TAM}. The transgene generates a MycER fusion protein that acts as a growth promoter in the cells under the control of 4-hydroxy tamoxifen (4-OHT) and confers phenotypic and genotypic stability of the CTX0E03 cells through long term expansion culture. Myc dependent cell replication is curtailed by removing 4-OHT in cultures. The hNSCs were obtained by early expansion of a single isolation from a 12 week foetal cortical neuro-epithelium. The CTX0E03 cell

line has undergone cell expansion and banking and long term storage in liquid nitrogen in accordance with Good Manufacturing Practice (cGMP). CTX-DP is manufactured under GMP from cryopreserved CTX0E03 cells as an Advanced Therapy Investigational Medicinal Product (ATIMP) 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 $5x10^4$ cells/µL. The diluent, 'HTS-FRS (Biolife Solutions, Bothell, USA)' is made up of ions, buffers, impermeants, colloid, metabolites and an antioxidant. The final formulation is devoid of 4-OHT and growth factors, restoring the cells' capability to differentiate. For every treated subject, CTX-DP was manufactured in a commercial GMP facility on the day of the surgery, transported to the hospital pharmacy under strict temperature control (2-8 ^{0}C) and implanted intra-cerebrally within 3 hours of transfer to room temperature in the operating theatre. Cell implantation was targeted to the putamen ipsilateral to the infarct since this was equivalent to the site of implantation in rodent studies, and in addition there is prior clinical experience confirming the safety of this approach for similar volumes of cells.

Surgical Procedure

Patients were reviewed by the study neurosurgeon at a pre-admission visit for discussion. Patients were admitted a day before surgery for clinical assessments, surgical consent and anaesthetic review. On the day of surgery, following a qualified person's quality approval of the CTX-DP, patients underwent CT head under general anaesthesia with a Leksell Stereotactic frame fitted (Elekta Instruments, Sweden). The operating surgeon identified suitable targets and trajectories within the basal ganglia of the affected side using pre-operatively acquired magnetic resonance imaging (MRI) (T1weighted 3D). These images were then fused with the stereotactic CT dataset using BrainLab iStereotaxy software and co-ordinates for the targets and entry points generated. A single 15mm burr-hole situated according to the calculated co-ordinates was fashioned using a craniotome. The first 2 cohorts ($2 x10^6$ & $5 x10^6$ dose) had a single injection tract to deliver cells. The 3rd ($10x10^6$ dose) and 4th ($20 x10^6$ dose) cohort required 2 and 4 tracts respectively. A maximum of 100µL was delivered per tract at the rate of 5µL/min in 20uL boluses at each of 5 points separated by 1mm along the tract. A sterile stainless steel implantation cannula (inner diameter= 0.35mm, outer diameter= 0.9mm, length= 235mm; manufactured and CE marked as a Class III medical device by ReNeuron, based on a design described

by Kondziolka et al[23]) with a luer hub was mounted within a Backlund injection needle (Elekta, Sweden) and attached to a 250µL Hamilton syringe (CE marked by ReNeuron as a sterile, class I medical device). Operative times (first incision to last stitch) ranged from 50 to 140 minutes. Patients were observed in the recovery ward until fully awake and stable physiologically before being returned to a neurosurgical ward.

Brain Imaging

Brain MRI was performed on a 3-Tesla GE-Signa-Excite-HDxt (General Electric, Milwaukee, USA) scanner. The protocol for structural brain imaging included T1W sagittal FLAIR (Time to Echo (TE) 8.5ms, Time to repetition (TR) 2.5s, Inversion time (TI) 920ms), T1W IR-FSPGR 3-dimensional (TE1.5ms, TR7.2ms, TI500ms), T2W PROP Fast Spin Echo (TR5s,TE109.2ms), T2* gradient echo (TE22ms, TR670ms, flip angle 10º) and T2W FLAIR (TE140ms, TR10s, TI2250ms, slice thickness 5mm, slice gap 1.5mm) sequences. These were acquired at day -56, day -21, M1, M3, M12 and M24. Additional T1w 3D post gadolinium and T2w 3-dimensional FLAIR (TE128.3ms, TR6000ms, TI1857ms) were acquired after January 2014 following scanner software upgrade. An experienced neuroradiologist reviewed all images.

Diffusion tensor imaging (DTI) was acquired at multiple (D-21, M1 and M12) time points to measure longitudinal change in fractional anisotropy (FA), a surrogate marker of white matter integrity, around the needle tracts. One acquisition of DTI images (TR11s, TE87.1ms, matrix 128x128, FOV240, 1.8x1.8x5 mm voxels, 34 directions with b values 0 and 1000 s/mm) was collected. DTI pre-processing and region-of-interest analyses are included in supplementary information.

Immunological Monitoring

Patients did not receive any immunosuppressive therapy. Venous blood was obtained for analysis of HLA Class I and II antibodies against CTX0E03 pre-treatment and at M1, M3, M6, M12 and M24. Allo-antibody positive patients were excluded prior to implantation.

Statistical Analysis

Adverse events and change in NIHSS neurological function were recorded. Functional outcome data are reported as either median and interquartile range (Q1, Q3) or mean

and standard deviation (SD). All statistics were done using SAS v9.3, Microsoft Excel 2010 and Minitab 16. Change in FA on DTI is reported using the Cohen's d effect size.

Role of Funding Source

The sponsors of the study contributed to study design but had no role in patient selection, recruitment, data collection, follow-up and imaging analysis. They reviewed the trial report before submission for publication. All authors had full access to the data. The responsibility for submission was that of the corresponding author, agreed by the DSMC chair.

Results:

Thirteen male patients were recruited between September 2010 and January 2013, of whom 2 were, excluded pre-implantation, one due to a seizure, and the other for the presence of a possible allo-antibody. Eleven received CTX-DP. This report covers the period up to median follow-up post implantation of 44 months (range 33 to 60 months), with the last recruited patient completing 33 months. Baseline demographics and stroke characteristics are listed in Table 1. A lesion overlap map showing the distribution of cerebral infarcts is shown in figure 2. Individual scans are available in the web-appendix (figure 9).

Adverse Events

All patients were discharged home on day 2 after surgery. Serious adverse events (SAE) are summarised in Table 2 (non-serious adverse events are described in table 4 in the web-appendix). All SAEs were related to the neurosurgical procedure, or to incidental or known medical conditions. One new ischaemic stroke, an occipital infarct not present on day -56 or day -21 brain imaging, was noticed retrospectively on the pre-surgical CT, but identified clinically only after new visual symptoms were described by the subject some weeks later. A superficial malignant melanoma occurred in one subject with chronic sun exposure history. No event was considered attributable to CTX-DP.

Screening for cellular rejection

All CTX-DP implanted patients were HLA negative before and after intervention.

Functional Outcome Measures

Individual patient data showing changes in NIHSS, Ashworth arm and leg scores, Barthel Index, and EQ-5D over time are shown in Figure 3: all functional measures change from baseline (figure 6) and median change by dose cohort (figure 7) are available in online web-appendix. Pre-operative neurological deficits and spasticity were stable in all patients. After CTX-DP implantation, improvements over time were noted in NIHSS, summated Ashworth scores for arm and leg and Barthel Index. Disability as measured by modified Rankin scale at 1 year, was unchanged in 7/11 patients and improved by 1 grade in 4 patients and at 2 years, was unchanged in 7/11, worsened by 2 grades in 1/11 and improved by 1 grade in 3/11 patients. Patient-reported overall health state as measured by the visual analogue sub-score of the EQ-5D improved by median 18 (-5, 30) at 12 months compared to baseline.

Brain Imaging

Qualitative: Five patients (P2, P3, P4, P7 and P9) showed hyper-intensity around the needle injection tract on T2w FLAIR images. Hyper-intensity was first seen at M1 and persisted at M24 (figure 4a). Two further patients (P1 and P8) had subtle increase in pre-existing peri-infarct white matter T2w FLAIR hyper-intensity between M1 and M12 (figure 4b). No changes were seen in the remainder of the patients. No clinical association with these changes was observed. The DSMC's qualitative safety review of all scans concluded no significant increase in T2w hyper-intensities over time.

Quantitative: Mean FA on an axial ROI was reduced at 1 month (post implantation) compared to baseline since voxels within the injection tract contributed zero values. At month 12 compared to month 1, four patients (P2, P4, P7, P9) showed reduced FA in 17/28 sampled slices (n=4) and increased FA in 9/28 slices (figure 5). All slices showed reduced FA in 1 patient (P3). In 4/9 slices increased FA was closer to putamen and 5/9 slices were closer to cortex.

Discussion:

This "first-in-man" study offers preliminary data on the feasibility, tolerability and cell-related safety of stereotactic intra-cerebral injection of the genetically modified human neural stem cell line CTX0E03-DP in patients with chronic ischaemic stroke.

We observed 4 asymptomatic procedural SAEs in 4 of 11 patients, consistent with safety data for brain stereotactic procedures generally.[24] Unlike previous trials in stroke of teratocarcinoma-derived neuronal cells [25,26] and foetal porcine cells[27], we did not observe any post-operative seizures. In one patient a seizure event, 10 months after implantation, was likely precipitated by alcohol withdrawal. Superficial melanoma was diagnosed on histology (pT1a N0 M0)[28] in 1 patient, 6 months after elective excision of a painful mole that had been present in a sun-exposed region (pinna) for >10 years. This patient had previously been prescribed antimetabolite skin creams for sun-related skin injury. The majority of other adverse events were due to systemic co-morbidities including falls and elective procedures that required hospital admissions. This profile is expected in disabled stroke survivors with multiple comorbidities.[29]

Hyper-intensity on T2 weighted FLAIR MRI was observed around the needle tract in 5 patients at some point during the follow-up period. In general, this may be attributable to various causes including localised inflammation, graft-host reaction, gliosis or dysmyelinosis. Studies of longitudinal imaging in patients following stereotactic procedures for functional reasons are lacking, so it is unclear whether this imaging feature is related specifically to cell injection. Increased FA after cell implantation as was observed in several axial slices along the tract has been related to increased myelination in some conditions, [30, 31] suggesting potential improvement in microstructural white matter. Planned post-mortem pathological studies may in time offer additional data to characterise this finding.

In animal models, stem cells of various kinds are associated with better neurological outcomes after focal brain ischaemia. Human neural stem cells have neural cell differentiation potential in addition to paracrine effects, and have most commonly been developed as allogeneic therapy, giving the potential flexibility of implantation in acute or sub-acute periods without dependence on successful cell harvest, extracorporeal cell expansion in a laboratory from days to weeks and uncertain dosing inherent in autologous cell therapies. Stereotactic intracranial injection ensures delivery of the intended cell dose to the target site adjacent to the ischaemic damage, replicating the conditions of animal studies of CTX-DP and offering a strategy more likely to yield proof-of-concept for cell therapy than less invasive routes. IV or IA administration might be safer, but animal data indicate that these routes result in

negligible cell engraftment in the brain[10] and are therefore reliant on diffuse paracrine or even peripherally mediated therapeutic effects.[32]

Exploratory indices of efficacy were secondary end-points. Given small patient numbers, a heterogeneous population, and the open-label, single arm design, no reliable conclusions can be drawn about the effects of cell implantation on neurological or functional change. It was notable, however, that despite selection of chronic, stable patients at late stages after stroke, the majority of participants showed some improvement across several indices of function, including in 4 individuals (median 32.5 months since stroke; range 21-51) moving across a modified Rankin Scale threshold. Whether attributable to cell implantation or to other factors, such as engagement with trial evaluations and increased generic medical input, change in this population suggests that trials of intervention at late stages of stroke, when recovery is not generally believed to be attainable, may be worthwhile. Anecdotal accounts described reduced spasticity, minor return of finger movement at phalangeal joints, improved visual perception and better bed-to-chair transfers, and are supported by changes 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 post-implantation deterioration. Other indices of neurological function are likely to offer better sensitivity to neurological functional change 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[33] for in-vitro control of cell number replication, only males were considered for this stage of trial. However, together with no preclinical evidence of in-vivo cell cycle switching observed and safety data from PISCES, future studies will not be limited to male patients only.

Patients were not administered immunosuppressive drugs since non clinical studies of CTX0E03 found no evidence of cell survival and efficacy requiring immunosuppression, in vitro studies for MHC-DR and MHC-ABC showed low protein expression for CTX0E03 and to minimise the risk of post-stroke infections which are independently associated with poor outcome.

The putamen was chosen for implantation based on preclinical data as the closest intact subcortical neuronal cluster and preferable to white matter injections that can cause pressure-related further axonal injury. Dose selection was extrapolated by scaling up from efficacious doses in rats and an ascending dose design selected to allow cautious dose increments after safety review. Inclusion of appropriate concurrent controls and measures to ensure blinding will be essential for future efficacy-focussed investigations. The value of including control groups in early phase clinical investigations involving invasive procedures in small numbers of severely disabled subjects is debated. A non-operated control group, although considered, was not pursued as it was thought unlikely to provide valid control data, especially given stroke lesion heterogeneity and small patient numbers. A placebo surgery control group raises ethical concerns about exposure to surgical and anaesthesia risks, and may be unacceptable to patients.[34]

Limitations: A small sample size by design limits the number of patients being exposed to each dose level, particularly only two patients receiving the highest dose due to cell production issues. Any adverse events of low incidence may not therefore have been identified. Safety was assessed over a 2 year period, but it is conceivable that longer term safety issues might occur, and lifelong surveillance is being undertaken. The open label design and lack of control subjects mean that exploratory efficacy data should be regarded with extreme caution. It is possible to exclude the possibility that any neurological change over time might result from stereotaxic injection rather than cell implantation, although such effects have not been observed in animal models with placebo injection.

In conclusion, we observed no adverse events after treating 11 chronic stroke patients with intracerebral implantation of CTX hNSC and the longitudinal clinical observations suggest that this novel cell therapy for ischaemic stroke is feasible, safe and would warrant a larger, phase 2 trial.

Panel: Research in Context

Systematic Review: We searched the PubMed database 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”, excluding articles concerning mesenchymal stem cells, bone marrow derived cells, animal studies and non-

ischaemic stroke. We found no studies that have investigated intracranial delivery of neural stem cells alone. One study[35] compared and reported intra-cisternal delivery of a combination of human foetal neural stem progenitor cells of unspecified origin and MSCs with IV MSCs alone in 6 patients between 1 week and 2 years after stroke. Intracranial delivery of autologous cells in stroke has been reported for teratocarcinoma-derived cells.[26] There are several published and on-going studies investigating IV delivery of autologous MSCs which have several differences compared to NSCs including timing, mechanism of action and delivery.

Interpretation: Our study is the first report of the intracranial administration of human neural stem cells in chronic ischaemic stroke patients. These results are a significant addition to the current literature because of the novel potential treatment for stroke patients, however further research in carefully selected patients is needed.

Contributors:

KM was chief investigator who designed and managed the study. DK was the co-investigator who recruited patients, collected and analysed data, wrote the first draft and subsequent versions with input and key revisions by all authors. JS and KP developed the stem cell product and as ReNeuron representatives sponsored the trial. LD was the neurosurgeon who performed all surgeries. WS was the research nurse who co-ordinated patient visits. CH and AM managed trial statistics. JM and CS managed imaging data acquisition and safety reporting. PB chaired the Data and Safety Monitoring Committee and helped design the study. All authors reviewed and approved the final report.

Conflicts of Interest:

DK has received travel grants from Guarantors of Brain, Jim Gatheral and Mac Robertson scholarship. JM, WS, CS and LD have no conflicts of interest. CH and AM's university employer have received funding from ReNeuron. JS and KP are employees and stock holders of ReNeuron. JS has a patent cell Lines issued to ReNeuron, and a patent neural transplantation issued to ReNeuron. KP has a patent US 7,416,888 B2 issued. PB has received honoraria from ReNeuron. KM has received trial funding from ReNeuron for PISCES and for the ongoing Phase 2 stroke stem cell trial.

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Data & Safety Monitoring Committee (DSMC) committee: Prof.Philip Bath DSc (DSMC Chair, University of Nottingham, UK), Prof.Joanna Wardlaw MD (Neuroradiologist, University of Edinburgh, UK), Prof.Ian Whittle MD (Neurosurgeon, University of Edinburgh, UK), Dr.Christopher Weir PhD (Biostatistician, University of Edinburgh, UK)

Table 1: Baseline demographic data

Patient	Dose of cells	Age (years)	Months since stroke	Infarct Hemisphere; Vascular territory	Risk Factors	NIHSS	mRS	BI
P1	2 million	68	14	Left Cortical, MCA	Smoking, high cholesterol	8	4	12
P2		82	21	Right subcortical, MCA	Smoking, hypertension, family history stroke & diabetes	9	4	10
P3		78	51	Left Subcortical, MCA	Smoking, family history diabetes	6	4	11
P4	5 million	75	32	Right cortical, PCA	Smoking, hypertension, h/o myocardial infarction	6	3	14
P5		69	33	Right Cortical, MCA &ACA	Smoking, hypertension, high cholesterol, diabetes mellitus	10	4	9
P6		61	12	Right Cortical, MCA	Smoking, high cholesterol, family history of stroke & diabetes	8	4	12
P7	10 million	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 mellitus	7	3	13
P10	20 million	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
Median (Q1, Q3)		68 (61, 75)	32 (14, 44)			7 (6, 8)	3(3, 4)	12 (11, 14)

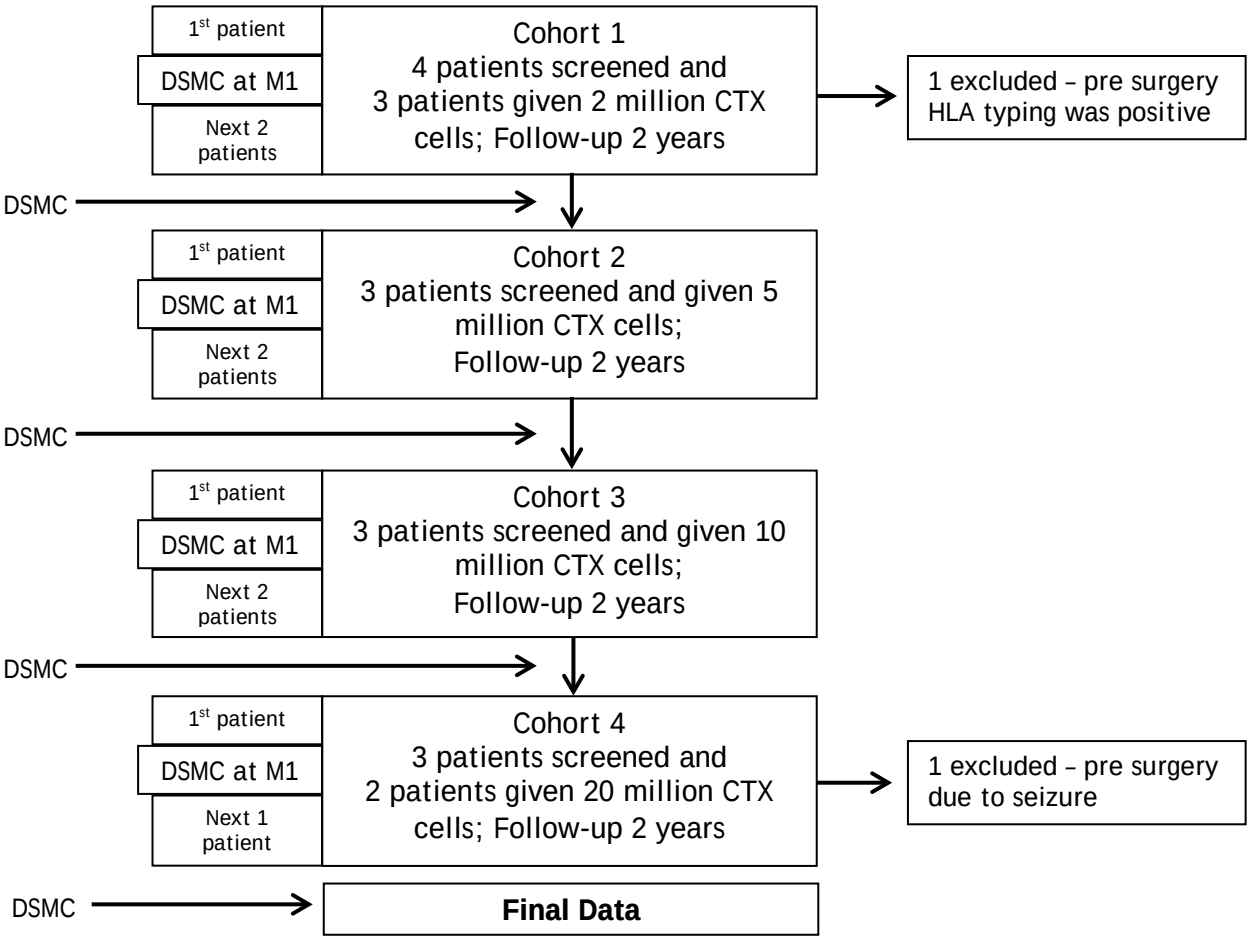
MCA= Middle Cerebral Artery; NIHSS= National Institute of Health Stroke Scale; mRS= modified Rankin Scale; BI= Barthel Index

Table 2: Serious Adverse Events

Event	Cohort	Time after surgery (months)	Attributed Cause	SUSAR
1 month Peri-operative				
Extradural Haematoma (asymptomatic)	1	1	Procedure	Yes
Subdural haematoma (asymptomatic)	1	1	Procedure and anticoagulant use	Yes
Right Occipital infarct (pre-surgical onset)	3	0	Withholding anti-platelets prior to surgery	-
From 1 to 6 months				
Cystoscopy - Elective surveillance procedure	1	6	Hospitalisation	-
Minor bleed at the burr hole on MRI (2subjects)	1 & 2	1	Procedure	-
Malignant melanoma – Left Ear Pinna	3	6	Pre-stroke high risk	-
6 months and beyond				
Diverticulitis – flare up	1	7	Pre-stroke risk	-
Hematemesis	1	8	Pre-stroke risk	-
Perforated sigmoid diverticulum	1	16	Pre-stroke risk	-
Colonoscopy for altered bowel	2	8	Pre-stroke risk	-
Seizure	3	10	Alcohol withdrawal	-
Alcohol withdrawal syndrome	3	12	Regular alcohol use	-
Collapse – Low Sodium	3	18	Acute on chronic hyponatremia	-
Gastroenteritis	3	23	Infection	-
Community acquired pneumonia	4	11	General infection risk	-

SUSAR= Sudden Unexpected Serious Adverse Reaction; MRI= Magnetic Resonance Imaging

Figure 1: Trial Patient Flow



DSMC= Data Safety Monitoring Committee; CTX= CTX0E03 stem cells

Figure 2: Spectrum of Ischaemic lesions of all 11 subjects (overlapped)

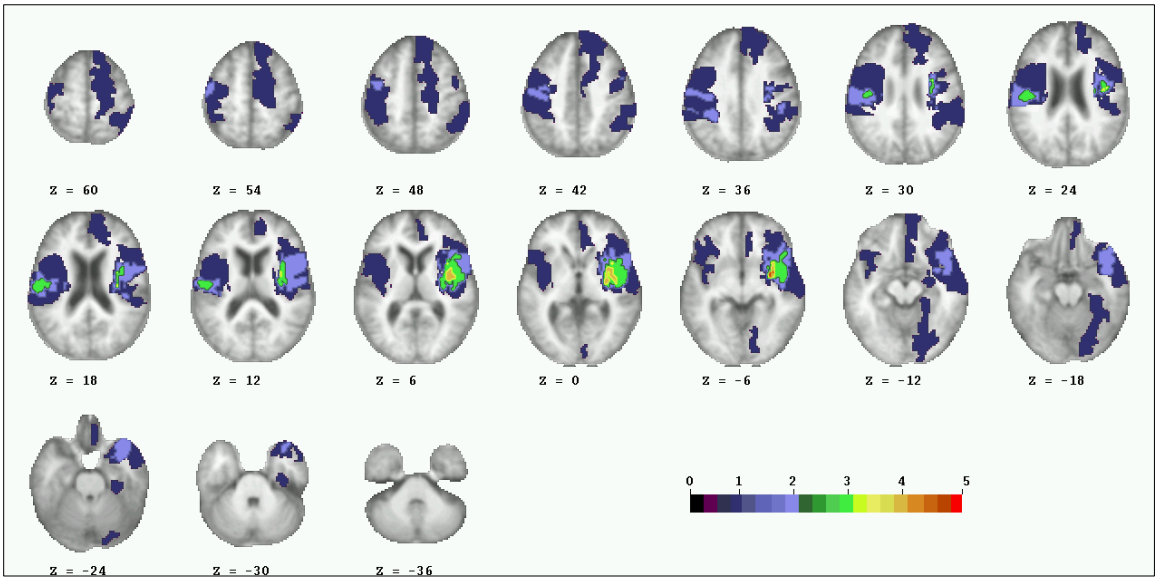
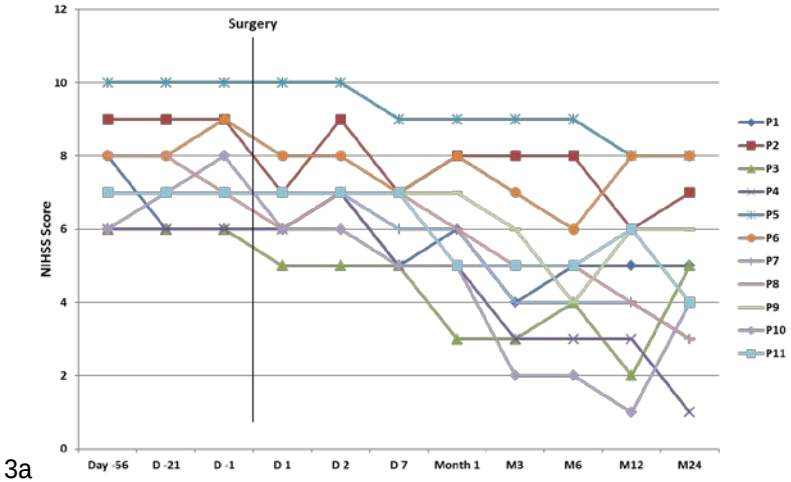


Figure 3: Functional Outcome Measures of all patients.



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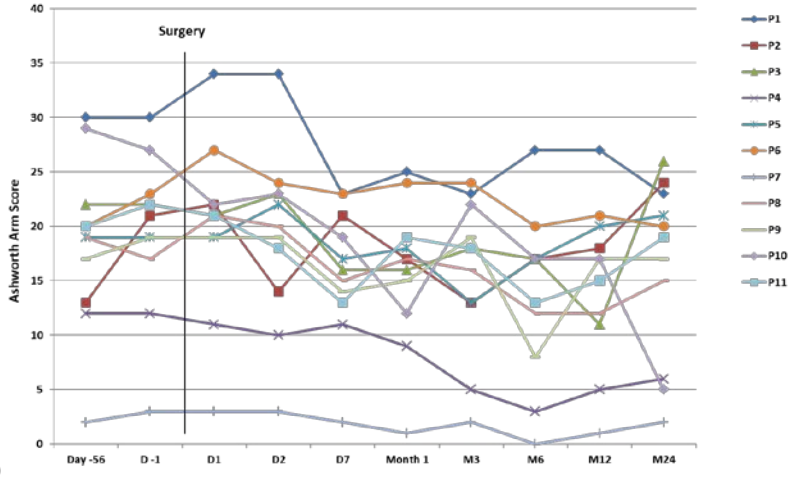
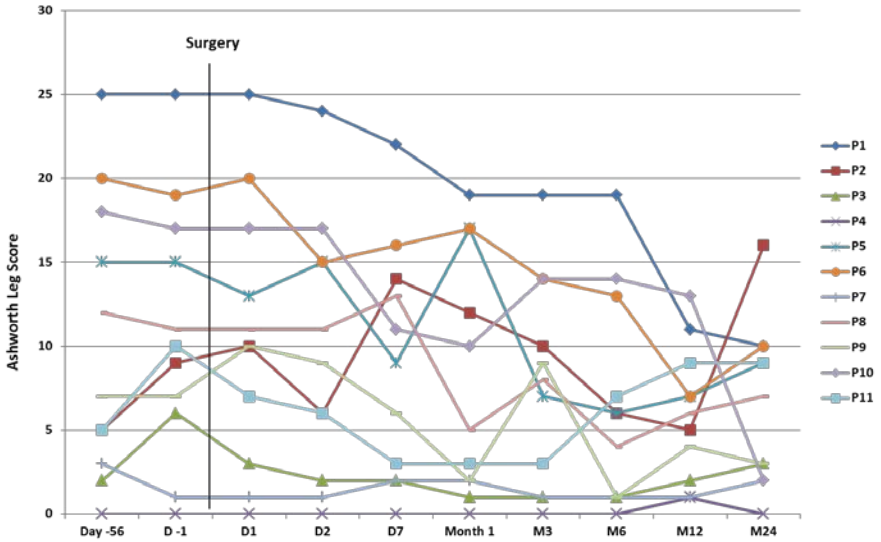
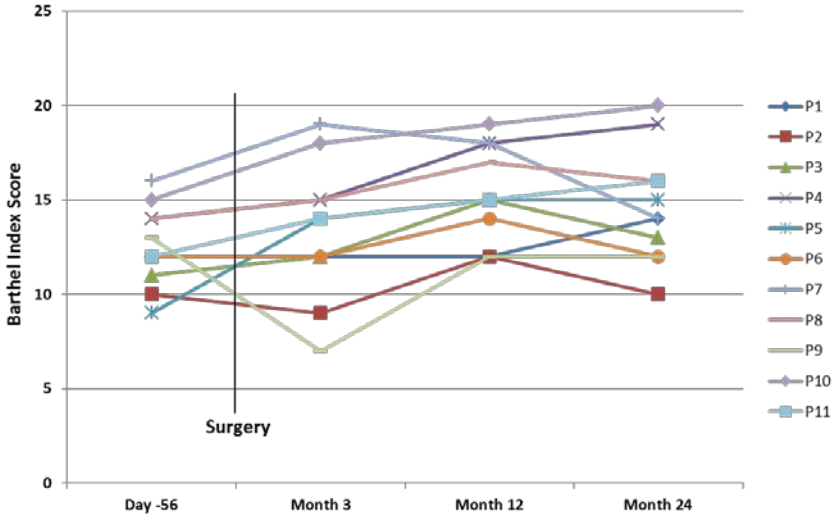


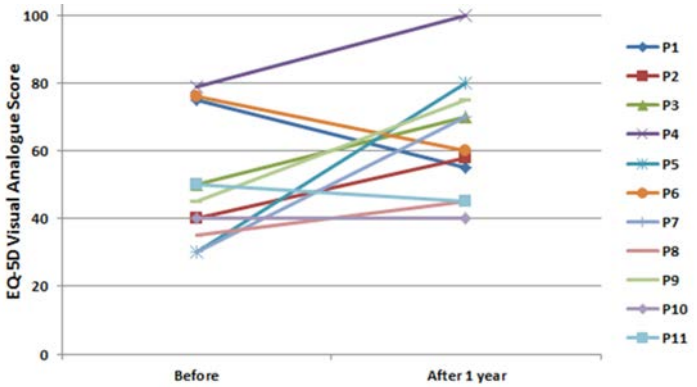
3b

3c





3d

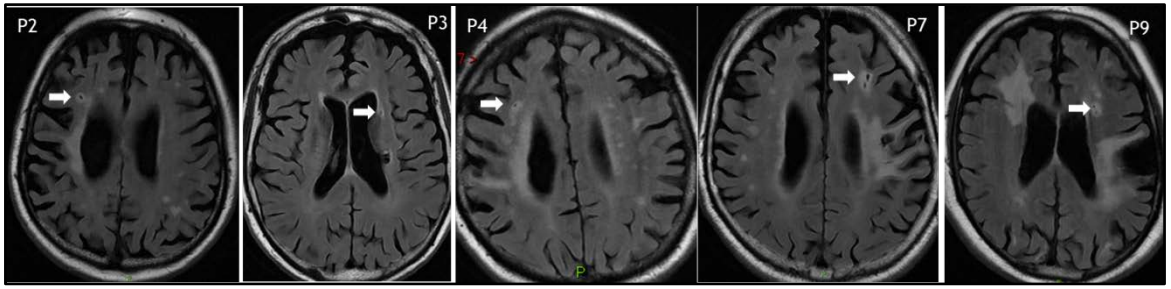


3e

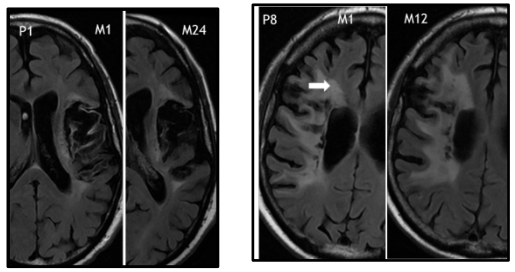
Line plots of all individual patients at D-56 (left) and M12 or M24 (right) for each figure is shown. 3a. NIHSS measures neurologic deficits. 3b. Arm spasticity measured using Ashworth scale. 3c. Leg spasticity measured using Ashworth scale. 3d. Barthel Index measures activities of daily living. 3e. EQ-5D Visual Analogue Scores measures the patient reported overall health state.

Figure 4:

a

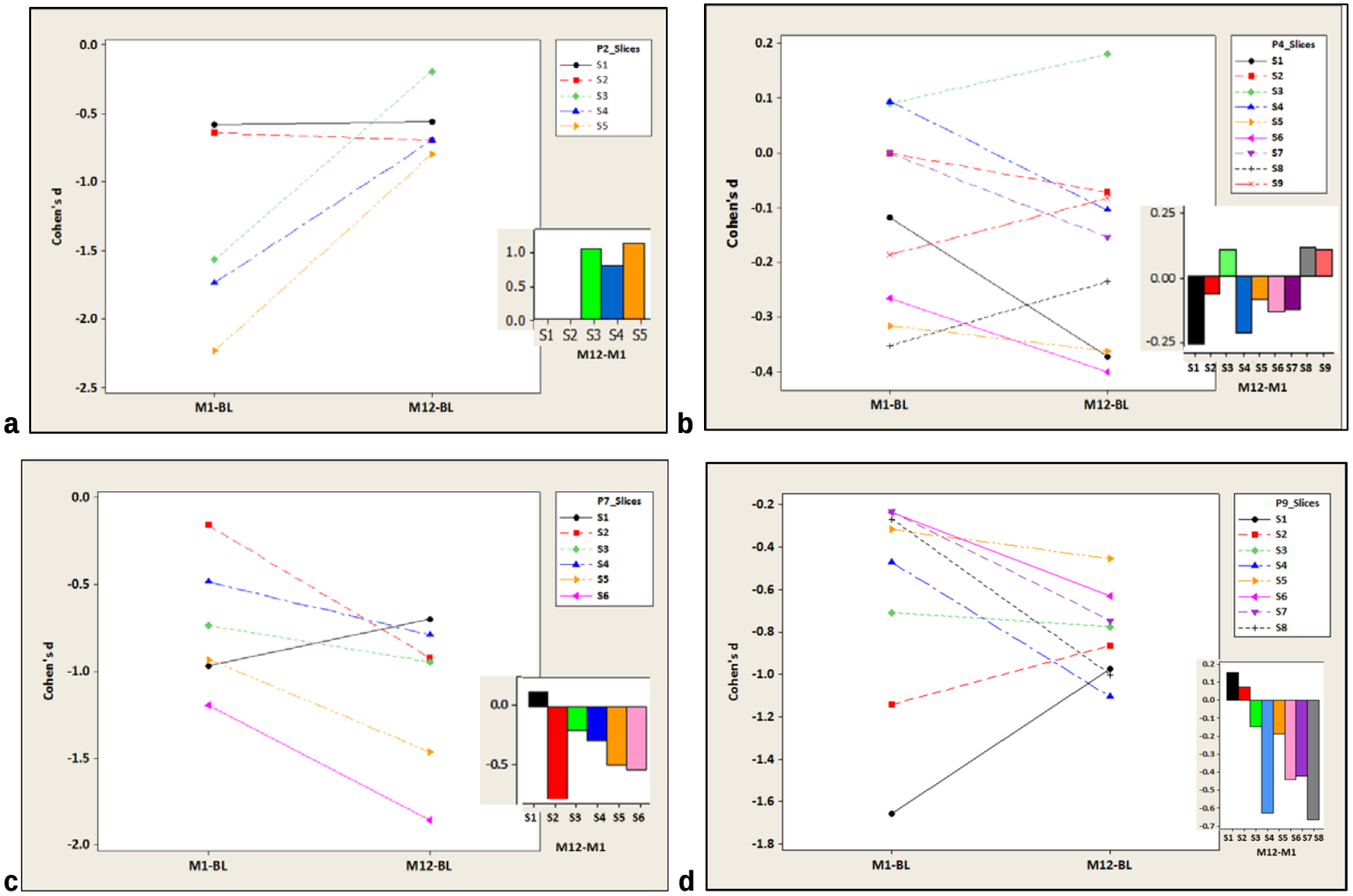


b



7a. Hyper-intensity around injection tract in T2W FLAIR sequences in 5 patients (P2, P3, P4, P7, P9) with injection tract distinct from the lesion or pre-existing gliosis (representative axial cut) 7b. In 2 patients (P1 & P8) increased peri-infarct white matter hyper-intensity is seen at M24 for P1 and M12 for P8.

Figure 5: Line plot of change in Cohen's d values of different axial brain slices (S1 to S9) from month 1 (M1) to month 12 (M12) compared to baseline (BL) for patients P2 (5a), P4 (5b), P7 (5c) and P9 (5d). The bar graph illustrates the post intervention change between the months M1 and M12.



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