ReNeuron Limited

Pencoed Business Park, Pencoed, Bridgend, CF35 5HY

CLINICAL TRIAL PROTOCOL

Trial Number: RN01-CP-0002

Version Number: 10.0

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Trial Title: A Phase II Efficacy Study of Intracerebral CTX0E03 DP in Patients with Stable Paresis of the Arm Following an Ischaemic Stroke.

EudraCT Number: 2012-003482-18

Product: CTX0E03 DP

Development Phase: Phase II

Chief Investigator:
Professor Keith Muir Tel +44 (0)(0)141 4515892

Sponsor Personnel:

Chief Medical Officer (office hours):
Dr Julian Howell Tel +44 +(0)7983 552 209

Pharmacovigilance Provider:

SAE Reporting: 24-hour cover:
ProductLife Limited
Tel +44 (0)1223 402660
Fax +44 (0)1223 413689
Email:safety@productlife-group.com

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1. Amendments to Previous Versions

1.1 Protocol Amendment (Version 9.0 to 10.0)

a) Revision to duration of long term safety evaluation

The requirement to follow all subjects who receive treatment with CTX0E03 DP for the remainder of their life as part of the long-term safety evaluation has been amended.

The amendment follows a comprehensive review of the technology employed in the development of the CTX0E03 cell line, current FDA and EMA guidelines, and available safety profile of CTX0E03 DP (following intracerebral treatment in 34 patients with follow-up ranging so far from 11 months to 7 years).

The amendment has also given important consideration to the difficulties in the practicalities of the sponsor and Registries following participating subjects for extended durations, and the design of follow-up observations to ensure that they are appropriate to detect potential delayed adverse events. Specifically, the risk of delayed adverse events is considered low for a number of reasons:

i) The retroviral transfection of the CTX0E03 cells was a single event in the derivation of the cell line. Replication Competent Retrovirus testing has been performed at several points in the manufacturing process and demonstrated absence of the retrovirus
ii) Sponsor data has confirmed that the CTX0E03 cells do not harbour the MoMLV *gag-pol* gene. This data is considered as evidence that CTX0E03 cells cannot replicate a c-mycER^{TAM} transducing retrovirus.
iii) The CTX0E03 cell line has the retrovirus inserted into a gene with no known oncogenic function. The nature of the insertion is unlikely to give rise to gene disruption or activation.
iv) The insertion does not fall near any endogenous retroviruses, whose activation might conceivably induce oncogenic activation.
v) There are no other genes other than that into which the insertion falls that are sufficiently close or of sufficient oncogenic potential to raise concerns regarding oncogenic activation.
vi) The CTX0E03 cells have maintained a normal human diploid karyotype and no chromosomal aberrations from early (P10) to late (P45) passage.
vii) Data has demonstrated that re-exposure of growth arrested/differentiated CTX0E03 cells to 4-OHT *in vivo*, does not return them to a proliferative state.
viii) Study results have shown that endogenous steroid hormones do not activate the expressed c-MycER^{TAM} protein *in vitro* indicating that such agents would not activate the technology and drive CTX0E03 cells to a proliferative state after administration.
ix) Studies *in vivo* have found that the c-mycER^{TAM} expression is silenced through methylation after administration, adding a further safety factor to the technology.
x) The potential therapeutic effect of the CTX0E03 DP arises from an immunomodulatory, neurogenic and angiogenic action following administration. There is no modification of host (patient) cell genome as a result of administration of CTX0E03 DP.
xi) Two in vivo tumourigenicity studies were performed with concomitant tamoxifen administration alongside CTX0E03 administration in order to confirm the above-mentioned findings. The results of these studies was that concomitant administration of tamoxifen with CTX0E03 cells did not result in an increased incidence of tumor compared to animals treated with CTX0E03 alone, tamoxifen alone or untreated control animals.
xii) The risk of oncogenic activation as a consequence of insertional mutagenesis can never be considered to be zero, since there must remain oncogenic genes and mechanisms yet to be discovered. Nonetheless, given the state of current knowledge,

it appears reasonable to conclude that the risk of oncogenic activation as a consequence of insertional mutagenesis in the CTX0E03 cell line is very low.

xiii) Data demonstrate that a significant number of the implanted CTX0E03 cells undergo apoptosis shortly after administration

xiv) From these findings, it is reasonable to conclude that the risk of CTX0E03 cells transforming, migrating, or otherwise having the potential to develop ectopic tissue that would require long-term safety monitoring is very low.

Based upon the above features of the technology, the sponsor considers that CTX0E03 DP does not present safety concerns that justify a life-long safety monitoring duration as included in the current protocol. The sponsor considers that a 5 year safety follow-up duration is sufficient to detect potential delayed adverse events in subjects enrolled in this study, and that this will additionally minimise unnecessary subject burden, site administration and promote better subject compliance.

The following sections have been amended:

Section 9.3 Withdrawal of Patients from Treatment or Assessment

Section 11.4.13: Registry Follow-up

Section 11.4.14: Annual GP follow up

Appendix 1: Trial Evaluation Schedule

b) The removal of requirement for site to contact GP annually

The requirement for sites to contact GPs annually following the 12 month (post treatment) on study period has been removed. The required information, including incidence of cancer, primary site of tumour and death and cause of death (if applicable), will be obtained from the relevant national cancer registry, thus negating a duplication in effort and data. This data will now be collected for a period of 5 years in the first instance, as described above.

The following sections have been amended:

Section 11.4.14: Annual GP follow up

Appendix 1: Trial Evaluation Schedule

c) Administrative and other changes

In addition to the above, the protocol has undergone typographical and administrative changes to aid clarity.

1.1 Protocol Amendment (Version 8.0 to 9.0)

Safety Follow up Assessments

Brain imaging post- implantation safety assessment requirements have been amended to allow CT scan to be conducted for patients that have developed a contraindication to MRI

Post-treatment MRI scans at Visit 9 (day 180) and Visit 10 (day 365) are for safety evaluation as part of long term follow-up. In the event that a subject becomes contraindicated for MRI scan, Safety follow up CT Scans can now be conducted in place of the scheduled post-treatment MRI scan (only for patients who become contraindicated to MRI following enrolment in the study). As a result the following sections have been updated:

Section 11.4.5: Scans

Appendix 1: Trial Evaluation Schedule

Ethical Conduct of the Trial

The protocol wording has been revised to make clear that the study is run in accordance with all current applicable Clinical Trials Regulations, and to allow for alterations in study conduct to be made in accordance with any updated clinical trials regulations. The following sections have been updated:

Section 4.2: Ethical Conduct of the Trial

Patient Medical Records

The protocol has been updated to specify that the funcational assessment rating scales bound within the CRF and used to document subjects' ARAT, RFA, BI, NIHSS and FMA scores are source data. The following section has been updated:

Section 17.8: Patient Medical Records

Administrative and other changes

In addition to the above, the protocol has undergone administrative changes to the following sections:

Front page: Update to Chief Investigator telephone contact number

Front page: Updated PharmacovigiaInce safety reporting email address

1.2 Protocol Amendment (Version 7.0 to 8.0)

Primary endpoint measurement will be taken at Day 90 (Month 3) (formerly Day 180 (Month 6))

The primary endpoint will now be assessed at Day 90 (Month 3) (formerly Day 180 (Month 6)), but the primary efficacy measure, ARAT, will not change. ARAT and the other supportive efficacy measures (NIHSS, RFA and BI) will continue to be assessed at Day 28, 90, 180 and 365.

Review of the 2 year data arising from the RN01-CP-0001 study (A phase I safety study in patients with stable ischaemic stroke) has suggested a positive response by Month 3 (by measurement of NIHSS) in patients who received a single dose of CTX0E03 DP cells administered by direct intrastriatal implantation into the putamen. A 3-month time to response has also been observed in data emerging from other ongoing clinical studies evaluating the safety and efficacy of cell therapy in patients with chronic motor deficit due to ischemic stroke. An earlier endpoint review will thereby best position the primary ARAT efficacy measurement for a response at Month-3 in chronic stroke patients whilst continuing to record ARAT and other efficacy measures by various methods at the protocol specified timepoints.

The following sections have been amended:

Section 2.0: Trial Synopsis

Section 8.1: Primary Objective

The inclusion of an additional sensorimotor functional assessment as a secondary endpoint: Fugl-Meyer Assessment (FMA)

The Fugl-Meyer Assessment (FMA) is a stroke-specific, performance-based impairment index. It is designed to assess motor functioning, balance, sensation and joint functioning in patients with post-stroke hemiplegia. This universally recognised assessment is used to determine disease severity, describe motor recovery, and to plan and assess treatment.

The addition of the FMA on both upper and lower limbs as a secondary efficacy measure will enable an enhanced overall evaluation of sensorimotor response as measured by an increased number of collective efficacy measures (ARAT, NIHSS, RFA, BI and Fugl-Meyer), although ARAT will be retained as the primary measure. This will thereby improve the reliability of the final benefit/risk assessment of the CTX0E03 DP treatment under investigation.

The following sections have been amended:

Section 2.0: Trial Synopsis

Section 3.0: List of Acronyms, Aacronyms, bbreviations and Definition of Terms

Section 8.2: Secondary Objectives

Section 9.1: Description of Overall Trial Design

Section 9.4: Re-screening of Patients

Section 11.1: Efficacy Measurements

Section 11.4.12: The Fugl-Meyer Assessment

Section 26.0: References

Appendix 1: Trial Evaluation schedule

The following new sections has been added:

Appendix 6: Fugl-Meyer Assessment

Change to the overall study design

The study design has been changed from a "Simon Two-Stage design" to a single-cohort design (i.e. up to the end of the currently described Stage 1).

Ongoing evaluation of data and reports of late-phase recovery in stroke indicated that the 'pass/fail' approach of the Two-Stage design could miss smaller, but clinically meaningful, improvements in motor impairment. For this reason the Fugl-Meyer Assessment has been added, which is more sensitive to change and includes lower limb.

The change in study design and the early completion of Stage I of the study will also facilitate the transition to a larger randomised Phase III study based on the enhanced evaluation of sensorimotor response and pending safety profile, and thus offer continued access of this novel treatment to patients in an area of high unmet need.

The following sections have been amended:

Protocol cover page

Section 2.0: Trial Synopsis

Section 5.0: Investigators and Trial Administrative Structure

Section 6.5: Rationale for Study Design

Section 9.1: Description of Overall Trial Design

Section 13.1: Determination of Sample Size

Section 13.3: Interim Analyses

Section 14.0: Data Safety Monitoring Boards (DSMB)

The change to the study design has had an impact on the following parameters:

1) Total number of patients to be treated

Consequent to the changes made to the study design, at least 21 patients (instead of the 41 proposed in the Simon Two-Stage design) will be enrolled. Patients will receive CTX0E03 DP (20 million cells) by stereotaxic intra-striatal implantation ipsilateral to the location of the supratentorial ischemic stroke.

Baseline and all post-treatment evaluations will remain unchanged (other than the additional FMA mentioned above).The study will continue to be overseen by an independent DSMB at the pre-determined intervals stated in the protocol.

The following specific section has been amended:

Section 9.1: Description of Overall Trial Design

2) Removal of Interim analysis

Consequent to the changes made to the study design i.e. a single cohort of 21 patients, there is no longer the necessity for an interim analysis to be performed as the study will terminate after the completion of the 21st patient.

The following specific section has been amended:

Section 13.3: Interim Analyses

3) Change to the Statistical Analyses

Consequent to the changes made to the study design, the statistical calculations have been revised to describe how the data collected from Stage 1 of the study will be analysed and provide a sufficient basis for efficacy evaluation.

The following specific sections have been amended:

Section 8.1: Primary Objective

Section 13.1: Determination of Sample Size

Section 14: Data Safety Monitoring Board (DSMB)

Inclusion of females of child-bearing potential (FOCBP)

There is no evidence that CTX0E03 cells may be teratogenic or induce teratogenicity; the contraceptive measures that are being introduced are standard for investigational products that do not have a known safety profile in pregnancy.

Females of child-bearing potential (FOCBP) or within 2 years of last menstrual cycle, may be included in the study if they are using two reliable methods of contraception e.g. oral contraceptive and condom, intra-uterine device (IUD) and condom, diaphragm with spermicide and condom) for the duration of the study.

Sexually active male subjects in relationships with FOCBP must be willing to use a reliable method of contraception (e.g. barrier and spermicide or as described above) for the duration of the study.

CTX0E03 cells are genetically manipulated to produce a stable cell lines for clinical use by the fusion of c-Myc plus a modified oestrogen receptor fusion protein and regulated by a synthetic drug, 4-hydroxytamoxifen (4-OHT). The decision to include FOCBP in the study is based upon toxicology, tumourgenicity, studies of the viability of implanted cells and molecular biology data (accumulated by ReNeuron) that demonstrates that CTX0E03 cells are not reactivated by secondary exposure to tamoxifen or 4-0HT, the active metabolite of tamoxifen), the drug tamoxifen or endogenous oestrogen or other steroids that could be encountered following implantation in patients. Additionally, data demonstrating transgene silencing by methylation of the c-mycER^{TAM} promoter element once the cells are injected into tissue is also available.

The following sections have been amended:

Section 2.0: Trial Synopsis

Section 3.0: List of Acronyms, Abbreviations and Definition of Terms

Section 9.2.2: Exclusion Criteria

Section 11.2: Safety Measurements

Section 11.4.3: Pegnancy Tests

Appendix 1: Trial Evaluation Schedule

Update to Summary of Clinical Experience

The summary of clinical experience has been updated with data obtained from the ongoing CTX0E03 DP clinical studies.

The following section has been amended:

Section 6.4: Summary of Clinical Experience

Section 7.2.4: Allergic Response to Allogeneic Cells

Section 7.2.5: Unknown Risks Associated with Early Clinical Trials

Section 10.7: CTX0E03 DP Administration

Patient Visit and Treament Windows

It is recognised that the selected patient population may have concurrent illness and disability that may impede the strict adherence to the protocol-specified patient visit and treatment windows. In these exceptional circumstances, any potential visit or treatment window deviations must be notified in advance to the Chief Medical Officer, who will review each deviation on a case by case basis, taking account of all current eligibility requirements, patient safety and data integrity.

The following new section has been added:

Section 9.5: Patient Visit and Treatment Windows

Use of Botulinum Toxin, Phenol and Antispasticity Medications

Further definition has been provided for the use of oral and injectable antispasticity medications, botulinum toxin and phenol.

The following sections have been amended:

Section 2.0: Trial Synopsis

Section 9.2.2: Exclusion Criteria

Section 10.15: Prior and Concomitant Therapy

AEs/SAEs, Follow-ups and Pregnancy Reporting

Safety follow-up and reporting have been updated and new details regarding the follow-up of SAEs and the reporting of pregnancy have been added to the protocol.

The following sections have been amended:

Section 11.3.1: Follow Up of AEs

Section 11.3.2: Serious Adverse Events (SAEs)

Section 11.3.4: Reporting SAEs

Section 11.3.7: Serious Unexpected Seerious Adverse Reactions (SUSARs)

Section 11.4.14: Annual Family Doctor Follow-up

Section 18.10: Safety Reporting

The following new sections have been added:

Section 11.3.3.: Follow-up of SAEs

Section 11.3.5: Pregnancy Reporting

Administrative and other changes

In addition to the above proposed changes, the protocol has undergone a number of administrative changes, including removal of anomalies, edits for consistency of terminology and correction of grammatical and spelling errors.

Foremost changes have been made to the following sections:

Section 3: List of Accronyms, Abbreviations and Definition of Terms

Section 4.2: Ethical Conduct of the Trial

Section 9.2.2: Exclusion Criteria

Section 10.7: CTX0E03 DP Administration

Section 11.3: Adverse Events (AEs)

Section 11.4.5: Scans

Section 17.2: R&D Review

Section 19.0: Data Management, Statistical Analyses and Final Report

Section 24.0: Protocol Approval

Section 25.0: Protocol Acceptance

Section 26.0: References

1.3 Protocol Amendment (Version 6.0 to 7.0)

The following changes have been made to the Clinical Trial Protocol:

Qualifying stroke functional assessments

The period for conducting the qualifying stroke functional assessments i.e. measurement of stable paresis of the arm (conducted on visits 1 and 2), has been altered to allow more time for patients to reach functional stability.

Following consent, the qualifying functional assessments (NIHSS, ARAT, RFA, BI) occurring on visit 1 and visit 2 may take place anytime between Day 28 (Month 1) and Day 300 (Month 10). The visit 1 and 2 functional assessments will occur on 2 independent occasions, separated by a minimum of 28 consecutive days. Patients whose functional assessment scores satisfy the study inclusion criteria on both occasions will be deemed eligible for entry into the study.

The corresponding visit 1 and 2 safety assessments e.g. temperature, ECG, biochemistry, haematology etc., will not change and will be taken concurrently at the appropriate visit.

The following sections have been amended:

Section 9.1: Description of Overall Trial Design

Appendix 1: Trial Evaluation Schedule

Extension of Patient Consent Period

Consequential to the changes made to the qualifying stroke functional assessments period, the time at which informed consent may be obtained has been adjusted. To align with the rescheduled timings, consent may now occur between Day 28 (Month 1) and Day 270 (Month 9).

The following sections have been amended:

Appendix 1: Trial Evaluation Schedule

Study Drug Administration Period

Consequential to the changes made to the timing of the qualifying stroke functional assessments, the period during which eligible patients may receive study treatment has been adjusted. However, the treatment window remains unchanged and study drug administration will continue to occur within 3 months of Visit 2 i.e. within 3 months of demonstrable stable paresis of the arm.

The following sections have been amended:

Appendix 1: Trial Evaluation Schedule

Revisions to Eligibility Criteria

1) Modified National Institute of Health Stroke Scale (NIHSS) Score (Inclusion Criterion #5 and Exclusion Criterion #2)

Patients with an NIHSS score of 4 will now also be eligible for the study (formerly NIHSS scores of only 2 or 3 were eligible).

The inclusion of patients with an NIHSS score of 4 will enable patients most disabled by stroke to enter the study. It is considered scientifically valid and ethically justifiable to include those patients with an NIHSS score of 4 as these patients may also derive significant benefit from improved movement and strength, which is considered appropriate as there are no alternative treatment options for this patient population.

2) Removal of Upper Age Limit (Inclusion Criterion #4)

Patients aged ≥40 years (previous protocol version: age 40≥89 years) who are deemed sufficiently healthy and competent by the Investigator to undertake the study assessments, general anaesthesia and the study operative procedure, may be enrolled into the study.

In an increasingly elderly population, and in an area of high unmet need, using age alone is considered a weak predictor of suitability for surgery/anaesthesia and post-operative recovery, and further, as an exclusion criterion, may inadvertently discriminate against patients who would otherwise be eligible for the study.

3) History of Malignant Disease (Exclusion Criterion #9)

Patients with previous history of malignant disease (excluding any history of malignant brain tumours or brain metastasis) may now enter the study if they have been cancer free for at least 5 years. Patients who have any history of non-melanoma skin cancer are, however, eligible for this study.

A history of malignant disease e.g. breast, colon, prostate cancer, will be frequent in this population and a significant number of patients in this category could derive benefit but are currently excluded. If the patient has been invasive cancer free for at least 5 years and the Investigator has no concurrent safety concerns, these patients will now be eligible for the study.

4) Diabetic Patients (Exclusion Criterion #14)

Patients with diabetes will only be excluded if they have uncontrolled diabetes e.g. history of hypo- or hyper-glycaemic events requiring hospital admission over previous 6 months.

A static measurement of HbA1c alone is not considered a reliable indicator of diabetic stability in stroke patients as HbA1c levels are often raised post-stroke due to poor control in hospital/home or dietary changes. Therefore the requirement to measure HbA1c levels in diabetic patients will be removed and replaced with a criterion to exclude patients with uncontrolled diabetes.

The following sections have been amended:

Section 2.0: Trial synopsis

Section 9.2.1: Inclusion criteria

Section 9.2.2: Exclusion criteria

Section 11.2: Safety measurements

Section 11.4.4: Laboratory safety tests

The following section has been removed:

Appendix 3: Haemoglobin A1c conversion

Re-screening of Patients

Patients who have been screened under an older version of the protocol may be re-screened for entry to the study if thought by the investigator to be suitable and meet all current eligibility requirements.

The protocol amendment has been designed to include a broader cross-section of stroke patients in an area of high unmet need. There is no increased risk for patients who were previously ineligible, and so these patients can be re-considered for the study.

Stroke functional assessment data (NIHSS, ARAT, RFA, BI) previously collected as part of screening data or for the observational study (RN-CS-0001) on a previous occasion may be used as a qualifying functional assessment (i.e. visit 1) if the data is less than 12-months old. In such instances, this data should be copied and transcribed into the patients CRF.

The following sections have been amended:

Section 4.3: Patient Information and Consent

The following section has been added:

Section 9.4: Re-screening of patients

Administrative and other changes

In addition to the above proposed changes, the protocol has undergone a number of administrative changes, including removal of anomalies, edits for consistency of terminology and correction of grammatical and spelling errors.

The following sections have been updated:

Section 6.5 Rationale for study design

Section 9.2: Selection of trial population

Section 10.2: Packaging and labelling

Section 10.7: CTX0E03 DP administration

Section 10.8.1: Drug product handling procedure training

Section 10.8.2: Patient Preparation

Section 11.4.5: Scans

Section 11.4.7: Immunological response to CTX0E03 DP

Section 24.0: Protocol approval

1.4 Protocol Amendment (Version 5.0 to 6.0)

The following changes have been made to the Clinical Trial Protocol:

Exclusion Criterion 8 (cardiovascular events)

Clarify the definition of excluded 'cardiovascular events' within the last 3 months to indicate that only cardiovascular events considered to increase the risk of the study procedure (anaesthetic) should result in the patient being excluded.

Section 2	Trial Synopsis
Section 9.2.2	Exclusion Criteria

Anticoagulation

Clarification regarding the use of antiplatelet and anticoagulation medication prior to surgery. This will ensure that in the event the hospital does not have a written practice or in the event that the Investigator or anaesthetist consider that the particular medical needs of the patient require management of anticoagulation or antiplatelets at variance with hospital practice, that the investigator documents the rationale for the variance.

Section 10.15	Prior and Concomitant Therapy
Appendix 1	Trial Evaluation Schedule

1.5 Protocol Amendment (Version 4.0 to 5.0)

The following changes have been made to the Clinical Trial Protocol:

Consent Window

The consent window has been widened to allow patient consent to occur anytime between stroke occurrence and Visit 2 (Day 56 ±7) provided the specified criteria are met. This will allow the site staff more time to approach and consent potential patients. The following sections have been amended:

Appendix 1 Trial Evaluation Schedule

Functional assessment scores (NIHSS, ARAT, RFA and BI)

Patients' functional assessment scores must satisfy the study inclusion criteria at the time of consent.

Functional assessments at Visit 1 (Day 28 ±7 post-stroke) need not be performed provided these data are already available having been collected at the corresponding visit (Day 28 ±7 post-stroke) in ReNeuron Observational Study (Protocol RN-CS-0001). This will reduce the number-of/need-to repeat functional assessments for patients who have recently undertaken these assessments.

Pre-surgery functional assessments (Visit 3) should not be repeated if the previous functional assessment was performed within the last 7 days. This will reduce the number-of/need-to repeat functional assessments for patients who have recently undertaken these assessments.

The following section has been amended:

Section 2 Trial Synopsis

Section 9.2.1 Inclusion Criteria

Appendix 1 Trial Evaluation Schedule

Allo-antibody testing

Testing for allo-antibodies at screening can be performed at any time post-consent and provided the results are available prior to administration of CTX0E03 DP. This will allow earlier detection and exclusion of patients with positive allo-antibodies. The following section has been amended:

Appendix 1 Trial Evaluation Schedule

Concomitant Medication

Exclusionary drugs should be identified at Visit 1 (Day 28 ±7) [formerly Visit 2 (Day 56 ±7)] to enable the early identification of patients who may not be suitable for the study. The following sections have been amended:

Appendix 1 Trial Evaluation Schedule

Surgery Window

The surgery window (Visit 4) has been widened. Surgery and CTX0E03 DP administration may now be performed between Days 57-112 (formerly Days 57-84) to maximise IMP

availability/shelf-life and aid surgical theatre scheduling at participating sites. The following sections have been amended:

Appendix 1	Trial Evaluation Schedule

Clinical Trial Supplies

The requirement to invert the vials multiple times has been removed given that all of the product sample will be taken up into the syringe so homogeneity of drug product cell suspension during syringe loading is maintained. This will improve the recoverable volume of drug product from the vial.

Correction to the wording of syringe loading cannula to syringe loading needle. Consequently also clarification of wording in Protocol regarding the number of cannulae used (i.e. 1 implantation cannula and 1 syringe loading needle).

The following sections have been amended:

Section 10.8.1	Drug Product Handling Procedure Training
Section 10.8.2	Patient Preparation
Section 10.8.3	Loading the Syringe

Administrative Corrections

In addition to the above proposed changes, the Protocol has been corrected for anomalies, consistency of terminology and correction of grammatical and spelling errors.

1.6 Protocol Amendment (Version 3.0 to 4.0)

The following changes have been made to the Clinical Trial Protocol:

Amend Cover Page Responsibilities and Contact Details

The cover page has been amended to provide updated contact details for key study functions and personnel.

Inclusion & Exclusion Criteria

Following review of the study inclusion and exclusion criteria by the participating Principal Investigators and ReNeuron, the following changes have been made to aid recruitment into the study:

- Inclusion criterion 2 has been amended to remove reference to the vascular anatomy associated with the infarct. It is advised that eligibility should not be restricted by or based on specific vascular anatomy (which varies widely between individuals), but instead should be based on brain anatomy and neurological impairment.
- Inclusion criterion 5 has been amended to remove the requirement of "first" stroke.
- Inclusion criterion 7 has been clarified to include an appropriate anatomical target for cell implantation when assessing eligibility for neurosurgery.
- Exclusion criterion 12 has been deleted.
- Exclusion criterion 13 (now 14) has been amended to correct a typographical error and to clarify that measuring of HbA1c levels is only applicable to patients with diabetes. Patients who have poorly controlled diabetes should be excluded from the study.

The following sections have been amended:

Section 2	Trial Synopsis
Section 9.2.1	Inclusion Criteria
Section 9.2.2	Exclusion Criteria
Section 11.4.4	Laboratory Safety Tests

In addition, two exclusion criteria have been added:

- Requirement for antiplatelets and/or anticoagulants including heparin, warfarin or other anticoagulants/medication that cannot be interrupted to allow surgery.
- Requirement for intermittent botulinum toxin therapy, phenol or oral antispasticity medications (antispasticity medications are acceptable if they have been taken regularly for at least one month prior to consent).

The following sections have been amended:

Section 2	Trial Synopsis
Section 9.2.2	Exclusion Criteria
Section 10.15	Prior and Concomitant Therapy

Visual Inspection of IMP

A visual inspection of the IMP is to be performed prior to administration. Reference is made to the 'Pharmacy Guidelines' (document title changed from the Pharmacy and Dosing Guidelines).

The following sections have been updated:

Section 10.6	CTX0E03 DP Quarantine and Release
Section 10.7	CTX0E03 DP Administration

Clinical Trial Supplies

HypoThermosol-FRS (HTS-FRS) will be supplied in either 10 mL or 100 mL bottles. The specific volume of supplied HTS-FRS has been omitted from the Protocol.

The following sections have been updated:

Section 10.8.1	Drug Product Handling Procedure Training
Section 10.8.2	Patient Preparation

The disinfectant 'Trigene', supplied by ReNeuron, has changed its name and is now called 'Distel'.

The following section has been updated:

Section 10.10	IMP Handling, Spillage and Accidental Exposure

Surgery Guidelines

More than one burr hole may be necessary to deliver CTX0E03 DP along 4 trajectories.

The following section has been updated:

Section 10.8.2	Patient Preparation

A typographical error has been corrected in the syringe loading instruction. Drug Product should be drawn up to the 225 microL graduation mark.

The following section has been updated:

Section 10.8.3	Loading the Syringe

SUSAR reporting

Expedited reporting on a CIOMS I form is no longer permitted; only electronic formats should be used.

The following section has been updated:

Section 11.3	Adverse Event (AEs)

Unscheduled Visits

Unscheduled visits may be performed, if clinically indicated, to record additional relevant data.

The following section has been added:

Section 11.4.14	Unscheduled Visits

Data Safety Monitoring Board

After the first patient is treated, a treatment hold rather than a recruitment hold, will be observed until data are reviewed by the DSMB and an opinion/recommendation is issued. During this safety review period further patients may be screened but will not receive treatment.

The following section has been updated:

Section 14 Data Safety Monitoring Board (DSMB)

Trial Evaluation Schedule

An additional imaging time point has been added to the schedule at one year post CTX0E03 implantation. A detailed medical history will be conducted at Visit 1.

In addition, the Protocol will allow investigative centres to adopt local pre-surgery work-up practices.

- **Imaging:** Sites may elect to perform a single pre-surgery MRI scan where MRI-compatible stereotaxic frames are used. This will lessen patient burden by reducing pre-surgery MRI scanning from 2 (Visit 3 and Visit 4) to a single occasion (Visit 4).
- **Anaesthesia**: Anaesthesia assessment may be carried out at any point between Visit 3 and Visit 4 pre-surgery

The following section has been updated:

Appendix 1 Trial Evaluation Schedule

Administrative Corrections

In addition to the above proposed changes, the Protocol has been corrected for consistency of terminology and correction of grammatical and spelling errors.

1.7 Protocol Amendment (Version 2.0 to 3.0)

The following changes have been made to the Clinical Trial Protocol:

Add/amend Contact details. The cover page has been updated to include the name and contact details for the Pharmacovigilance Provider; SAE reporting/Medical Advice & 24 hour cover.

Clarification of Primary Objective:

The last bullet point of the Primary Objective has been reworded for grammatical consistency. The following sections have been updated:

Section 2	Trial Synopsis
Section 8.1	Primary Objective

Clarification of Exclusion Criteria

Following review of the Protocol, the following exclusion criteria have been added:

16. Organ transplant recipient.
17. In the opinion of the Investigator, sustained consumption of alcohol or drugs at a level likely to be injurious to health.

This is in line with other CTX0E03 DP clinical trials. In addition, exclusion criterion 11 has been clarified to use consistent terminology. Valproate drugs are excluded. The following sections have been updated:

Section 2	Trial Synopsis
Section 9.2.2	Exclusion Criteria
Section 10.15	Prior and Concomitant Therapy

Safety Variables

Brain imaging post implantation has been included as a safety assessment in line with the schedule of assessments. In addition, HbA1c has been added as safety variable and the Trial Evaluation Schedule to provide consistency with exclusion criterion 13. The following sections have been updated:

Section 2	Trial Synopsis
Section 11.2	Safety Measurements
Appendix 1	Trial Evaluation Schedule

Status Update of Phase I Trial (RN01-CP-0001)

The Phase I study is currently ongoing with all patients now treated. Participants will continue to be followed up as part of the RN01-CP-0001 study out to 10 years post cell implantation. An update to the clinical experience of CTX0E03 DP reflecting the current status of the RN01-CP-0001 study is included in this Protocol amendment. In addition, data accrued up to and including 3 months post the last patient treated on the RN01-CP-0001 study have been reviewed by the DSMB in support of the Phase II Clinical Trial Authorisation (CTA) application to the UK Regulatory Agency. The following sections have been updated:

Section 6.4	Summary of Clinical Experience
Section 6.5	Rationale for Study Design
Section 6.5.1	Dose Selection

Clarification/ Correction to Non-Clinical Studies

Toxicology studies were performed in naïve mice, not mouse models of hind limb ischaemia. The following section has been corrected:

Section 7.1.4.1 Single Dose Toxicity

Allergic Response to Allogeneic Cells

Clarification of the amount of time patients should be observed following stereotaxic surgery. The following section has been updated:

Section 7.2.4 Allergic Response to Allogeneic Cells

Withdrawal of Patients from the Study

Patients will be withdrawn from the study if they test positive for Human Leucocyte Antigen (HLA) during their screening assessments and prior to CTX0E03 DP administration. The following section has been updated:

Section 9.3 Withdrawal of Patients from Treatment or Assessment

Post Treatment Sterility, Mycoplasma and Endotoxin Results

A frozen version of the same formulation of CTX0E03 DP has been developed for clinical use. As a result of an extended shelf life, the results of sterility, mycoplasma and endotoxin tests will be known before CTX0E03 DP is administered to patients. The following section has been deleted:

Section 9.4 Management of post treatment sterility, mycoplasma and endotoxin results

CTX0E03 DP; Frozen Product

A frozen version of the same formulation of CTX0E03 DP has been developed for clinical use. The following sections have been reviewed and updated to reflect the management of a cryo-preserved product:

Section 10 Investigational Medicinal Product

Appendix 2 Example of Drug Labels

IMP Handling, Spillage and Accidental Exposure

The following section has been added to include instructions on IMP handling, spillage and accidental exposure:

Section 10.10 IMP Handling, Spillage and Accidental Exposure

In addition, the following sections have been amended to clarify that all materials exposed to CTX0E03 DP should be handled as clinical waste containing GMOs.

Section 10.11 (formerly 10.10) Disposal of Unused UMP

Section 10.12 (formerly 10.11) Disposal of Waste Products

Laboratory Safety tests

An inconsistency between Section 11.4.4 and the Trial Evaluation Schedule has been rectified. Cholesterol, uric acid and creatine kinase will not be evaluated in this trial.

MRI Scans

The following section has been clarified to broaden the scope of MRI sequences where clinically indicated:

Section 11.4.5 Scans

Central Laboratory Testing

The following section has been amended to inform sites that “allo-response” testing will be carried out using a central laboratory in this study:

Section 11.4.7 Immunological Response to CTX0E03 DP

Life Long Follow-Up

The following section has been clarified to describe the process of annual family doctor follow-up:

Section 11.4.13 Annual Family Doctor Follow-up

Safety Reporting

The following section has been amended to clarify the responsibilities of ReNeuron and the Pharmacovigilance Provider in reporting SAEs and SUSARs

Section 18.10 Safety Reporting

Administrative Corrections

In addition to the above proposed changes, the Protocol has been corrected for consistency of terminology, correction of grammatical and spelling errors, addition of references, updates to referenced guidelines updates to the List of Acronyms and rearrangement of text paragraphs within sections for ease of reading.

1.8 Protocol Amendment (Version 1.0 to 2.0)

The following changes have been made to the Clinical Trial Protocol:

Add/amend contact details

The cover page has been updated to include the names and contact details for:

- Chief Investigator
- ReNeuron's Medical Monitor
- Pharmacovigilance (PV) provider; SAE reporting

Inclusion and Exclusion criteria

Following review of the clinical Protocol, the below inclusion exclusion criteria have been updated for consistency and clarification:

An inconsistency between inclusion criterion 5 and the Trial Evaluation Schedule has been rectified. To be eligible for the study, patients must have presented with their first stroke within the past 4 weeks (at time of consent).

Two bullet points (Inclusion criterion 5) have been amended: the NIHSS motor arm score of 2 has been defined and bullet point 4 has been deleted and appropriately redefined as an exclusion criteria i.e. NIHSS upper limb score of 4 (Exclusion criterion 2).

Exclusion criterion 12 has been updated to clarify that patients who have experienced (documented) fever within 7 days of the planned surgery will be excluded from the study.

The following sections have been updated:

Section 2 Trial Synopsis

Section 9.2.1 Inclusion Criteria

Section 9.2.2 Exclusion Criteria

Pregnancy test

An inconsistency between exclusion criterion 14 i.e. requirement to perform a pregnancy test and the safety variables to be evaluated in the study has been rectified. A pregnancy test need only be performed where female participants are between 2-4 years post last menstrual period. Female patients who are less than 2 years post last menstrual period will be excluded from the study. The following sections have been updated for consistency:

Section 2. Trial Synopsis – Safety Variables

Section 11.2 Safety Measurements

Investigators and Trial Administrative Structure

Section 5: Investigators and Trial Administrative Structure has been amended to clarify key administrative structures of the study, i.e. DSMB and Trial Steering Committee (TSC).

Status update of Phase I trial (RN01-CP-0001)

The Phase I study is currently ongoing with all patients now treated. Participants will continue to be followed up as part of the RN01-CP-0001 study extension out to 10 years post cell implantation. An update to the clinical experience of CTX0E03 DP reflecting the current status of the RN01-CP-0001 study is included in this Protocol amendment. The following sections have been updated:

Section 6.4 Summary of Clinical Experience

Description of CTX0E03 DP

Following a change in the CTX0E03 DP manufacturer, the description of the product has been amended to reflect a more accurate visual description. The following sections have been updated:

Section 7 Biological Properties of IMP

Section 10.1 CTX0E03 Drug Product (DP)

Management of post treatment sterility, mycoplasma and endotoxin results

The results of several manufacturing tests (sterility, mycoplasma and endotoxin) will not be available until after cell implantation. The following section has been added to the Protocol to provide guidance on the management of positive sterility, mycoplasma and/or endotoxin test results post cell implantation. Guidance is in line with the RN01-CP-0001 Phase I study.

Section 9.4 Management of post treatment sterility, mycoplasma and endotoxin results.

Pre-surgery scan

The following section has been amended to clarify that MRI may be used to determine stereotaxic coordinates as opposed to CT scan where local procedures permit.

Section 10.8.2Patient Preparation

Pre-surgery medication stop

The following sections have been amended to clarify that antiplatelet medications (in addition to anticoagulants) should be stopped 7 days prior to surgery:

Section 10.14 Prior and Concomitant Therapy

Appendix 1 Trial Evaluation Schedule

SAE reporting

In line with a new Pharmacovigilance (PV) provider, the following section has been amended to instruct sites to report all SAEs to the PV provider and also that SUSARs will be reported to the DSMB in line with CA and REC reporting timelines:

Section 11.3 Adverse Events (AEs)

Physiotherapy

Following consultation with an expert in post-stroke physiotherapy, the following section has been amended to further clarify the expected frequency and amount of physiotherapy post CTX0E03 DP injection.

Immunological testing and patient management

Following initial review of the proposed study by the MHRA, the Competent Authority requested an amendment to the proposed Protocol addressing the following:

"Allo-antibodies are being measured at baseline and at 28 days post-implantation. The Protocol must also clearly state action that will be taken in the event of a positive result in a patient. It is expected that this will follow the management of a positive result as per Protocol for study RN01-CP-0001" (Phase I study).

The following section has been amended in line with the MHRA's request:

Section 11.4.7 Immunological Response to CTX0E03 DP

Additional DSMB Safety Review

Following a request to the MHRA and Gene Therapy Advisory Committee to amend the Phase I study (RN01-CP-0001) from a 12 patient study to an 11 patient study, as a result of the CTX0E03 DP Contract Manufacturing Organisation going into receivership, ReNeuron propose an additional DSMB review after the first patient treated in this proposed Phase II study. A recruitment hold will be observed until the DSMB review one month data post CTX0E03 DP administration on the first patient treated in this study. The DSMB membership is common to both the Phase I and Phase II stroke studies, thereby providing continuity of safety review. In addition, a Trial Steering Committee comprising of clinical experts in stroke and ReNeuron will be convened to guide the study based on emerging data. The following section has been amended in-line with this proposal:

Section 14 Data Safety Monitoring Board (DSMB)

The Trial Evaluation Schedule

In consultation with the Chief Investigator, the Trial Evaluation Schedule (Appendix 1) has been amended to better clarify pre-surgery work-up and post surgery assessments. The following changes have been made:

- Addition of the Day -8 “antiplatelet and anticoagulant stop” telephone call reminder to participants for consistency with Section 10.14 Prior and Concomitant Therapy

- Addition of a Day -5 to Day -1 pre-surgical workup period in preparation for surgical implantation of CTX0E03 cells (surgery and anaesthesia assessments). Surgical workup may be performed over several days, dependent on neurosurgeon and anaesthetist availability, MRI scheduling and the number and type of routine pre-surgical evaluations to be performed. Participants will be admitted to hospital the day before surgery

- Post surgery safety evaluations (vital signs, ECG, routine blood and urinalysis) are to be performed in the first 48 hours post CTX0E03 DP injection

- Stroke assessment scales (NIHSS, ARAT, RFA and BI) are to be performed during the pre-surgery workup period to reconfirm patient eligibility if previous assessment exceeds 7 days

- Addition of a post surgery MRI at day 180

In addition, an inconsistency between the Trial Evaluation Schedule and section 11.4.7 Immunological Response to CTX0E03 DP, has been rectified. Blood samples will be tested at screening and at 28 days post implantation.

Administrative corrections

In addition to the above proposed changes, the Protocol has been corrected for consistency of terminology and correction of grammatical and spelling errors throughout.

2 Trial Synopsis

Trial Number:	RN01-CP-0002
Trial Title:	A Phase II Efficacy Study of Intracerebral CTX0E03 DP In Patients with Stable Paresis of the Arm Following an Ischaemic Stroke.
EUDRACT Number.	2012-003482-18
Product:	CTX0E03 Drug Product (DP)
Phase:	Phase II
Investigators/Centres:	A multicentre study
Objectives: Primary:	• To determine whether a sufficient proportion of patients experience response of their paretic arm following treatment with CTX0E03 DP at a dose level of 20 million cells to justify a subsequent randomised study. o Response will be defined as a minimum improvement of 2 points in test number 2 of the Action Research Arm Test (Grasp a 2.5 cm^3 block and move it from the starting position to the target end position) in the affected arm 3 months after injection of CTX0E03 DP. This would represent an improvement from a pre-treatment state in which the patient was unable to grasp and reposition the block as required to a post-treatment state in which the patient could accomplish the task as specified within 60 seconds and would represent recovery of useful function in a previously paretic arm.
Secondary:	• To assess the efficacy of intracranial CTX0E03 DP in restoring upper limb function following an ischaemic stroke using the Action Research Arm Test (ARAT) (Yozbatiran *et al.,* 2008). • To assess the efficacy of intracranial CTX0E03 DP in restoring function following an ischaemic stroke using the National Institutes of Health Stroke Scale (NIHSS) (Anemaet, 2002; Brott *et al*., 1989; Lyden *et al*., 2001). • To assess the efficacy of intracranial CTX0E03 DP in restoring patient's functional independence following an ischaemic stroke using the Rankin Focused Assessment (RFA) (Saver *et al*., 2010) version of the Modified Rankin Scale (mRS). • To assess the efficacy of intracranial CTX0E03 DP in improving patient's ability to execute activities of daily living following an ischaemic stroke using the Barthel Index (BI) (Mahoney & Barthel, 1965). • To assess the efficacy of intracranial CTX0E03 DP in sensorimotor recovery of the affected limb following an ischaemic stroke using the Fugl-Meyer Assessment (Fugl Meyer, et al, 1975; Gladstone et al, 2002, www.rehabmeasures.org (Feb 2016)). • To assess the safety and tolerability of intracranial CTX0E03 DP in patients following an ischaemic stroke.

Trial Design:	Open, single arm design
Number of Patients:	At least 21 patients will be recruited.
Inclusion Criteria:	1. Written informed consent or witnessed informed consent in the event that the patient is unable to sign informed consent due to paresis of the affected arm. 2. Supratentorial ischaemic stroke. 3. Male or female. 4. Age 40 years or more. 5. Stroke, at time of consent, satisfying the following criteria: • Modified NIH Stroke Scale (NIHSS) Motor Arm Score of 2 (some effort against gravity), 3 (no movement against gravity) or 4 (no movement) for the paretic arm post ischaemic stroke at visit 1 and 2. • Clinical diagnosis of stroke confirmed by physician using neuro-imaging (computerised tomography or magnetic resonance imaging). • A Score of 0 or 1 for test 2 of the Action Research Arm Test (Grasp a 2.5 cm^3 block and move it from the starting position to the target end position) at visit 1 and 2 using the affected arm. 6. Ability to comprehend verbal commands. 7. Eligible for neurosurgery, including appropriate anatomical target for cell implantation.
Exclusion Criteria	1. Prior history of stroke resulting in permanent moderate to severe disability (i.e. Rankin Scale greater than 2) (other than the presenting ischaemic stroke). 2. Stroke due to haemorrhage. 3. History of neurological or other disease resulting in significant functional impairment of the paretic arm impairing potential ability to pick up, lift and place a 2.5 cm^3 block (e.g. Parkinson's disease, motor neuron disease, arthritis, Dupuytren's contracture or fixed anatomical abnormality). 4. Any contraindications to MRI including presence of a cardiac pacemaker (excluding MR-conditional cardiac pacemaker), metal fragments in eye etc. 5. Uncontrolled blood pressure defined as systolic blood pressure ≥180 mm Hg or diastolic blood pressure ≥110 mm Hg (patients are only to be excluded if an initial value exceeding these limits is repeated on retesting over several days). 6. Patient with a severe comorbid disorder, not expected to survive more than 12 months. 7. Acute cardiovascular events other than the presenting ischaemic stroke (e.g. myocardial infarction, recent coronary intervention for symptomatic cardiac disease) considered by the Investigator or the anaesthetist responsible for the patient to place the patient at increased anaesthetic risk, 3 months prior to planned injection of CTX0E03 DP. 8. History of malignant disease (except for non-melanoma skin cancer) within the previous 5 years or any history of malignant brain tumours or brain metastasis. 9. Current treatment with tamoxifen.

	10. Patients taking valproate drugs for any indication in whom it is not considered appropriate to discontinue the valproate for a period of one week prior and four weeks post neurosurgery. Patients in whom valproate is switched to an alternative agent during this period may be included. 11. Requirement for antiplatelets and/or anticoagulants including heparin, warfarin or other anticoagulants/ medication that can not be interrupted to allow surgery. 12. Requirement for intermittent (stop/start date from 1-month prior-to and 3 month post- CTX0E03 DP administration) use of oral antispasticity medications (oral antispasticity medications are acceptable if they have been taken regularly for at least one month prior to CTX0E03 DP administration). 13. A history of uncontrolled diabetes e.g. history of hypoglycaemic or hyperglycaemic events requiring hospital admission over previous 6 months. 14. Females of childbearing potential (FOCBP) (or within 2 years of last menstrual cycle) must have a confirmed negative pregnancy test at time of treatment and agree to use two reliable methods of contraception (e.g. oral contraceptive and condom, intra-uterine device (IUD) and condom, diaphragm with spermicide and condom) for the duration of this study 15. Sexually active males with partners who are FOCBP must be willing to use a reliable method of contraception (e.g. barrier and spermicide or as described above) for the duration of this study. 16. Considered unlikely to be able to attend for all follow-up visits. 17. Organ transplant recipient 18. In the opinion of the investigator, sustained consumption of alcohol or drugs at a level likely to be injurious to health.
Duration of Treatment:	• Single implantation of CTX0E03 DP • Post-implantation follow-up of patients to 12 months. • Where national cancer registries permit, patients will be flagged for 5yr follow-up for new diagnosis of cancer and survival.
Supplies:	CTX0E03 DP 20 million cells and excipient
Criteria for Evaluation: Efficacy Variables: Safety Variables:	 • Action Research Arm Test • National Institutes of Health Stroke Scale • Rankin Focused Assessment • Barthel Index • Fugl-Meyer Assessment • Pregnancy test (FOCBP and wihin 2 years of last menstrual cycle) • Medical history (baseline or since last visit) • General physical examination • Temperature • Pulse rate and rhythm • ECG • BP

	• FBC • LFTs • Serum urea and electrolytes • Adverse Events • Brain imaging post-implantation
Statistical Methods:	All data generated during the study will be listed, tabulated and summarised. Descriptive analysis will be used.

3 List of Acronyms, Abbreviations and Definition of Terms

4-OHT	4-hydroxy tamoxifen
ADR	Adverse Drug Reaction
AE	Adverse Event
ARAT	Action Research Arm Test
ATC	WHO Anatomical Therapeutic Chemical classification
BP	Blood Pressure
CA	Competent Authority/ies
CI	Confidence Interval
CLI	Critical Limb Ischaemia
CRF	Case Report Form
CROs	Contract Research Organisations
CT Scan	Computerised Tomography Scan
CTR	Clinical Trial Report
CTX0E03 DP	CTX0E03 Drug Product
CV	Curriculum Vitae
DNA	deoxyribonucleic acid
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
EIA	Environmental Impact Assessment
EudraCT	European Union Drug Regulatory Authority Clinical Trial Database
EU	European Union
FBC	Full Blood Count
FMA	Fugl-Meyer Assessment
FOCBP	Females of Childbearing Potential
GCP	Good Clinical Practice
GFAP+	Glial Fibrillary Acidic Protein positive
GMP	Good Manufacturing Practice
GPvP	Good Pharmacovigilance Practice
HbA1C	Glycosylated haemoglobin A1c
HLA	Human Leucocyte Antigen
IB	Investigator Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
i.m.	Intramuscular
IMP	Investigational Medicinal Product
IUD	Intrauterine device
LFTs	Liver Function Tests
NIHSS	National Institutes of Health Stroke Scale
NHS	National Health Service
MCA	Middle Cerebral Artery
MCAo	Middle Cerebral Artery occluded
MedRA	The Medical Dictionary for Regulatory Activities
MRI	Magnetic Resonance Image/ing
mRS	Modified Rankin Scale
4-OHT	4-hydroxytamoxifen
PIS	Patient Information Sheet
PV	Pharmacovigilance
QP	Qualified Person
REC	Research Ethics Committee
RFA	Rankin Focused Assessment
SAE	Serious Adverse Event

SAS	Safety Analysis Set
SI	Statutory Instrument
SOPs	Standard Operating Procedures
SUSAR	Suspected Unexpected Serious Adverse Drug Reaction
$tcPO_2$	Transcutaneous oxygen tension
U&Es	Serum urea and electrolytes

4 Ethics

4.1 Regulatory and Independent Ethics Committee Review

Before any patient undergoes any per Protocol assessment procedure or treatment, the Protocol must be approved in writing by all relevant Competent Authorities (CA) and Research Ethics Committees (REC) as required by the laws and established practice of the country in which the patient is treated.

CA(s) and REC(s) will be kept informed of any new safety data that may adversely affect the benefit-risk ratio of the product being tested as required by relevant laws and regulations.

4.2 Ethical Conduct of the Trial

The trial will be carried out in accordance with the SOPs of ReNeuron Ltd and/or those of the CROs and institutions involved, which are designed to ensure adherence to ICH GCP, GMP and GPvP and any other relevant applicable Clinical Trials Regulations.

4.3 Patient Information and Consent

Patients may only be included in the trial if they provide written informed consent in advance using a Patient Information Sheet and Informed Consent Form (PIS/ICF) approved by the appropriate REC.

All signed PIS/ICFs should be retained by the Investigator for a period of 30 years from the date of the Clinical Trial Report (CTR), or from the date of early termination of the trial. They should be available for inspection by the Monitor.

Potential participants should be given detailed information relating to the trial by either the Investigator or a designated member of her/his staff. The Investigator or a medically qualified Sub-Investigator must ensure that the patient is fully informed about the trial and the product being tested and that they understand the information they have been given. The patient must then sign and date the PIS/ICF in the presence of the Investigator or Sub-Investigator who must also sign and date the form. If the patient is not physically able to sign the form a witness may sign on their behalf. The patient should be given a copy of the form to keep and the original should be retained by the Investigator. A copy should be retained for the patient's medical records.

The PIS/ICF may need to be revised if there is a substantial Protocol amendment or if new safety information becomes available during the trial that may change the benefit-risk ratio of the product. In these circumstances or if a patient is re-screened (see Section 9.4 Re-screening of Patients) then a patient may be asked to sign a new or updated consent form.

The Investigator should inform the participant's General Practitioner of their proposed participation in the trial in writing. A copy of the standard letter to be used will be submitted to the relevant REC as part of the approval process.

5 Investigators and Trial Administrative Structure

The trial will be a multicentre study conducted in the UK. Safety data will be monitored by an independent Data Safety Monitoring Board (DSMB) (see Section 14) at predetermined intervals. In addition, the study will be guided by clinical experts in stroke and ReNeuron, who may generate hypotheses regarding optimal patient selection based on data emerging during the study. Collectively, this committee may propose amendments to the Protocol including the statistical section.

6 Background Information

6.1 Clinical Background and Rationale

In industrialised countries stroke is the second or third most common cause of death and the primary cause of morbidity and long-term disability (Olsen *et al.*, 2003). Stroke affects 795,000 people each year in the USA (Lloyd-Jones *et al.*, 2009). Approximately 80% of strokes are ischaemic in origin (Saito *et al.,* 1987). Of stroke survivors, 30% are unable to walk without assistance and 26% are dependent in activities of daily living. Up to 30% are left permanently disabled and 20% require institutional care at 3 months after onset (Duncan *et al.*, 2005).

Non-clinical studies suggest that treatment with appropriate stem cells may increase recovery from stroke even if the treatment is administered some weeks or months after the stroke (Veizovic *et al.,* 2001; Shen *et al.*, 2006). The mechanism of action is not fully understood. However, increasing evidence suggests that cytokines, growth and other factors secreted by stem cells in response to injury influence inflammation, neural plasticity and neovascularisation and may trigger enhanced repair (Chamberlain *et al.,* 2007).

The ultimate goal for many stroke patients is to achieve a level of functional independence that enables them to return home and reintegrate into community life as fully as possible. The object of this study is to determine if treatment with CTX0E03 DP at a dose of 20 million cells will increase the number of patients able to achieve this goal by increasing useful function of the post-stroke paretic arm.

6.2 Relevant Epidemiological and Prognostic Data

Longitudinal studies show that almost all stroke patients experience at least some predictable degree of functional recovery in the first six months post-stroke. However, the non-linear pattern as a function of time is not well understood. Several mechanisms are presumed to be involved, such as recovery of penumbral tissues, neural plasticity, resolution of diaschisis and behavioural compensation strategies. Rehabilitation is believed to modulate this logistic pattern of recovery, probably by interacting with these underlying processes. Prediction models that are adjusted for the effects of time after stroke onset suggest that outcome is largely defined within the first weeks post-stroke (Kwakkel 2004).

Although functional outcome is related to baseline clinical syndrome (best with lacunar infarct and worst with total anterior circulation infarct), patients who improve early have a more favourable functional outcome regardless of clinical syndrome. Patients who fail to show significant recovery (measured by the Scandinavian Stroke Scale) by day 10 retain significant disability at day 90 (Sprigg *et al.*, 2007).

Several studies have sought to determine prognostic factors for recovery after a stroke resulting in hemiparesis involving the upper limb. A study of 102 patients with stroke following middle cerebral artery occlusion found that after the first week the strongest clinical factor that predicts outcome of dexterity at 6 months is severity of paresis of the arm. In addition, it was found that the optimal prediction of outcome of dexterity can be made within the first month after stroke by measuring motor recovery of the upper limb (Kwakkel *et al.,* 2003). At the end of week 4, a probability of 94% was found in those patients who had a Fugl-Meyer upper extremity score of $\geq$19 points. In contrast, the

chance to achieve some dexterity at 6 months dropped to only 9% in those patients who failed to achieve this level of motor performance within 4 weeks. No further improvement in accuracy of prediction was found after 4 weeks, suggesting that long-term outcome of dexterity can already be optimally predicted within this time frame. In agreement with previous reports (Duncan *et al*., 1992; Heller *et al*., 1987; Sunderland *et al.,*1989) this latter finding suggests that the time window for predicting the return of dexterity is limited to only 1 month after onset.

6.3 Summary of Previous Non-Clinical Studies

Animals in which the middle cerebral artery is occluded (MCAo) are affected in the same anatomical location (i.e. basal ganglia and sensorimotor cortex) as humans with an MCAo stroke, producing the same core functional deficits such as unilateral paralysis and neglect. A validated MCAo rat model of ischaemic stroke that reflects stroke patient presentation has been used for non-clinical studies (Laing *et al.,* 1993; Virley, 2005; Longa *et al.,* 1989).

CTX0E03 cells have been shown to ameliorate stable neurological deficits in a rodent MCAo model of cerebral ischaemia following transplantation adjacent to the infarcted region (Stroemer *et al*., 2009).

Two studies using MCAo stroked rats have demonstrated long term improvements in sensorimotor function following intracerebral CTX0E03 implantation. In the first study (GFi5) CTX0E03 cells from early stage cell banks were implanted 3-4 weeks after MCAo. Animals were immunosuppressed using methylprednisolone and cyclosporine A. Transplantation of CTX0E03 cells in this model of stroke caused statistically significant improvements in both sensorimotor function and gross motor asymmetry at 6-12 weeks post-grafting. In addition, cell migration and long-term survival *in vivo* were not associated with significant cell proliferation (Pollock *et al*., 2006). A second study (GFi10) using CTX0E03 Drug Product demonstrated a cell dose response effect. Again, animals were immunosuppressed using methylprednisolone and cyclosporine A, albeit for the first two weeks.

Statistically significant dose-related recovery in sensorimotor function deficits (bilateral asymmetry test in the mid- and high-dose groups and rotameter test after amphetamine exposure in the high-dose group) was found in the CTX0E03 cell implanted groups compared to the vehicle group. In-life functional improvements correlated with cell dose although these improvements did not correlate with survival of CTX0E03 cells measured at postmortem. There was differentiation of CTX0E03 cells into oligodendroglial (8%) and endothelial phenotypes (6%). MCAo-induced reduction of neurogenesis in the subventricular zone (SVZ) was partially restored to that observed in controls without MCA occlusion. No adverse CTX0E03 cell-related effects were observed during in-life observations or on tissue histology. These effects were seen at 3 months post implantation (Stroemer *et al*., 2009).

Additional pharmacodynamic data generated within a safety study (GFi9) demonstrated improvement in sensorimotor function at 6 months post implantation. Analysis of brain tissue indicated that CTX0E03 cell implantation may promote host cell neurogenesis in the SVZ.

In a further study (RN01-PT-0034) intraparenchymal implantation of CTX0E03 cells in the rat MCAo model improved sensorimotor dysfunctions (bilateral asymmetry test) and motor deficits (foot-fault test, rotameter). Importantly, analyses based on lesion topology (striatal versus striatal plus cortical damage) revealed a more significant improvement in animals

with a stroke confined to the striatum. No improvement in learning and memory (Morris water maze) was evident. In contrast to intraparenchymal implantation, intracerebroventricular implantation of cells did not result in any improvement. MRI-measured lesion, striatal and cortical volumes were unchanged in treated animals compared to those with stroke that received an intraparenchymal injection of suspension vehicle. Grafted cells only survived after intraparenchymal injection with a striatal plus cortical topology resulting in better graft survival (16,026 cells) than in animals with smaller striatal lesions (2,374 cells). Almost 20% of cells differentiated into Glial Fibrillary Acidic Protein positive (GFAP+) astrocytes, but <2% turned into FOX3+ neurons. These results indicate that CTX0E03 cell implants robustly recover behavioral dysfunction over a 3 month time frame and that this effect is specific to their site of implantation. Lesion topology is potentially an important factor in the recovery, with a stroke confined to the striatum showing a better outcome compared to a larger area of damage (Smith *et al*., 2012).

6.4 Summary of Clinical Experience

There are currently 2 other ongoing clinical trials with CTX0E03 DP in two indications: ischaemic stroke and limb ischaemia. To date 11 patients have received CTX0E03 DP intracerebrally up to a dose of 20 million cells in the First-in-Human RN01-CP-0001 study, a further 9 patients have received CTX0E03 DP intracerebrally at a dose of 20 million cells in this RN01-CP-0002 study and 3 patients have received CTX0E03 DP intramuscularly at a dose of 20 million cells (in cohort 1) in the limb ischaemia study, RN09-CP-0001.

RN01-CP-0001 is a Phase I safety study, comprising of four dose cohorts assessing 2, 5, 10, or 20 million cells. Patients participating in the study are male aged >60 years with a history of ischaemic stroke between 6 months and 5 years prior to implantation and with residual disability. No patient received immunosuppression. Each patient received a single dose of cells administered by direct intrastriatal injection in the putamen. All 11 patients have been treated and continue to be followed up. Data up to and including 3 months post the last patient treated were reviewed by the independent DSMB who recommended progressing CTX0E03 DP to Phase II assessment.

RN09-CP-0001 is a single centre Phase I ascending dose safety study to investigate 3 cohorts of patients with peripheral artery disease receiving intramuscular injections at 20 million, 50 million or 80 million CTX0E03 DP cells into the affected limb. It has completed its 1st cohort and treated 3 patients with CTX0E03 DP at a dose of 20 million cells, with a minimum and maximum enrollment of 9 and 18 patients, respectively.

There have been a total of 2 possible cell-related serious adverse events and 1 possibly-related immune response reported in the patients treated with CTX0E03 DP to date. Adverse events related to the surgical procedure, including an asymptomatic sub-dural bleed, extradural haematoma at site of entry, burr hole site bleeds, and superficial scalp infections at the implantation wound site have been reported. Please refer to section 7 of the Investigator Brochure for further information.

Sustained, modest reductions in neurological impairment as measured by the National Institutes of Health Stroke Scale (NIHSS) and spasticity as measured by the Summated Ashworth Scale for affected upper and lower limbs were observed in patients in study RN01-CP-0001 compared with their stable pre-treatment baseline.

6.5 Rationale for Study Design

The Phase I ascending dose study was designed primarily to assess safety. It is important to determine whether CTX0E03 DP has the potential to induce functional improvement in patients with residual paresis following a stroke. Given the need for stereotaxic implantation, such a treatment will only be justified if it results in sufficient recovery leading to an improvement in a patient's level of independence. Minor changes in neurological rating scales would be insufficient to justify this intervention. This study will evaluate the safety and efficacy of intracerebral CTX0E03 DP at a dose level of 20 million cells in patients with paresis of an arm following supratentorial ischaemic stoke. Eligible patients will have no useful function of the paretic arm a minimum of 28 days after the ischaemic stroke (a modified NIH Stroke Scale Motor Arm Score of 2-4 for the affected arm – i.e. some movement against gravity to no movement). Published literature (Duncan *et al.*, 1992; Heller *et al.*, 1987; Sunderland *et al.,* 1989) suggests that the probability of such patients recovering useful function of an arm which remains severely paretic at these time-points is less than 5%. The aim of this study is to determine whether it is sufficiently likely that treatment CTX0E03 DP at a dose level of 20 million cells improves the recovery to justify a subsequent larger prospectively controlled study. Useful recovery will be defined as the ability to use the previously paretic arm to pick up a 2.5cm^3 block from a table top, lift it and reposition it an a higher surface (test item 2 of the Action Research Arm Test). Such an increase in function would be expected to facilitate other basic tasks such as feeding oneself. No therapy to date has offered such efficacy. Despite the encouraging improvements in rating scales of neurological deficit observed in the Phase I study it is not known if CTX0E03 DP will be able to induce this degree of recovery.

6.5.1 Dose Selection

The Phase I ascending dose safety study of the same formulation CTX0E03 DP is evaluating doses of 2, 5, 10 and 20 million cells per implantation per patient by direct injection into the striatum.

Non-clinical studies in the rat MCAo stroke model have demonstrated dose related efficacy. Allometric scaling suggests that an equivalent dose in man is 20 million cells.

Non-clinical studies have not indicated the dose ceiling above which there is no further increase in efficacy. However, the volume of material that can be injected into the brain suggest that the highest practical dose of CTX0E03 DP in man for stroke is 20 million cells.

7 Biological Properties of IMP

CTX0E03 DP is an off-white, opaque, sterile suspension composed of CTX0E03 cells at a passage of ≤37 and formulated in HypoThermosol (HTS-FRS) at a concentration of 5x10^4 cells/µL. The CTX0E03 cell line incorporates a proprietary c-mycER^{TAM} conditional immortalisation technology allowing for suitable scale-up manufacturing and clinical application. Growth factors and 4-Hydroxytamoxifen (4-OHT) used in the manufacturing process have been omitted and are not part of the final formulation.

7.1 Non-Clinical Findings

7.1.1 Pharmacodynamics

Non-clinical evaluation of CTX0E03 stem cells in the rat MCAo ischaemic stroke model has shown efficacy of CTX0E03 cells in a reproducible and dose related manner. Efficacy is observed whether animals were immunosuppressed or non-immunosuppressed.

Studies of CTX0E03 cells in rat models of ischaemic stroke have demonstrated:

- Improvements in sensorimotor function following intracerebral CTX0E03 implantation.
- Increased host cell neurogenesis in the sub ventricular zone.
- Restored collagen IV to almost normal levels.

In addition, studies of CTX0E03 stem cells in mouse models of hind limb ischaemia have demonstrated the following cell related effects:

- Increased blood flow at sites of peripheral ischaemia as assessed by laser Doppler flowmetry.
- Increased $tcPO_2$.
- Increased blood flow measurement using fluorescent microspheres.
- Increased small arteriolar and capillary density.
- Reduced necrosis of digits.
- Gene expression of several cytokines, chemokines and growth factors linked with angiogenesis was found enhanced in the cell treated group (ReNeuron Study Report RN09-GE-0024).

Further details of these studies can be found in the Investigator Brochure (IB).

The mechanism of action of stem cells in general, and CTX0E03 in particular, in enhancing recovery following ischaemia is not well characterised. It is believed that CTX0E03 cells release factors that change and regulate the activity of other cells and "coordinates" elements of tissue response and repair. The response of CTX0E03 likely involves signalling of CTX0E03 cells to other cells involved in tissue repair. Stem cells are also known to be potent modulators of the immune system.

7.1.2 Pharmacodynamic Interactions

Studies *in vitro* have confirmed that re-exposure of CTX0E03 cells implanted into allogeneic tissue to 4-OHT does not return differentiated CTX0E03 cells to a proliferative state. Methylation analysis performed on implanted CTX0E03 in ischaemic rat brain showed the CMV promoter underwent methylation resulting in down-regulation of c-mycER^{TAM} (Stevanato *et al*., 2009). Similarly, exposure of CTX0E03 cells to endogenous steroid hormones or to the drug tamoxifen as might occur *in vivo* does not activate the c-mycER^{TAM} technology in cells, and does not lead to inappropriate cell proliferation.

Valproic acid, a drug used for the treatment of epilepsy and mood stabilisation is a histone deacetylase inhibitor that can result in secondary demethylation of methylated DNA (Milutinovic *et al*., 2007).

An *in vitro* study (ReNeuron Study GN01-GE-0050) investigated the effects of valproic acid on the possible demethylation of the c-mycER^{TAM} gene in CTX0E03 cells which had been cultured up to 4 weeks in a "non-proliferative" condition (i.e. without 4-OHT and growth factors). The 4-week non-proliferative culture condition was chosen as the more appropriate *in vitro* assay to represent the *in vivo* case scenario, where the CTX0E03 cells

had not been subjectsubjectedsubject to recent 4-OHT exposure. Following exposure to valproic acid, reactivation of cell proliferation was measured by cyquant, and c-mycER^{TAM} gene reactivation was measured by real-time PCR (qPCR). CTX0E03 cells were treated for either an additional 24 hours or 1 week in the presence of valproic acid (1 µM or 40 µM) and compared with untreated controls. The addition of valproic acid to a cell culture which had been maintained in non-proliferative state for 4 weeks did not cause significant c-mycER^{TAM} gene reactivation or cell growth (ReNeuron Study GN01-GE-0051).

This study demonstrated that although valproic acid can maintain and reactivate c-mycER^{TAM} expression in CTX0E03 cells, it is unlikely to do so after 4 weeks of cells being cultured in non-proliferative conditions. Given that administered cells will be in non-proliferative conditions once *in vivo,* it may be assumed that any medicine containing sodium valproate, if given 4 weeks after cell administration, would not reactivate the c-mycER^{TAM} gene and cause cell proliferation. To exclude any potential risk patients who have taken sodium valproate within the previous week will be excluded from inclusion into the clinical trial and the use of valproate drugs will not be allowed 4 weeks post cell administration.

7.1.3 Pharmacokinetics

Conventional absorption, metabolism and excretion studies which normally comprise the pharmacokinetic assessment of biological or medicinal products are not appropriate for this product. However, biodistribution and cell survival post implantation to determine whether cells migrate from the implantation site to other tissues and/or organs is a key consideration for clinical development of CTX0E03 DP.

7.1.3.1 Absorption

Not relevant for this product.

7.1.3.2 Distribution and Cell Survival

Results of distribution studies following implantation of CTX0E03 cells are listed in the IB.

These findings that CTX0E03 cells stay localised at or near the site of implantation with little or no migration of cells to peripheral tissues indicate the low risk of AEs to patients from stray cells. This is important because once implanted there is no means to monitor the CTX0E03 cells in the brain or peripheral tissues.

The incidence of CTX0E03 cell survival in non-clinical models decreases rapidly in the first few days following implantation. None of the cells surviving time were proliferating. The longest period of cell survival observed in rat MCAo model was 12 months. Cell proliferation is observed in CTX0E03 cells for up to 6 months post implantation in the brain. From the studies presented, the level of proliferation of implanted CTX0E03 cells is similar to that seen in host brain and is not associated with any evidence of tumour formation. Beyond 6 months implantation, the incidence of cell survival in individual animals decreases substantially and none of the cells surviving to these times are proliferating.

7.1.3.3 Metabolism and Excretion

Traditional studies designed to evaluate the metabolism and excretion of chemicals and biological products are not applicable to transplanted cells.

7.1.4 Toxicology

7.1.4.1 Single Dose Toxicity

Single dose studies using CTX0E03 cells were conducted predominantly in rats and mice. Study duration varied from days to 12 months. Studies were conducted in normal healthy rats as well as in rat models of stroke (900,000 cells in the model of middle cerebral artery occlusion) and naïve mice–(maximum of 2,500,000 cells). Mouse studies were conducted in both immunocompetent and immunodeficient strains as well as in non-diabetic and uncontrolled diabetic (secondary to acute streptozotocin toxicity) mice. In addition, routine blood chemistry was monitored in life. No abnormal behavioural results were observed. No CTX0E03 cell related adverse events were identified in any of the studies. The only exception occurred in one study (RN09-GE-0026) using a different formulation of cells from that proposed for this clinical study. In that single study, increased mortality was noted when the alternative formulation was administered to mice with uncontrolled diabetes immediately following surgery to ligate the main artery supplying the upper hind limb. Further, no excess mortality was noted with the cells if treatment was delayed till seven days following surgery. (The validity of this study is discussed in fuller detail in the pharmacology section of Investigator Brochure).

Brain and peripheral organ pathology and histopathology were also assessed in tissues from non-human primates; again there were no CTX0E03 DP related adverse events noted.

7.1.4.2 Repeat Dose Toxicity

No repeat-dose studies have been conducted with CTX0E03 cells as the proposed clinical regimen is for administration of CTX0E03 cells on a single occasion.

7.1.4.3 Genotoxicity

No genotoxicity studies have been conducted with CTX0E03 cells.

7.1.4.4 Tumourigenicity

A range of tumourigenicity studies have been completed in MCAo rats, non-human primates and NOD-SCID mice. Studies in the latter 2 species were uninformative in this respect due to lack of CTX0E03 cell survival post implantation.

In the MCAo rat studies, cell survival, proliferation index and phenotype have been monitored. CTX0E03 cell proliferation was comparable to endogenous neurogenesis seen in host brain.

No CTX0E03-related tumour was observed in studies of up to one year duration. All available data support the view that CTX0E03 DP does not present a tumour risk following implantation into the brain or muscle.

In addition, ReNeuron have shown that long term (9 months) treatment of animals with tamoxifen had no impact on CTX0E03 cell survival and proliferation and there were no CTX0E03 DP- plus or minus tamoxifen- related reports of tumour formation.

A research study has also demonstrated that the c-mycER^{TAM} transgene in the CTX0E03 DP cells is down regulated following implantation *in vivo* thus reducing the likelihood of inappropriate proliferation of the cells post implantation.

7.1.4.5 Reproductive and Development Toxicity

No reproductive and developmental toxicity studies have been conducted with CTX0E03 cells.

7.1.4.6 Other Toxicity Studies

No other toxicity studies other than those described above have been undertaken.

7.2 Risks Evaluation

All risks listed in the IB have been considered in the design of this study. The following particular risks merit discussion regarding the design of this study:

7.2.1 Tumourigenicity

Non-clinical tumourigenicity studies have all been negative. In the absence of 4-OHT, c-mycER^{TAM} is inactive. Following the final stage of manufacture of CTX0E03 DP that occurs in the absence of 4-OHT, non-clinical studies have demonstrated that re-exposure to 4-OHT does not result in reactivation of c-mycER^{TAM}. Non-clinical studies indicate that the c-mycER^{TAM} gene expressed in CTX0E03 cells in culture medium during production is silenced when the Drug Product is used *in vivo*. Individually and combined, these studies suggest no added risk to patients participating in this study.

7.2.2 Surgical Complications / Intracranial Bleeding

Stereotaxic intraparenchymal delivery offers some advantages in cell therapy for stroke, ensuring that large numbers of cells are adjacent to the site of ischaemic tissue damage and avoiding any concern that cells may fail to cross the blood-brain barrier. This route of administration opens the possibility of later intervention following stroke, beyond the acute phase when blood-brain barrier permeability is increased. Delivery by intraparenchymal injections may impose some anatomical restrictions since some sites are unlikely to be safely amenable to stereotaxic surgery however these are not the subject of this study. The predictable risk associated with intraparenchymal injection is intracranial bleed. There is considerable experience with stereotaxic neurosurgerical delivery of gene or cell therapies and deep brain stimulation. Procedural complications rates, principally intracerebral haemorrhage are usually in the range of 1-2% (Muir *et al.,* 2011) and seizures 2.4% (Coley *et al*., 2009).

7.2.3 Exacerbation of Myocardial Ischaemia or Sudden Death

Non-clinical studies did not indicate any risk of cardiac ischaemia or cardiac toxicity associated with CTX0E03 cells. Other than the Phase I ascending dose safety study assessing CTX0E03 DP administration following stroke no clinical studies have assessed the safety of stem cells in patients following a stroke. Numerous clinical reports in the published literature have reported the safety of stem cells in clinical trials for another condition due to atherosclerotic vascular disease: critical limb ischaemia. Case reports, single arm open studies (Gopall *et al*., 2010; Lenk *et al*., 2005; Sprengers et al., 2008) (Lawall *et al.,* 2011) and a double-blind placebo controlled study (Powell *et al*., 2008) suggest that treatment of CLI with autologous stem cells may offer a net benefit to patients with CLI. In addition, intra-cardiac injections of human endothelial progenitor cells have been studied in patients following myocardial infarction or as an experimental intervention in patients with a history of compromised cardiac output with apparently favourable results (Devanesan *et al.,* 2009). It is noted however that the “Therapeutic Angiogenesis using Cell Transplantation” study (Tateishi-Yuyama *et al*., 2002) reported two sudden deaths of unknown origin within 24 weeks out of 25 patients treated, and Miyamoto reported one case of sudden death of unknown origin in a 30-year-old patient without any previous cardiac history (Miyamoto *et al*., 2006). A separate study comparing i.m. injection of bone marrow derived mesenchymal stem cells versus bone marrow derived mononuclear cells for the treatment of CLI reported that three of 41 enrolled patients withdrew from the trial due to death from myocardial infarction within 4 weeks of enrolment into the trial. Although not clear, the text implies that these patient had not yet been treated with cells and are indicative of the high incidence of atherosclerotic co-

morbidity in this population (Lu *et al*., 2011). While the available data regarding the effect of stem cells in general and CTX0E03 in particular suggest the potential for a positive effect in patients with vascular disease, the DSMB will review all serious adverse events as they occur. The balance of available data indicates the potential for significant benefit in this population while the evidence suggesting risk is small.

7.2.4 Allergic Response to Allogeneic Cells

The immunogenic potential of CTX0E03 cells appears low. CTX0E03 DP demonstrates efficacy in non-clinical models of stroke and CLI in immune-competent animals and immune-suppressed animals without the need for immunosuppressive drugs in the immune-competent animals. The efficacious dose of cells is similar in both animal populations. Moreover, HLA expression is low on CTX0E03 cells. In the on-going studies CTX0E03 DP is injected directly into the putamen of stroke patients without immunosuppression, ongoing laboratory assessment of allo-responses has not indicated an allogeneic response to date. While it is recognised that the brain is an "immune-privileged" site, and that a different response may occur when allogeneic cells are injected intramuscularly, there have been no immune reactions observed following the 3 patients who have received CTX0E03 DP cells injected intramuscualarly. Given the non-clinical data, the low expression of HLA and interim data from the ongoing studies, it is believed that the risk of an allergic response is small. Given the need to closely monitor patients following stereotaxic surgery to inject the allogeneic cells, patients treated in this study must be observed for a minimum of 12 hours after implantation of the cells under the supervision of a healthcare professional with access to immediate resuscitation facilities and the experience and supplies necessary to treat anaphylaxis or an intracranial adverse event. Moreover, a patient's HLA-reactivity status will be determined both before and after implantation of CTX0E03 DP (see Section 11.4.7).

7.2.5 Unknown Risks Associated with Early Clinical Trials

Unknown risks cannot be excluded. It is also recognised thatpatients with a history of ischaemic stroke are at increased risk of myocardial infarction, ischaemia induced arrhythmias, critical limb ischemia, deep vein thrombosis, falls, pneumonia and further cerebrovascular events. Against this background, it is important that the safety of CTX0E03 is evaluated in a controlled clinical trial as soon as sufficient evidence of efficacy is obtained to justify a larger study. Use of an independent DSMB will permit ongoing risk assessment of all serious adverse events as they occur and of event rates at appropriate intervals throughout the study.

The Investigator's Brochure contains a list of completed non-clinical studies and findings from the ongoing clinical study. The investigator should review the IB before treating any patient.

8 Trial Objectives

8.1 Primary Objective

- To determine whether a sufficient proportion of patients experience response of their paretic arm following treatment with CTX0E03 DP at a dose level of 20 million cells to justify a subsequent randomised study.
    - Response will be defined as a minimum improvement of 2 points in test number 2 of the Action Research Arm Test (Grasp a 2.5 cm^3 block and move it from the starting position to the target end position) in the affected arm 3 months after implantation of CTX0E03 DP. This would represent an improvement from a pre-treatment state in which the patient was unable to grasp and reposition the block

as required to a post-treatment state in which the patient could accomplish the task as specified within 60 seconds and would represent recovery of useful function in a previously paretic arm.

8.2 Secondary Objectives

- To assess the efficacy of intracranial CTX0E03 DP in restoring upper limb function following an ischaemic stroke using the Action Research Arm Test (ARAT) (Yozbatiran *et al.,* 2008).
- To assess the efficacy of intracranial CTX0E03 DP in restoring function following an ischaemic stroke using the National Institutes of Health Stroke Scale (NIHSS) (Anemaet, 2002; Brott *et al.*, 1989; Lyden *et al.*, 2001).
- To assess the efficacy of intracranial CTX0E03 DP in restoring patient's functional independence following an ischaemic stroke using the Rankin Focused Assessment (RFA) (Saver *et al.*, 2010) version of the Modified Rankin Scale (mRS).
- To assess the efficacy of intracranial CTX0E03 DP in improving patient's ability to execute activities of daily living following an ischaemic stroke using the Barthel Index (BI) (Mahoney & Barthel, 1965).
- To assess the efficacy of intracranial CTX0E03 DP in sensorimotor recovery of the affected limb restoring function following an ischaemic stroke using the Fugl-Meyer Assessment (Fugl Meyer et al, 1975; Gladstone et al, 2002).
- To assess the safety and tolerability of intracranial CTX0E03 DP in patients following an ischaemic stroke.

9 Investigational Plan

9.1 Description of Overall Trial Design

This is a an open (non-blinded), single arm study to screen for evidence of efficacy. The study will be overseen by an independent DSMB.

Baseline evaluations as listed in Appendix 1: Trial Evaluation Schedule will be performed at visit 1 and 2 (i.e. between day 28 (±7) and day 300 (+7)) following an ischaemic stroke prior to injection of CTX0E03 DP ("day 0"). Subsequent investigations will be performed as listed in the same appendix.

At least A minimum of 21 patients will be enrolled to receive CTX0E03 DP (20 million cells) by stereotaxic intra-striatal injection ipsilateral to the location of the supratentorial ischemic stroke. Evaluation of the efficacy of CTX0E03 DP will be by review of the change in scores of the sensorimotor assessments (ARAT, NIHSS, RFA, BI and Fugl-Meyer) at pre-determined intervals compared to baseline.

It is recognised that no intervention to date has been demonstrated to produce marked recovery in an established paretic arm post-ischaemic stroke. There is no consensus in the field regarding the optimal study design for screening efficacy in this indication.

9.2 Selection of Trial Population

Only those patients who meet all of the criteria listed below will be invited to participate in this study.

The investigator should maintain a log of patients considered for the study but excluded due the inclusion or exclusion criteria or due to lack of availability of study drug. The log

should include the age of the patient and the reason(s) why the patient was not eligible for the study. The log should be maintained in the site file.

A patient must meet the following inclusion criteria to participate in this study:

9.2.1 Inclusion Criteria

1. Written informed consent or witnessed informed consent in the event that the patient is unable to sign informed consent due to paresis of the affected arm.
2. Supratentorial ischaemic stroke.
3. Male or female.
4. Age 40 years or more.
5. Stroke, at time of consent, satisfying the following criteria:
   - Modified NIH Stroke Scale (NIHSS) Motor Arm Score of 2 (some effort against gravity), 3 (no movement against gravity) or 4 (no movement) for the paretic arm post ischaemic stroke at visit 1 and 2.
   - Clinical diagnosis of stroke confirmed by physician using neuro-imaging (computerised tomography or magnetic resonance imaging).
   - A Score of 0 or 1 for test 2 of the Action Research Arm Test (Grasp a 2.5 cm^3 block and move it from the starting position to the target end position) at visit 1 and 2 post-stroke using the affected arm.
6. Ability to comprehend verbal commands.
7. Eligible for neurosurgery, including appropriate anatomical target for cell implantation.

9.2.2 Exclusion Criteria

1. Prior history of stroke resulting in permanent moderate to severe disability (i.e. Rankin Scale greater than 2) (other than the presenting ischaemic stroke).
2. Stroke due to haemorrhage.
3. History of neurological or other disease resulting in significant functional impairment of the paretic arm impairing potential ability to pick up, lift and place a 2.5 cm^3 block (e.g. Parkinson's disease, motor neuron disease, arthritis, Dupuytren's contracture or fixed anatomical abnormality).
4. Any contraindications to MRI including presence of a cardiac pacemaker (excluding MR-conditional cardiac pacemaker), metal fragments in eye etc.
5. Uncontrolled blood pressure defined as systolic blood pressure ≥180 mm Hg or diastolic blood pressure ≥110 mm Hg (patients are only to be excluded if an initial value exceeding these limits is repeated on retesting over several days).
6. Patient with a severe comorbid disorder, not expected to survive more than 12 months.
7. Acute cardiovascular events other than the presenting ischaemic stroke (e.g. myocardial infarction, recent coronary intervention for symptomatic cardiac disease) considered by the Investigator or the anaesthetist responsible for the patient to place the patient at increased anaesthetic risk, 3 months prior to planned injection of CTX0E03 DP.
8. History of malignant disease, (except for non-melanoma skin cancer) within the previous 5 years or any history of malignant brain tumours or brain metastasis.
9. Current treatment with tamoxifen.
10. Patients taking valproate drugs for any indication in whom it is not considered appropriate to discontinue the valproate for a period of one week prior and four weeks post neurosurgery. Patients in whom valproate is switched to an alternative agent during this period may be included.
11. Requirement for antiplatelets and/or anticoagulants including heparin, warfarin or other anticoagulants/ medication that cannot be interrupted to allow surgery.
12. Requirement for intermittent (stop/start date from 1-month prior-to and 3 month post-CTX0E03 DP administration) use of oral antispasticity medications (antispasticity

medications are acceptable if they have been taken regularly for at least one month prior to CTX0E03DP administration).

13. A history of uncontrolled diabetes e.g. history of hypoglycaemic or hyperglycaemic events requiring hospital admission over previous 6 months
14. Females of childbearing potential (or within 2 years of last menstrual cycle) must have a confirmed negative pregnancy test at time of treatment and agree to use two reliable methods of contraception (e.g. oral contraceptive and condom, intra-uterine device (IUD) and condom, diaphragm with spermicide and condom) for the duration of this study
15. Sexually active males with partners who are FOCBP must be willing to use a reliable method of contraception (e.g. barrier and spermicide or as described above) for the duration of this study.
16. Considered unlikely to be able to attend for all follow-up visits.
17. Organ transplant recipient.
18. In the opinion of the Investigator, sustained consumption of alcohol or drugs at a level likely to be injurious to health.

9.3 Withdrawal of Patients from Treatment or Assessment

A patient will be discontinued from the trial if:

1. They withdraw their consent.
2. They develop any physical, neurological or psychological disease before CTX0E03 cell implantation that may affect their ability to complete the trial as determined by the Investigator.
3. They test positive for Human Leucocyte Antigens (HLA) expressed on the CTX0E03 cell line as part of their screening assessments and prior to cell administration. Test results must be available prior to cell administration.
4. It is deemed necessary for clinical reasons (e.g. significant concomitant illness).

If a patient prematurely withdraws from the trial after CTX0E03 DP injection and all remaining trial follow-up visits cannot be completed, they should immediately have as many tests for day 365 time-point (Appendix 1: Trial Evaluation Schedule) conducted as possible with their consent. Consent will have already been given by the patient to use data already collected, and all documentation and case report form (CRF) visit pages must be completed up until the date of withdrawal. All adverse events must be documented in the CRF. Consent will also have been given by the patient to be flagged by the relevant national cancer registry (if one exists in that country) for 5 year follow-up.

The trial may be discontinued on the advice of the DSMB or if so requested by ReNeuron, the relevant national CA and the relevant REC. In the event that a CA, REC, the DSMB or ReNeuron advise discontinuing the study, the CA(s) and REC(s) of all participating jurisdictions will be informed. In the event that a CA or REC in one country recommends discontinuing the study, but other CAs, RECs and the DSMB determine that it is appropriate to continue the study in the remaining countries, these countries may continue.

In reaching their recommendations the DSMB and ReNeuron will consider all the safety data and issues including the following:

- Any Serious Adverse Event(s) that, in their opinion constitutes a sufficiently great safety hazard as to warrant stopping the trial.
- Safety and/or tolerability issues with CTX0E03 DP which come to light after the trial starts.

9.4 Re-screening of Patients

Patients who have previously been screened under an older version of the protocol or for the ReNeuron observational study (RN-CS-0001) may be re-screened for entry to the PISCES II study if considered by the Investigator to be suitable and meet all current eligibility requirements.

Stroke functional assessment data (NIHSS, ARAT, RFA, BI, FMA) previously collected as part of screening data or for the ReNeuron observational study (RN-CS-0001) on a previous occasion may be used as a qualifying functional assessment (i.e. Visit 1) if the data is less than 12-months old. In such instances, this data should be copied and transcribed into the patients CRF.

9.5 Patient Visit and Treament Windows

It is recognised that the selected patient population may have concurrent illness and disability that may impede the strict adherence to the protocol-specified patient visit and treatment windows. In these exceptional circumstances, any potential visit or treatment window deviations must be notified in advance to the ReNeuron Chief Medical Officer, who will review each deviation on a case by case basis, taking account of all current eligibility requirements, patient safety and data integrity.

10 Investigational Medicinal Product (IMP)

10.1 CTX0E03 Drug Product (DP)

CTX0E03 DP is a formulation containing a human neural stem cell line developed by ReNeuron.

CTX0E03 DP is an off-white, opaque, sterile suspension. It is composed of CTX0E03 cells at a passage of ≤37. The cells are formulated in HypoThermosol (HTS-FRS) at a concentration of 5 x 10^4 cells/µL. HTS-FRS is made up of ions, buffers, impermeants, a colloid, metabolites and an antioxidant.

CTX0E03 DP is supplied, transported and stored cryo-preserved at <-135ºC in a cyro-shipper. CTX0E03 DP must be used before the expiry date and time listed on the label. Once removed from cryo-storage, CTX0E03 DP should be rapidly thawed at 37ºC, kept at room temperature (15-25ºC) and must be administered to the patient within 3 hours of thaw.

10.2 Packaging and Labelling

One pack of study drug (CTX0E03 DP) will be prepared per study patient and shipped to the study Pharmacist.

Each vial (cryotube) will be labelled in accordance with GMP Annex 13 and any local regulatory requirements. The immediate small container label on the cryovial will contain the following minimum details:

- name of sponsor
- pharmaceutical dosage form, route of administration, quantity of dosage units and the name/identifier and strength/potency

- batch number
- a trial reference code
- the trial patient identification number

For each 20 million cell dose, two vials will be placed inside a secondary container. This container will be labelled in accordance with GMP Annex 13 and any local regulatory requirements. The secondary container label on the cryobox will contain the following minimum details:

- name, address and telephone number of the sponsor
- pharmaceutical dosage form, route of administration, quantity of dosage units and the name/identifier and strength/potency
- the batch code number to identify the contents and packaging operation
- a trial reference code
- the trial patient identification number which includes the investigator/site reference
- directions for use
- "For clinical trial use only"
- the storage conditions
- period of use (use-by date and expiry date)

10.3 Ordering Manufacture and Shipment of IMP

CTX0E03 DP will be manufactured in batches at intervals depending on the rate of patient enrolment. Investigators will be informed by regular email updates when each batch of material is scheduled for release. Investigators should complete the study drug order form and append this to an email to stroke2.drug.order@reneuron.com. ReNeuron will schedule the earliest possible despatch date for the study drug and will inform the Investigator and Pharmacist.

10.4 Shipping (Transport /Handling) of IMP

CTX0E03 DP will be transported by courier from the site of manufacture/ storage site to the Investigator site pharmacy in a cryo-shipper at <-135 ºC. The process will be subject to continuous temperature monitoring. The cryo-shipper will be delivered directly to the Pharmacist who will securely store until ready for use.

10.5 Storage and Drug Accountability of IMP

CTX0E03 DP must be stored at <-135ºC. Once removed from cryo-storage, CTX0E03 DP should be rapidly thawed at 37ºC. Once thawed, CTX0E03 DP must be kept at room temperature (15-25ºC) and must be administered to the patient within 3 hours post thaw.

All CTX0E03 DP supplies used to conduct this trial must be maintained under adequate security and stored under the conditions specified on the label until administration to trial patients or returned to ReNeuron or its agent. An accurate running inventory of CTX0E03 DP will be kept by the Pharmacist and will include the shipping documents and the date and time each dose of CTX0E03 DP is dispensed. The Investigator agrees not to supply the CTX0E03 DP to any persons not entered into this trial.

10.6 CTX0E03 DP Quarantine and Release

CTX0E03 DP will be provided to the site under temperature controlled quarantine.CTX0E03 DP will remain under quarantine until data from the temperature

monitor(s) have been downloaded and sent to ReNeuron's Quality Assurance manager for review and final release. Once CTX0E03 DP has been released for use it may be taken out of the cryo-shipper, rapidly thawed at 37ºC to room temperature (15-25ºC) and dispensed. Management of the CTX0E03 DP post arrival at the clinical site and release from quarantine will be fully documented in 'Pharmacy Guidelines' and accompanying drug management form to be in place prior to treatment of the first patient. Staff trained to follow this procedure will document the time of removal from the cryo-shipper, time of thaw to ambient temperature, dispensing to the clinical team and administration to the trial participant. This procedure will ensure that the CTX0E03 DP is used within 3 hours from the time of thawing and in accordance with the label.

Once the Pharmacist has been informed that CTX0E03 DP has been released from quarantine and the patient is in the operating theatre ready for injection of CTX0E03 DP, the Pharmacist will thaw and dispense the CTX0E03 DP for injection.

10.7 CTX0E03 DP Administration

The contents of the cryotube must be visually inspected (for absence of particles and matching colour description) prior to administration, as for all injectables. Refer to the 'Pharmacy Guidelines' in case the product is not an off-white, opaque suspension, free of any particulate matter.

Each patient will receive an intracranial implantation of 400 µL CTX0E03 DP containing $20x10^6$ cells in sterile suspension on a single occasion in this trial.

No comparator or placebo will be used.

CTX0E03 DP will be implanted under general anaesthesia by a neurosurgeon experienced in stereotaxic intracranial implantation. Stem cell delivery will be performed using a technique used successfully in two previous clinical trials to implant stem cells intracranially by Kondziolka (Kondziolka *et al.,* 2004) and in ReNeuron's Phase I and II trials (RN01-CP-0001 and RN01-CP-0002).

Each vial of CTX0E03 DP will contain 250 microL and the relevant dose will be drawn up into a sterile Hamilton glass syringe in the operating theatre. Two vials are needed for each 20 million cell dose.

Four target trajectories around the stroke site will be chosen on the basis of the pre-operative structural MRI scan. Under general anaesthesia, a Leksell (or equivalent) stereotaxic frame will be fixed to the patient's head and a plain CT scan of the head obtained. CT images will be fused with the pre-operative MRI images, allowing stereotaxic co-ordinates for each of the trajectories under consideration to be obtained.

A burr-hole craniotomy (or more than 1 craniotomy if required) will be fashioned at an appropriate point on the patient's skull and the cells implanted using the ReNeuron Implantation Cannula and connected to a Hamilton glass syringe as originally described by Kondziolka (Kondziolka *et al.,* 2004).

For a 20 million cell dose, 5 deposits of 1 million cells/20 microL volume will be made along each of four trajectories at a rate of 5 microL/min. The total time to implant the maximum dose (20 million cells) is estimated to be approximately 2 hours, including the time to reload the syringe and to change position of trajectories.

The dose and volume administered on each dosing occasion will be recorded. If implantation is not completed within the 3 hour expiry time then administration should cease and the lower dose administered recorded.

After completion of the stereotaxic injections the cannula will be withdrawn and the cranial wound(s) closed.

10.8 Surgery Guidelines

Administration of the CTX0E03 DP to the patient will involve the following personnel

- Neurosurgeon
- Anaesthetist
- Scrub nurse
- Circulating nurse
- Radiographer
- Recovery nurse

10.8.1 Drug Product Handling Procedure Training

All operating room staff who might be called upon to assist in the patient dosing procedure will be trained in the handling of the drug product. All training will be documented and retained for the site file.

The training will involve a demonstration of the handling procedure as set out below. Training equipment will comprise of a Hamilton glass syringe, a syringe loading needle and an implantation cannula. In addition, a CTX0E03 DP cryotube drug product container containing 250 microL liquid and a bottle containing liquid (tap water is sufficient for this training procedure) will be used to simulate the drug product and excipient. All theatre staff will also be trained as appropriate in checking the drug product.

10.8.2 Patient Preparation

On the day of surgery (Day 0) the patient will be taken to the anaesthetic room. A white board or equivalent in the operating theatre will be set up to record timings of various procedures and information as per the Drug Management Form for the Case Report Form (CRF) and as described below. The patient will be given a general anaesthetic and the stereotaxic frame fixed to the skull. The patient will then be taken for a CT scan to enable the stereotaxic coordinates for the implant to be determined. Use of MRI as an alternative imaging modality for stereotaxic frame placement is acceptable in centres where this is standard practice.

The CT scan data will reside on the scanner system and will be transferred to a Brain-Lab, or similar, stereotaxic Planning Station. These 2 electronic records of the scan will be archived. At a suitable time a copy of the scan for each patient will be requested on CD for the CRF by the Monitor.

The patient will be returned to the operating theatre in anticipation of the treatment. The implantation coordinates will be calculated using the CT scan and documented. At this time the product will be delivered into the operating theatre and be available for use. A burr hole(s) will be drilled through the skull and the guide cannula positioned on the stereotaxic frame.

The drug product will be transported to the operating theatre in a zip-lock bag by a member of the study team along with an unopened bottle of the excipient HypoThermosol-FRS (HTS-FRS) supplied to pharmacy direct from the manufacturer (BioLife Solutions Inc.).

Paperwork accompanying the product will include a Drug Management Form, a cryobox overview showing the grid position of each vial to ensure traceability of each batch and a patient prescription. The label on the outer packaging of the drug product will provide the

expiry time information at 15-25°C for the drug product, and this will be noted on the white-board of the operating theatre by a member of the theatre staff in order to ensure that treatment of the patient will be completed before the expiry time. The circulating nurse will remove the drug product vials from the outer packaging. The tamper-evident seal will be removed from the HTS-FRS bottle.

The dose of CTX0E03 DP planned for this trial is 20 million cells in 400 microL volume of vehicle. The CTX0E03 DP will be drawn up into a 250 microL Hamilton glass syringe with a luer lock fitting, through a syringe loading needle, and delivered via an implantation cannula. Each of these components will be provided by ReNeuron as individually packaged and sterile for single use only. For each patient two loading needles, two implantation cannulae and two syringes will be required. Spare syringes and cannulae will be available in the operating theatre.

The 20 million-cell dose will be drawn as 2 x 200 microL volumes from each of the two immediate product containers.

10.8.3 Loading the Syringe

The product container should be held upright at all times and should not be inverted or flicked. The syringe will be loaded as follows:

1. The neurosurgeon will attach a sterile stainless steel syringe loading needle to the sterile 250 microL Hamilton glass syringe.

2. The circulating nurse will open the container of sterile excipient (HTS-FRS). The neurosurgeon will aseptically draw the excipient up into the syringe through the syringe loading needle. Using rapid movements, the neurosurgeon will use the syringe plunger to draw the excipient in and out of the syringe several times until there is no longer any air bubbles present in the syringe/loading cannula. This will take approximately 10 movements of the syringe. The final expulsion will leave ~5-6 microL of excipient in the loading needle with no air in the system.

3. The circulating nurse will close the container of excipient, and open one immediate container containing CTX0E03 DP and hold it upright. The neurosurgeon will insert the syringe loading needle to the bottom of the tube, and carefully withdraw the syringe plunger slowly to the 225 microL graduation of the syringe. The circulating nurse will then discard the used drug product immediate container into a biohazard container. The outer packaging of the drug product should be retained for collection by the research nurse as a record of drug accountability.

4. The neurosurgeon will remove the syringe loading needle from the syringe, and replace it with the cell implantation cannula. The neurosurgeon will prepare the first 10 million cell dose by depressing the syringe plunger until it reaches the 205 microL gradation. Expelled excess drug product should be collected and wiped from the end of the cell implantation cannula using a sterile swab or patty. When the first 10 million cells are delivered the neurosurgeon will repeat steps 1-3 above using a second sterile loading needle and syringe to draw up the cells from the second 250 microL of product in the second immediate container.

A second sterile implantation cannula is also used in step 4 above to deliver the second 10 million cell dose.

10.8.4 Administration of the Product

The syringe plus cell implantation cannula loaded with drug product will be positioned on the stereotaxic frame and the implantation cannula threaded through the guide cannula.

CTX0E03 DP will be injected into the putamen ipsilateral to the side of the ischaemic

stroke. The final coordinates of the cell implantation cannula are determined on a per patient basis by the neurosurgeon based on the CT and MRI scans of the patient. Exact co-ordinates will be determined depending upon the site of ischaemic disruption noted on pre-implantation scans. Scans performed as part of the standard management of the patients as well as any scan performed due to the patient's participation in this study may be used by the surgeon in planning optimum co-ordinates within the putamen for injection of the study material.

The product will be delivered manually into the target area of the brain in 20 microL deposits at a rate of 5 microL/min pausing for at least 20 seconds between bolus deposits. For each 20 microL deposit, the product will be delivered by depressing the syringe plunger to deliver 5 microL volumes over 1 minute intervals. Timings will be directed by the theatre nurse using a start/stop clock. Delivery times of each 20 microL deposit will be recorded on the white board. Delivery of the dose is completed when the plunger reaches the 5 microL gradation on the syringe. The deepest implant will be delivered first and the cannula withdrawn for subsequent boluses along any single trajectory.

Five 20 microL deposits will be placed along each needle tract. A total of four needle tracts will be used per patient to deliver at total of 20 million cells to the ipsilateral putamen.

Administration of the dose specified must be within three hours of CTX0E03 DP being brought to room temperature. The times of start and completion of dose administration will be noted on the white board along with the volume of drug product administered as shown below. For the dose planned, in the event that one of the immediate containers is compromised then a maximum dose of 10 million cells will be administered. In the event that the product reaches the expiry time the dosing will be stopped and volume administered recorded on the white board.

10.8.5 Documenting Product Handling During Surgery

Primary information on expiry time of the product, patient ID and other relevant information will be recorded on the white board in accordance with the Drug Management Form. This will be set up with a list of information to be captured by the clinical research nurse prior to the start of treatment. At the end of surgery these data will be photographed by the clinical research nurse for incorporation into the Case Report Form.

If during surgery there are any patient adverse events that are specifically related to a malfunction or breakage in the cell implantation cannula, such events must be reported to the Sponsor immediately (i.e. within 24 hours).

10.8.5.1 Example White Board Set-Up

Data to be listed on theatre white-board and ancillary form.

STUDY NO:

SURGEON:

ANAESTHETIST:

STEREOTAXIC COORDINATES: TRAJECTORY 1, 2, 3, 4

IMP EXPIRY TIME:

PATIENT ID:

PATIENT No.

DOSE / VOLUME:

THEATRE TEMPERATURE MIN: MAX:

CANNULA LOT NO:

CANNULA EXPIRY DATE:

HAMILTON SYRINGE LOT NO:

HAMILTON SYRINGE EXPIRY DATE:

LOADING NEEDLE LOT NO.:

LOADING NEEDLE LOT EXPIRY DATE:

IMP DEPOSIT START TIME:

IMP DEPOSIT STOP TIME:

TIME OF DISPOSAL OF USED DRUG VIALS:

10.8.6 Drug Accountability

The Pharmacist will maintain drug accountability records on an on-going basis.

The Pharmacist will acknowledge receipt of the CTX0E03 DP, check and retain a copy of the manufacturer's QP release certificate. The CTX0E03 DP will be stored/ maintained in the cryo-shipper at <-135ºC until ready for use.

Management of the CTX0E03 DP post arrival at the clinical site, will be fully documented in a drug management form to be in place prior to treatment of the first patient. The secondary container must be retained for drug accountability purposes.

The Monitor should be permitted, at intervals, and upon request during the trial, to check the storage, dispensing procedures and records of the supplies. The Monitor will check drug accountability including the dose recorded as administered and that the administration was within the shelf-life of the product.

10.8.7 IMP Handling, Spillage and Accidental Exposure

Personnel who handle CTX0E03 DP or waste contaminated with CTX0E03 DP should protect themselves from contamination. All healthcare staff handling CTX0E03 DP must wear protective clothing, gloves and any other local hospital requirements as required. All disposable surgical supplies; including gloves, masks, gowns, dressings and swabs used during the implantation procedure will be destroyed by incineration according to hospital policy at the end of the procedure.

Spillages should be cleaned up with 'Distel' or locally approved disinfectant. All contaminated materials should be disposed of according to institutional guidelines for the disposal of genetically modified (recombinant) waste.

Although there is no clinical experience with accidental exposure or with inadvertent needle stick injuries, animal data do not suggest that there would be a deleterious effect. If contamination occurs, the site should be thoroughly cleaned with ethanol and the individual should be observed for any effects attributed to CTX0E03 DP.

10.8.8 Disposal of Unused IMP

Unopened and unused CTX0E03 DP provided to the Pharmacist should not be disposed of without prior written permission from the ReNeuron assigned Monitor.

The Monitor may request that unused material is returned to ReNeuron or its agent. If so, the Monitor will arrange transportation and provide instruction regarding packaging and labelling.

Alternatively the Monitor may provide written authority for the Pharmacist to dispose of the CTX0E03 DP. Once in receipt of written authority the Pharmacist should personally supervise and document disposal of the material in accordance with the hospital procedures for disposal of biological material. The CTX0E03 DP should be treated as clinical waste and deposited in a biohazard bin/container for subsequent routine disposal in accordance with local and national requirements for disposal of clinical waste containing GMOs.

10.8.9 Disposal of Waste Products

Part-used CTX0E03 DP (residual cells and syringes) provided to the location at which injections occurs, and all other contaminated material will be treated as clinical waste and deposited in a biohazard bin/container for subsequent routine disposal in accordance with local and national requirements for disposal of clinical waste containing GMOs. Full drug accountability will be maintained.

10.9 Method of Assigning Patients to Treatment Groups

This is an open non-comparative study. Eligible consenting patients will be treated in the order they present.

10.10 Blinding

This is an open non-comparative study. All patients receive the same treatment which is not blinded.

10.11 Prior and Concomitant Therapy

Antiplatelets and Anticoagulants:

Patients taking antiplatelets, warfarin or other anticoagulants must stop such medication as per local practice prior to surgery and will be advised of this study requirement at Visit 2. In the event the hospital does not have a written practice or in the event that the Investigator or anaesthetist consider that the particular medical needs of the patient require management of anticoagulation at variance with hospital practice, a file note should be prepared. The file note, prepared by the Investigator, should justify the proposed anticoagulant treatment plan, be placed in the Site File and a copy forwarded to the Sponsor. Patients will be contacted approximately a week before the scheduled surgery to remind them of planned changes in their anticoagulation medication. Patients who need to resume antiplatelet and/or anticoagulation therapy after surgery should usually do so after their Day 2 post injection assessment at the discretion of the Investigator.

Tamoxifen and Analogues:

Patients taking tamoxifen or tamoxifen analogues (e.g. raloxifene) at the time of consent are excluded from the study.

Valproate Drugs:

Valproate drugs are contraindicated 1 week prior to and 4 weeks post cell administration.

Botulinum Toxin and Antispasticity Medications:

Patients taking oral antispasticity medication **intermittently** (i.e. stop/start date from 1-month prior-to and 3 month post-CTX0E03 DP administration) are excluded from the study. Oral antispasticity medications are acceptable if they have been taken regularly for at least one month prior to CTX0E03 DP adminstration. Botulinum toxin, phenol or other injectable antispasticity medications are permitted to be used according to the clinical situation.

Other Medications:

All other products will be allowed on entry to the trial. Ideally the dose used should remain constant throughout the trial. Concomitant medications will be checked throughout the trial and any change in medication after the date on which the patient signs the informed consent form should be recorded in the CRF.

10.12 Physiotherapy

Prior to injection of CTX0E03 DP patients should continue to receive physiotherapy and occupational health support normally provided by the study site to any patient with a similar condition.

Following injection of CTX0E03 DP patients should receive regular physiotherapy (1.5 hours per week) including the paretic upper limb for a period of six weeks.

11 Efficacy and Safety Variables

The Trial Evaluation Schedule is summarised in the Appendices (Appendix 1: Trial Evaluation Schedule).

11.1 Efficacy Measurements

The following efficacy parameters will be recorded according to the Trial Evaluation Schedule presented (Appendix 1: Trial Evaluation Schedule).

- Action Research Arm Test
- National Institutes of Health Stroke Scale
- Fugl-Meyer Assessment
- Rankin Focused Assessment
- Barthel Index

11.2 Safety Measurements

The following safety parameters will be recorded according to the Trial Evaluation Schedule presented (Appendix 1: Trial Evaluation Schedule).

- Pregnancy test (all female patients of childbearing potential up to 2 years since last menstrual period)
- Medical history (baseline or since last visit)
- Concomitant medication
- General physical examination
- Temperature
- Pulse rate and rhythm
- ECG
- BP

- FBC
- LFTs
- Serum urea and electrolytes.
- Adverse Events
- Brain imaging post implantation

11.3 Adverse Events (AEs)

An AE is any untoward medical occurrence in a clinical trial patient administered a medicinal product and which does not necessarily have a causal relationship with this treatment.

An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medicinal product, whether or not related to the medicinal product.

AEs will be monitored throughout the trial at every visit from Visit 1. At each trial visit, the Investigator will assess whether any AEs have occurred, using a non-leading question such as 'How have you been feeling since your last visit?'. Patients will also be encouraged to spontaneously report AEs occurring at any other time during the trial.

All AEs, whether reported by the patient or observed by the Investigator will be documented on the AE page of the CRF, whether or not the Investigator concludes the event to be related to the drug treatment.

AEs will be described in the following way using the following verbatim AE terms:

Duration of AE:
- Start and stop date

Serious?:
- Yes or No.

Severity:
- Mild (does not influence activities of daily living)
- Moderate (sufficient to make patient uncomfortable and to influence activities of daily living)
- Severe (severe discomfort and disruption of activities of daily living)

Action taken:
- None - continued trial
- Withdrawn from trial
- Other

Therapy prescribed?:
- Yes or No (including non-drug therapy)

Ongoing at end / discontinuation of trial?:
- Yes or No (if Yes, follow-up within 30 days)

Outcome:
- Resolved
- Resolved with sequelae
- Death – if a patient dies during the trial the exact cause of death should be recorded in the CRF and a copy of any autopsy report provided to ReNeuron
- Not resolved
- Unknown

Relationship to the Investigational Medicinal Product (IMP)

- Definitely related
- Probably related
- Possibly related
- Unlikely related
- Not related

"Definitely related": an AE, including laboratory test abnormality, that follows a reasonable temporal sequence from administration of the IMP or which follows a clinically reasonable response on withdrawal (de-challenge) and that satisfies any of the following:

- Reappearance of similar reaction upon re-administration (re-challenge).
- Positive results in a drug sensitivity test (skin test etc.).
- Toxic level of the IMP revealed by measurement of drug concentrations in blood or another body fluid.

"**Probably related**": an AE, including laboratory test abnormality, that follows a reasonable temporal sequence from administration of the IMP or which follows a clinically reasonable response on withdrawal (de-challenge) and for which involvement of factors other than the IMP, such as underlying diseases, complications, concomitant drugs and concurrent treatment can reasonably be excluded. Re-challenge information is not required to fulfil this definition.

"**Possibly related**": an AE, including laboratory test abnormality, that follows a reasonable temporal sequence from administration of the IMP or which follows a clinically reasonable response on withdrawal (dechallenge), but which could also be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal may be lacking or unclear.

"**Unlikely related**": an AE, including laboratory test abnormality, with a temporal relationship to drug administration which makes a causal relationship improbable, and in which other drugs, chemicals or underlying disease provide plausible explanations.

"**Not related**": an AE, including laboratory test abnormality, which can be confidently explained by one or more other factors (e.g. alternative diagnosis confirmed by diagnostic evident), and for which there is no pharmacological or temporal basis for associating the AE with the drug.

11.3.1 Follow-up of AEs:

All patients experiencing AEs, whether considered associated with the use of the IMP or not, must be monitored until the symptoms subside and any clinically relevant changes in laboratory values have returned to baseline, or until there is a satisfactory explanation for the changes observed. The results of any additional diagnostic measures taken because of AE and not included in the Protocol should be attached to the CRF giving the date on which they were carried out.

If a patient discontinues the trial early, any AEs ongoing at the time of stopping the trial should be followed up within 30 days to confirm the status of the AE.

11.3.2 Serious Adverse Events (SAE)

The seriousness of an AE should be assessed initially by the Investigator. Adverse events are to be classified as either serious or non-serious. Any AEs that do not meet the definitions for serious, may be classified as non-serious.

An SAE is any untoward medical occurrence, or effect, that at any dose:

- Results in death.
- Is life threatening*.
- Requires inpatient hospitalisation or prolongation of existing hospitalisation.
- Results in persistent or significant disability/incapacity.
- Is a congenital anomaly or birth defect.
- Is an important medical event**.

*The term life-threatening refers to an event in which the patient was at risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death, if it was more severe.

**Important medical events are those that may jeopardise the patient or may require intervention to prevent one of the outcomes listed in the definition above. Medical judgement should be exercised in deciding whether an AE is serious in accordance with these criteria. If in doubt an AE should be classified as serious until further information is available.

The following should also be considered SAEs:

- Any suspected transmission via a medicinal product of an infectious agent.
- All cancers (malignancy) occurring at any time during the study period.
- Overdose (including the accidental administration of a medication in a dose in excess of that which had been prescribed) when it leads to an SAE.

Copies of the results of any additional diagnostic measures taken as a result of an SAE and where not stipulated in the Protocol, should be attached to the CRF. Copies of Hospital Discharge Summaries and any autopsy reports should also be obtained.

11.3.3 Follow-up of SAEs

All patients experiencing SAEs, whether considered associated with the use of the IMP/administration procedure or not, must be followed until any of the following occur:

- The event resolves
- The event stabilises
- The event returns to a pre-treatment value, if this is available
- The event can be attributable to agents other than the IMP or to factors unrelated to study conduct
- It becomes unlikely that any additional information can be obtained (patient or health care practioner unable or unwilling to provide additional information, lost to follow-up after demonstration of due diligence with follow-up efforts).

Any SAEs that are not resolved by the end of the study or have not been resolved upon discontinuation of the patient's participation in the study must nevertheless be closed with an outcome assessment.

11.3.4 Reporting SAEs

The Investigator should complete an AE form for all AEs, whether serious or not and leave this in the relevant CRF until it is collected by the Monitor. In addition a SAE form should be completed for every SAE occurring in a patient after providing informed consent.

All SAEs should be reported to the Pharmacovigilance (PV) provider. The telephone number and email for the PV provider appear on the front page of this Protocol.

The Investigator must notify the PV provider immediately of any SAE by telephone or email. In case of a verbal report this must be followed with written confirmation by fax / email within 24 hours of becoming aware of the event, using an SAE Report form. The Investigator will be provided with SAE Report forms at the start of the trial or upon request. These forms should be clearly labelled as 'initial notification'.

Any subsequent information should be supplied on another SAE Report form and clearly labelled as 'follow-up'. Follow-up forms should contain only essential patient and event identifying data, and then all new data. There should be no repetition of any data given in the initial notification.

11.3.5 Pregnancy Reporting

Pregnancy occurring in a study patient or a female partner of a male patient participating in the study, after the patient signs the informed consent through to his/her final study visit, should be reported to the PV provider as though it were an SAE on a Pregnancy Report form. Specific pregnancy notification and outcomes forms will be provided.

Although pregnancy itself is not technically an AE, all pregnancies must be followed to conclusion to determine their outcome. This information is important for both drug safety and public health concerns.

The Investigator should notify ReNeuron as soon as he/she becomes aware of the pregnancy and make every effort to follow-up and track the pregnancy through to completion or early termination of the pregnancy. If the outcome of the pregnancy meets the criteria for classification as a SAE (i.e., postpartum complications, spontaneous abortion, stillbirth, neonatal death, or congenital anomaly, including that in an aborted foetus), the Investigator should follow the procedures for reporting SAEs.

11.3.6 Adverse Drug reactions (ADR)

For each AE an assessment must be made of the probability that it was caused by the IMP. All cases judged by the Investigator or ReNeuron as having a "reasonable" suspected causal relationship to the IMP qualify as adverse reactions.

An Adverse Drug Reaction is an untoward and unintended response to an investigational medicinal product related to any dose administered.

An unexpected Adverse Drug Reaction is an adverse reaction, the nature or severity of which is not consistent with the applicable product information (i.e. the Investigator's Brochure).

11.3.7 Suspected Unexpected Serious Adverse Reactions (SUSARs)

A SUSAR is an AE considered to be serious, at least 'possibly related' to the medicinal product and unexpected (i.e. its nature and severity are not consistent with the referencesafety information in the IB).

ReNeuron will report any SUSARs occurring with the IMP to the relevant CAs, REC and DSMB in an expedited fashion. This will be done electronically. Fatal and life threatening

SUSARs will be expedited as soon as possible but no later than 7 calendar days from the date of ReNeuron's first knowledge of the event and relevant follow-up information will be subsequently expedited within an additional 8 days. All other SUSARs will be expedited no later than 15 calendar days of first knowledge of the event.

New events related to the conduct of the trial or the development of CTX0E03 DP, including SAEs which could be associated with trial procedures and could require a modification to the conduct of the trial, or present a significant hazard to the patient population, should be reported according to the existing timelines (Statutory Instrument 2010 No. 1882).

Further expedited reporting will occur according to the European Commission Detailed Guidelines on GCP Specific Advanced Therapy Medicinal Products.

11.4 History, Physical Examination and Investigations

These should be conducted according the Trial Evaluation Schedule (Appendix 1: Trial Evaluation Schedule).

11.4.1 Medical History

The medical history should include demographic data, relevant past and current conditions.

11.4.2 Physical Examination

The physical examination should include an examination of the cardiovascular, respiratory, abdominal, endocrine, central nervous and musculo-skeletal systems, eyes and skin. It must be carried out by the Investigator or a medically qualified Sub-investigator.

11.4.3 Pregnancy Tests

A pregnancy test should be conducted to determine eligibility for all FOCBP up to 2 years since their last menstrual period.

Any form of pregnancy test that is routinely used by the study site laboratory and is approved by the relevant national CA may be used.

A female with a positive pregnancy test may not be treated with the investigational medicinal product.

11.4.4 Laboratory Safety Tests

Haematology and clinical chemistry assays will be conducted at the laboratory of the hospital at which the patient is injected. The same laboratory using the same techniques, equipment and standards should perform all follow up laboratory investigations. Any change in the laboratory or standards should be noted on the case report form by the Investigator.

Haematology – haemoglobin (Hb), red blood cell count (RBC), white blood cell count (WBC) and differential (neutrophils, lymphocytes, monocytes, eosinophils, and basophils).

Biochemistry – Albumin, alkaline phosphatase, AST, ALT, chloride, creatinine, gamma-GT, LDH, potassium, sodium, total protein, urea (or BUN), bicarbonate, bilirubin, calcium, inorganic phosphate.

Urinalysis – pH; semi-quantitative 'dipstick' evaluation of glucose, protein, bilirubin, ketones. If the dipstick evaluation is abnormal, a microscopic examination including RBC/High power field, WBC/High power field, casts/Low power field should be performed. If casts are noted,

the type is to be specified. A midstream urine sample (~30 mL) will be obtained, in order to avoid contamination and allow a proper assessment.

The Investigator should review all laboratory reports promptly. Any clinically significant changes should be reported as Adverse Events (AEs).

11.4.5 Scans

A clinical diagnosis of stroke using neuro-imaging (MRI or CT scan) will be conducted between day 1-35 post stroke. In addition, an MRI scan will be conducted at Visit 3 (≤7 days prior to treatment), Visit 4 (day 0 (MRI or CT scan)) can be conducted for trajectory planning, Visit 9 (day 180 (+/-14)) and Visit 10 (day 365 (+/-30)). For patients who develop a contraindication to MRI following enrolment to the study, post implantation CT scans should be conducted at Visit 9 (day 180 (+/-14)) and Visit 10 (day 365 (+/-30).

MRI: MRI assessment should include sequences of brain imaging sufficient to assess the trial entry criteria. This should include as a minimum 1) T2 FLAIR and 2) Gradient echo or Susceptibility Weighted Imaging sequences. For the pre-surgical scanning, MRI should include T2 FLAIR, T1 3D structural scan and Gradient echo or Susceptibility Weighted Imaging sequences. MRI obtained for routine clinical assessment any time later than day 5 after the stroke that includes the minimum sequences can be used for eligibility assessment. Where possible, diffusion tensor imaging and a single resting state BOLD should also be captured. The same sequences should be acquired at follow-up visits. Additional sequences may be acquired if clinically indicated.

An approved MR-conditional cardiac pacemaker may be used in the MRI environment when used according to the manufacturers instructions.

CT: CT should be conducted according to normal institutional Protocols for the assessment of stroke and for planning of stereotaxic surgery . Post-procedure follow-up CT in those with contraindications to MRI should obtain thin-slice CT (ideally with slice thickness ≤1mm) of the whole brain.

11.4.6 ECGs

A standard 12 lead ECG will be recorded at the times stated in the Trial Evaluation Schedule and will be reported by the Investigator or a medically qualified Sub-investigator and/or the reporting service routinely used by the hospital.

The Investigator or a medically qualified Sub-investigator should review ECGs promptly and any clinically significant changes should be reported as an AE.

11.4.7 Immunological Response to CTX0E03 DP

Serum samples will be obtained from 5 mL clotted whole blood for the measurement of antibodies (“allo” antibodies) to selected human leucocyte antigens expressed on the CTX0E03 cell line.

Luminex bead technology will be used to assess presence of specific antibodies to CTX0E03 HLA antibodies using a commercial kit supplied by One Lambda Inc. that is designed to recognise all HLA serologically defined specificities. Neat serum will be assayed.

Samples will be collected at baseline and 28 days (+4) post injection of CTX0E03 DP.

The results of these tests will be made available to the Principal Investigator and ReNeuron’s Medical Advisor and must be available prior to CTX0E03 DP administration. A 2-working day turnaround for the sample taken 28 days after implantation will be observed for patient safety. Patients with antibodies to any of the CTX0E03 HLA antigens

at baseline will be excluded from the trial. In the event of detection of a positive allo-response post implantation, the DSMB will be alerted and the patient monitored. If the patient is well, no action will be taken but if the patient is unwell, their symptoms will be treated as appropriate. Outcome data from any patients showing allo-responses will be anecdotally compared if possible against data from patients showing no allo-response.

Serum samples will be sent to a central laboratory for analysis.

11.4.8 The Action Research Arm Test

Refer to Appendix 2: The Action Research Arm Test.

11.4.9 The National Institutes of Health Stroke Score

Refer to Appendix 3: The National Institutes of Health Stroke Score.

11.4.10 The Rankin Focused Assessment

Refer to Appendix 4: Measurement of Rankin Focused Assessment.

11.4.11 The Barthel Index

Refer to Appendix 5: The Barthel Index.

11.4.12 The Fugl-Meyer Assessment

Refer to Appendix 6: Fugl-Meyer Assessment

11.4.13 Registry Follow-up

All patients in this trial will be followed for 5 years after treatment with CTX0E03 DP. The relevant national cancer facility (where such a facility exists and agrees to assist with the study) will be approached to assist with obtaining the following data (where available): date of diagnosis of new cancer, site of primary, survival status; alive or dead, date of death. At the fifth anniversary of the date of injection of the first patient, the Sponsor will re-evaluate the safety profile of CTX0E03 DP and assess whether continued safety evaluation of patients will generate further meaningful and/or useful additional safety data. In which case follow up via the relevant cancer registry as above will continue for a further 5 years and reviewed at 5 yearly intervals.

Any incidence of cancer or death reported to sponsor via the registry will be reported to regulatory authorities annually within the DSUR/PSUR as per the applicable safety reporting regulations.

11.5 Unscheduled Visits

Unscheduled visits can be performed to record additional relevant data. Unscheduled visits are those not specified in the Protocol. A Protocol visit done outside the Protocol window is NOT considered “unscheduled”.

11.6 Pharmacokinetic Measurements

No pharmacokinetic sampling or measurements are included in this study.

11.7 Pharmacodynamic Measurements

Pharmacodynamic assessment is not included in this study.

12 Data Quality Assurance

When the trial is initiated, a representative of ReNeuron will thoroughly review the Protocol and CRFs with the Investigator and Trial site personnel. During the trial the Monitor will visit the Trial site regularly, subject to mutual convenience, to check the completeness of patient records, the accuracy of entries on the CRFs, the adherence to the Protocol, ICH GCP, and applicable legislation, the progress of enrolment, and also to ensure that the IMP is being stored, dispensed and accounted for according to specifications. The Investigator and key trial personnel must be available to assist the Monitor during these visits whenever possible.

The Investigator must give the Monitor access to relevant clinical records, to confirm their consistency with the CRF entries. No information in these records about the identity of the patients will leave the Trial site. ReNeuron will maintain confidentiality of all patient records.

Representatives of ReNeuron, CAs or other authorised bodies (e.g. REC) may visit the Investigator in order to perform a Quality Assurance audit. The Investigator will be given as much notice as possible of the audit and he/she or another assigned member of his/her staff must be present. Feedback from the audit will be made available to the investigator and any particular problems identified will be discussed with him/her.

13 Statistics and Data Management

13.1 Determination of Sample Size

The primary objective of this study is to determine whether a sufficient proportion of patients experience recovery (response) of their paretic arm following treatment with CTX0E03 DP to justify a subsequent randomised study.

By limiting recruitment to patients who have still marked paresis of the affected arm (inability to lift arm off table against gravity) the population is limited to patients in whom the probability of spontaneous improvement of the arm (sufficient to use it for feeding) is considered to be less than 5%.

At least 21 patients will be recruited and treated. All treated patients will be included in the efficacy analysis. The desired minimum response rate in a wider population is 20% based on the ARAT test. The actual response rate in the total number of treated patients will be presented at 1, 3, 6 and 12 months with the lower (1 sided) confidence intervals (CIs), as calculated to include 50, 60, 75, 80, 90 and 95% of the population means. The Clopper-Pearson method will be used. For example, an observed ARAT response rate of 7 in 21 patients (33%) will result in a lower 1-sided CI of 31, 29, 25, 23, 20 and 17% response rate at the CI listed above. A response rate of 5/21 patients will mean that a real response rate of 20% or greater cannot be excluded at the 50% CI.

The decision to move to a larger randomised study will be based on the overall evaluation of sensorimotor response as measured by ARAT, NIHSS and Fugl-Meyer; as well as the safety profile and benefit/risk.

13.2 Statistical and Analytical Plans

All data generated during the trial will be listed, reviewed and summarised. Descriptive analysis will be used.

AEs during the 12-month period post-implantation will be listed and summarised according to body system and preferred term, as classified in MedRA . The number and

percentage of patients suffering at least one event in each treatment arm will be reported. Events will be further listed according to type, severity, relationship with study medication, treatment required and outcome.

The subset of events classified as serious will be summarised in the same manner.

Laboratory data will be listed and summarised; changes from baseline in laboratory measurements will be listed and summarised.

Concomitant medications at baseline and during the study will be summarised according to the WHO Anatomical Therapeutic Chemical (ATC) classification, levels 1 (main group) and 2 (therapeutic subgroup).

- ***Analysis Populations***

The population for safety analysis (the Safety Analysis Set) will consist of all patients who received any dose of study medication.

Any patients who consent to the study but do not receive study treatment with CTX0E03 DP will be excluded from the primary analyses. Data on these patients will be presented as separate line-listing and will include the reason why the patient dropped out of the study before treatment.

- ***General Considerations***

Categorical variables will be summarised by the number and proportion of patients falling in each category. Continuous variables will be summarised using the mean, median, standard deviation, inter-quartile range, minimum and maximum values. There will be no imputation of missing data.

Demographic and baseline characteristics will be summarised. Listings of study withdrawals and Protocol violations will be provided. Study drug administration will be summarised by treatment group and overall.

Assuming the appropriate normality assumptions are met, continuous outcomes will be analysed at each specified time point using analysis of covariance (ANCOVA). The baseline value of each parameter will be used as a covariate in the analyses.

- ***Provision of Data to DSMB***

During the trial a summary of the available safety data will be prepared at predetermined milestones and as requested by the DSMB. These data will not necessarily have been entered into the formal trial database or cleaned.

- ***Software for Statistical Analysis***

All statistical analysis will be performed using either SAS, Stata for Windows and/or R for Windows.

13.3 Interim Analyses

None.

14 Data Safety Monitoring Board (DSMB)

The DSMB will include a minimum of one clinician expert in the management of stroke, one neurosurgeon and one medical statistician. The DSMB will be chaired by one of the clinical experts. No DSMB member will be an investigator in this study (either directly or via oversight of staff reporting to them).

The DSMB will review all safety data at the following predetermined intervals during the course of the study.

1. after the first patient has been enrolled and data are available on the one month post surgery follow-up.
2. after the first ten patients have been enrolled and data are available on the one month follow up post surgery on the tenth patient.
3. after at least 21 patients have been enrolled and data are available on the one month follow up post surgery on the 21st patient

After the first patient is treateda treatment hold will be observed until data are reviewed by the DSMB and an opinion / recommendation is issued. During this safety review period further patients may be screened but will not receive treatment.

For the DSMB reviewabove, screening and treatment of patients may continue while data are maturing on the previously treated patients. This means for example that the eleventh patient can be enrolled prior to the one month data on the tenth patient being available for DSMB review.

The DSMB may elect to add additional review time points at their discretion either in response to analysis reviewed previously or in response to new safety data (clinical or non-clinical).

It is recognised that no intervention to date has been demonstrated to produce marked recovery in an established paretic arm post-ischaemic stroke. There is no consensus in the field regarding the optimal study design for screening efficacy in this indication.

Details of all serious adverse events (SAEs) that occur in the study will be provided to members of the DSMB in time for each of their reviews of the safety data as outlined above. Any SAEs considered to be SUSARs, (suspected, unexpected serious adverse reactions), or other expedited reports, will be sent to all members of the DSMB as soon as possible. The DSMB will be sent any new clinical or non-clinical safety information on CTX0E03 DP that becomes available during the trial that may change the benefit risk ratio of the CTX0E03 DP.

The DSMB has the right to recommend that the trial be suspended, terminated or lower doses be given, if considered appropriate.

Meetings of the DSMB may be in-person or virtual (by telephone, videoconference or by email correspondence). Each member of the DSMB must provide his or her assessment to the Chairman either in a meeting or by email. All comments will be documented and a summary and recommendation prepared and signed by the Chairman that includes the recommendations of the majority of the DSMB and lists any contrary minority opinions.

The Chairman should issue the recommendations of the DSMB to ReNeuron within 24 hours of the meeting to allow ReNeuron to respond rapidly to the DSMB's recommendation.

15 Protocol Amendments

The Protocol may not be altered informally. The Investigator may not amend or deviate from the Protocol without prior discussion with ReNeuron unless the safety of a patient would otherwise be compromised. If it is agreed that changes to the Protocol are required, a revised Protocol will be prepared by ReNeuron.

Revised Protocols incorporating substantial amendments must be submitted to the relevant national CAs and the RECs and any other local review bodies that reviewed the Protocol for approval prior to implementation.

The Patient Information Sheet and Informed Consent Form (PIS/ICF) is a separate document. If there are changes in the amended Protocol that involve the patients, then these changes need to be documented in a revised Protocol and a new PIS/ICF must be prepared. This may be used once it has been approved along with the revised Protocol. This may involve the re-consenting of patients already in the trial, as well as using the new version for new patients.

Changes to any Investigator or study related staff (e.g. Monitor) will not require a Protocol amendment. Any such changes should be recorded when the Protocol is next amended for another reason.

16 Responsibilities of the Institution

Institution is the term used for the organisation (e.g. NHS Trust) that takes responsibility for the trial being conducted at the trial site.

16.1 Confidentiality

The Institution will ensure all information supplied to them or the Investigator by ReNeuron will be maintained in confidence and such information will be divulged only to the REC or similar committee or to those under an appropriate understanding of confidentiality by the recipient.

In order to maintain patient confidentiality, the only means of identifying data removed from the trial site should be patient initials, trial number(s) and date of birth. The Institution agrees that within local regulatory restrictions and ethical considerations, ReNeuron or any regulatory agency may consult and/or copy trial documents in order to verify data in the CRF.

16.2 Clinical Trial Agreement

A Clinical Trial Agreement (or equivalent) should be signed in duplicate for the trial site by ReNeuron and a representative of the Institution before the trial may commence. This Agreement will ensure that each party understands their obligations relating to the trial including the financial arrangements. A copy of the Agreement should be made available to the Investigator.

16.3 Archiving

See Section 22 Retention of Records.

17 Responsibilities of the Investigator

17.1 Curricula Vitae

A current CV (signed and dated within 12 months and including national professional registration numbers as appropriate) must be provided by the Investigator, all Sub-Investigators and other key personnel, especially those who will carry out primary endpoint measurements in the trial. CVs must show current appointment details and evidence of involvement in the relevant therapy area, or experience of the procedure(s) being carried out. All personnel who provide CVs must give written permission for their CV to be held on file by ReNeuron used in the Clinical Trial Report (which may be circulated outside the EU) or provided to auditors if requested.

17.2 Research and Development Review

It is the responsibility of the Investigator to request approval for the trial from their R&D department and other relevant departments where this is required.

17.3 Patient Identification

The Investigator must, whenever possible, conduct a feasibility review before initiation of the trial to ensure that there will be sufficient patients available for the trial.

It is the Investigator's responsibility to enter the details of all patients screened for the trial on a Screening Log and to ensure that this is kept updated during the trial. This Screening Log should be stored with the trial documents for a period of 30 years from the date of the Clinical Trial Report or the date of early termination of the trial.

It is the Investigator's responsibility to keep a confidential record of the identification of all the patients who took part in the trial. The Patient Identification Log should be stored with the trial documents for a period of 30 years from the date of the Clinical Trial Report or the date of early termination of the trial.

17.4 Informed Consent and Emergency Contact

The Investigator will obtain informed consent from trial patients in accordance with the Declaration of Helsinki before including them in the trial.

The Investigator should ensure patients enrolled in a trial are provided with contact addresses and telephone numbers where further information can be obtained. In addition patients will be issued cards which will state that the patient is participating in the trial and providing a 24-hour emergency contact number in the event of any medical problem during the trial.

The Investigator will ensure patients are told about any information that becomes available during the trial, which may be of relevance to them.

17.5 Trial Conduct

The Investigator should ensure he has sufficient time to conduct and complete the trial, and that he has adequate support staff and appropriate facilities (including laboratories and archive space) available for the duration of the trial.

The Investigator should ensure other trials do not divert essential patients or facilities away from the trial in hand.

17.6 Clinical Evaluations

The Investigator should ensure written informed consent is obtained from every patient participating in the trial and that physical examinations are carried out as required by the Protocol. These may be delegated to a medically qualified Sub-Investigator. Other clinical evaluations may be delegated to other key personnel. Personnel to whom trial procedures may be delegated will be recorded on the Centre Signature Log.

17.7 Case Report Form Completion

Case Report Forms (CRFs) will be provided by ReNeuron for each patient. They must be completed legibly in black ballpoint pen and corrections of data may only be made by crossing out the incorrect data and writing the correct values next to those crossed out. Incorrect data should never be obliterated. Each correction must be initialled and dated by the person making the correction. CRFs may only be completed by personnel listed on the Centre Signature Log as authorised to do so.

Completed CRFs will be collected by the Monitor assigned by ReNeuron and a copy retained by the Investigator.

It is the Investigator's responsibility to ensure that the data on the forms are accurate.

17.8 Patient Medical Records

The primary source documents for this study will be the patient's medical records and all rating scale CRFs used to directly record functional assessment scores for ARAT, NIHSS, RFA, BI, FMA throughout the study. If separate research records are maintained by the investigator(s) both the medical record and the research records may be monitored/audited for the purpose of the study.

17.9 Monitoring and Data Access

The Investigator must allow representatives assigned by ReNeuron to visit at regular intervals during the trial to monitor the progress of the trial. During such visits, the Investigator must provide adequate space for monitoring and allow the Monitor direct access to the patients' medical records and/or any other source data. The Investigator or Sub-Investigator should also be available at each monitoring visit to resolve any queries on the data.

17.10 IMP Accountability

All IMP supplies used to conduct this trial must be maintained under adequate security and stored under the conditions specified on the label until administration to trial patients or returned for destruction / destroyed. Drug accountability records must be maintained on an on-going basis. The Investigator agrees not to supply the IMP to any persons not entered into this trial. In certain circumstances the destruction of the IMP by the trial site may be permitted. Their destruction must be documented.

17.11 Quality Assurance Audit

Representatives from or on behalf of ReNeuron, the CA or other authorised bodies (e.g. REC) may visit the Investigator and any associated facilities in order to perform a quality assurance audit. The Investigator will be given as much notice as possible of the audit and he or another delegated member of his staff must be present. The audit findings will be discussed with the Investigator.

18 Responsibilities of the Sponsor

18.1 Regulatory Review

ReNeuron is responsible for obtaining a favourable opinion from relevant national CAs in order to conduct this trial, and for ensuring substantial Protocol amendments are submitted to those CAs for approval / information, as appropriate, prior to implementation.

ReNeuron will submit to the relevant CAs an End of Trial Declaration within 90 days of the last patient last visit, or within 15 days of an early discontinuation of the trial. A summary of the clinical trial must be submitted to the relevant CAs within one year of completion.

18.2 Ethics Review and Updates

ReNeuron is responsible for ensuring that final written approval to conduct the trial has been obtained from the appropriate RECs before the trial site is initiated, and that all substantial Protocol amendments are submitted for approval / information as appropriate, prior to implementation.

A progress report on the trial must be submitted to the RECs at least annually.

ReNeuron will submit to the RECs an End of Trial Declaration within 90 days of the last patient last visit, or within 15 days of an early discontinuation of the trial. A summary of the clinical trial must be submitted to the RECs within one year of completion.

18.3 Indemnity

ReNeuron will provide a letter of Indemnity/Certificate of Insurance for the trial site prior to commencement of the trial.

18.4 Clinical Trial Agreement

ReNeuron and a management representative of the Investigator site must sign, in duplicate, a Clinical Trial Agreement (including the financial arrangements) before the trial may commence. This Agreement will ensure that each party understands their obligations relating to the trial. A copy of the Clinical Trial Agreement should be made available to the Investigator.

18.5 Trial Supplies

ReNeuron will provide all information on the trial drug and all trial supplies as necessary when required by the Investigator, the CA or the REC. Full drug accountability records will be maintained throughout the trial.

18.6 Trial Site Assessment

ReNeuron must ascertain that the Investigator(s) have appropriate time and facilities to carry out the trial.

18.7 Training

ReNeuron will train all personnel involved with the trial to ensure they are conversant with the Protocol.

18.8 Monitoring

ReNeuron will identify and appoint appropriately qualified / experienced Monitors who will visit the trial sites to review the progress of the trial on an ongoing basis. These visits are to confirm that facilities remain acceptable, that the Protocol is being followed, the trial is being carried out according to ICH GCP, the data are being accurately recorded in the CRF, that IMP accountability is being carried out and to provide information and support to the Investigator.

18.9 Data Validation/CRF Inspection

The Monitor will require sight of all the documentation relating to the trial including any clinical source documents for each patient to ascertain that the data contained within the CRFs are a true and accurate record. Patient confidentiality will be maintained at all times and there will be no reference to patient names in any documents maintained by ReNeuron.

18.10 Safety Reporting

ReNeuron's Pharmacovigilance Provider will report any SUSARs or other expedited reports occurring in the trial to the relevant CA and via the Sponsor also to relevant REC(s) and the DSMB as outlined in Section 11.3.

ReNeuron and/or ReNeuron's Pharmacovigilance Provider will keep the Investigator and DSMB informed of all SAEs reported to them for the product under investigation, from anywhere in the world, for the duration of the trial at a frequency appropriate to the trial.

In addition, any new safety information that would adversely affect the safety of patients or the conduct of the trial will be reported by ReNeuron to the CAs, RECs, DSMB and Investigators. If the trial is to be suspended as a result of a SUSAR, or due to any urgent safety measure taken, the CA and REC will be notified as soon as possible and within 3 days of the decision.

ReNeuron will submit Safety Reports to the CAs and RECs annually or more frequently if so requested.

19 Data Management, Statistical Analysis and Final Report

ReNeuron or designees will be responsible for the data management and statistical analysis of the study data. Data management and statistical analysis may be out-sourced to a commercial or academic third party with experience in the analysis of clinical research data and familiar with relevant regulatory and GCP requirements to ensure accuracy of data management.

ReNeuron will be responsible for ensuring that the results of the trial are reported on accurately. The Chief Investigator will be asked to assist in the preparation of the Clinical Trial Report, and to sign the final version of the Clinical Trial Report to the effect that he has read and agreed with its conclusions. A copy of the Clinical Trial Report synopsis will be supplied to the Investigator, with the full report being available on request.

The Investigator is at liberty to perform his own analysis of his data at the end of the trial should he wish.

20 Documents Required Prior to Starting Trial

Prior to the initiation visit and before drug supplies are shipped to the Trial site, the following signed documentation must be obtained:

- A letter from the CA agreeing the trial may proceed.
- Written evidence of REC review and approval of the Protocol, the Patient Information Sheet and Consent Form and any other relevant documents.
- R&D approval from the trial site (where necessary).
- Signed Indemnity.
- Signed Clinical Trial Agreement.
- Signed Protocol.
- Current CV of the Investigator plus evidence of a current license to practice medicine.
- Signed financial disclosure forms (as required by CAs).
- Signed EU data protection form.
- The REC composition.

21 Use of Information and Publication

21.1 Confidential Information

All information concerning the IMP and ReNeuron's operational procedures, such as patent applications, formulae, manufacturing processes, basic scientific data and formulation information supplied by ReNeuron and not previously published are considered confidential and shall remain the sole property of ReNeuron. The Investigator agrees to use this information only in accomplishing this trial and will not use it for other purposes without written approval from ReNeuron.

All information obtained during the conduct of the trial will be regarded as the confidential property of ReNeuron. Written permission from ReNeuron is necessary prior to disclosing any information concerning the trial to any person(s) not involved in the trial.

21.2 Publication of Trial Results

The results of the trial should be communicated to the Investigator as soon as they are available.

ReNeuron respects the Investigator's freedom to publish clinical results, but would like to have the opportunity of commenting on the pre-publication text and timing of publication. Fifteen working days should be allowed to review an abstract and 60 days to review a paper for publication.

Where early publication would prevent ReNeuron obtaining protection to its rights, ReNeuron shall be entitled to require a delay in publication.

Acknowledgements for assistance should be included in all publications where appropriate.

It is understood by the Investigator that information from the clinical trial will be used by ReNeuron in connection with the development of the trial drug and therefore may be disclosed to CAs and any co-development partners worldwide.

22 Retention of Records

It is the responsibility of both ReNeuron and the Institution to ensure that all documents relating to the trial including records of drug disposition, signed consent forms, completed CRFs and all correspondence are retained under appropriate conditions for a period of 30 years from the date of the Clinical Trial Report. The Institution should let ReNeuron know in writing of any changes to arrangements for the maintenance of their trial related documents.

ReNeuron may pay an external archiving company to retain the Institution's documents but may not have any access to these documents.

23 Trial Termination

The trial may be terminated before the planned number of patients has been achieved for the following reasons:

- Safety and/or tolerability issues with the trial drug which have come to light since the trial started
- Slow patient recruitment
- Fraud
- Unacceptable procedures at the trial site
- ReNeuron's decision
- Investigator's decision (relating only to the Investigator's site)
- Force Majeure.

24 Protocol Approval

Protocol Author:

Signature..... Date 26 Sept 2017

Name: Esther Kitto

Chief Medical Officer:

Signature..... Date 29 SEPT 2017

Name: Dr Julian Howell

Chief Investigator:

Signature..... 5th OCT. 2017.

Name: Professor Keith Muir

25 Protocol Acceptance

I have read this Protocol and agree to abide by it in the conduct of the trial:

Investigator

Signature.....…….. Date.....

Name: [insert PI name]

26 References

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Appendix 1: Trial Evaluation Schedule

	Pre-surgery with CTX0E03 DP			Post-surgery with CTX0E03 DP (Day 0 = day of injection)								
	Visit 1 **[Day 28 to Day 270 (±7) post-stroke]**	**Visit 2** **[(Visit 1+ 28 days) to Day 300 (+7) post-stroke]**	**Visit 3** **Up to 7 days prior to surgery**	**Visit 4** **Day 0 (Visit 2 + ≤ 3 months)**	**Visit 4** **Day 0-2 (First 48 hrs post inject)**	**Visit 5** **Day 2**	**Visit 6** **Day 7 (±2)**	**Visit 7** **Day 28 (±4)**	**Visit 8** **Day 90 (±7)**	**Visit 9** **Day 180 (±14)**	**Visit 10** **Day 365 (±30)**	**Registry follow up period**
Consent	X											
Pregnancy test*			X									
Medical history	X											
Physical examination		X										
MRI (CT scan∅)	X (day 1-35 post stroke)		X*** (see also ^)							X	X	
Antiplatelet and Anticoagulant adjustment instructions		X	X∞									
Pre-surgery work-up (Surgery and Anaesthesia Assessment)			X§									
Hospital Admission			X (Day -1)									
Pre stereotaxic surgery scan (CT or MRI)				X***								
Injection of CTX0E03 DP				X								
In-patient observation					X							
Temperature and pulse		X			X	X	X					
ECG		X			X		X		X	X		
BP		X			X	X	X	X	X	X		
FBC, U&E, FTs		X			X		X	X	X	X		
Serum for allo antibody		X†						X				
Urinalysis		X			X		X	X				
NIHSS	X◊	X	X****			X	X	X	X	X	X	

	Pre-surgery with CTX0E03 DP			Post-surgery with CTX0E03 DP (Day 0 = day of injection)								
	Visit 1 **[Day 28 to Day 270 (±7) post-stroke]**	**Visit 2** **[(Visit 1+ 28 days) to Day 300 (+7) post-stroke]**	**Visit 3** **Up to 7 days prior to surgery**	**Visit 4** **Day 0 (Visit 2 + ≤ 3 months)**	**Visit 4** **Day 0-2 (First 48 hrs post inject)**	**Visit 5** **Day 2**	**Visit 6** **Day 7 (±2)**	**Visit 7** **Day 28 (±4)**	**Visit 8** **Day 90 (±7)**	**Visit 9** **Day 180 (±14)**	**Visit 10** **Day 365 (±30)**	**Registry follow up period**
ARAT, RFA, BI, FMA‡	X◊	X	X****					X	X	X	X	
AE reporting		X	X	X	X	X	X	X	X	X	X	
Concomitant therapy	X	X	X	X	X	X	X	X	X	X	X	
National registry (if available) **												X**

* Females of childbearing potential (or within 2 years of last menstrual cycle) must have a confirmed negative pregnancy test at time of treatment.
** All patients will be flaggedusing the relevant national cancer registry (in countries where this is available) for 5 years follow-up of events such as cancer and death.
*** 3 DTI imaging for trajectory planning
**** If performed within 7 days of Visit 2, no need to repeat
^ May be combined with visit 4 MRI where MRI-compatible stereotaxic frames are used
§ Anaesthesia assessment may be carried out at any point between Visit 3 and Visit 4 pre-surgery
ø CT Scan permissible at visit 1 (i.e. clinical diagnosis of ischaemic stoke by neuro-imaging), visit 4 (trajectory planning) and visits 9 and 10 (visits 9 and 10 only if MRI is contraindicated)
† May be performed anytime post-consent but results of allo-antibody testing to be available prior to CTX0E03 DP administration
‡ Fugl Meyer Assessment to be conducted pre-surgery at Visit 2 and 3 only
◊ Functional assessment scores collected at Day 28 ±7 post-stroke in the ReNeuron Observational Study (Protocol RN-CS-0001) may be used if available
∞ Telephone reminder of any planned changes to anticoagulation or antiplatelet medication

Appendix 2: The Action Research Arm Test

APPENDIX
Manual for Performing and Scoring the ARAT

OVERVIEW OF THE ACTION RESEARCH ARM TEST

The final Action Research Arm Test (ARAT) score is the sum of the scores from 19 tests spread across each of 4 subscales[6]: grasp, grip, pinch, and gross movement. Items in each subscale are arranged in a hierarchical order of difficulty, with the most difficult item in the subscale tested first, followed by the easiest tested second. This approach, outlined by Lyle, can increase efficiency of subject assessment, as normal performance on the most difficult subscale item predicts success for all of the remaining items in that subscale, which are easier tasks. Similarly, complete failure on performance of the easiest item predicts failure with all of the remaining items, which are more difficult tasks. With this approach, the ARAT takes about 5 to 15 minutes to administer.

The quality of movement for each of the 19 tests examined in the ARAT is scored on an ordinal 4-point scale, with 0 = no movement, 1 = the movement task is partially performed, 2 = the movement task is completed but takes abnormally long, and 3 = the movement is performed normally (see Table A1). These are Lyle's original terms, clarification of which could improve standardization of ARAT testing.

Another aspect of the ARAT that could be improved is specification of the amount of time used to define "abnormally long," which distinguishes a score of 2 versus 3. Another aspect of the ARAT that requires greater standardization is the source, material, weight, and size of the materials used for examining subjects, variability in which likely influences ARAT scores. In addition, many of the fine details of test administration are not stated in the original report and are open to interpretation, such as body position/posture, test item positioning, and a maximum time allowed to complete each ARAT test item. This could be an additional source of score variance across centers and time. These are among the issues considered herein.

ARAT MATERIALS

The basic testing materials, as originally outlined by Lyle,[6] are a chair without armrests, a table, various sized wooden blocks, a cricket ball, a sharpening stone, alloy tubes, a washer and bolt, 2 glasses, marbles, and ball bearings. Also required are 2 planks for placing the alloy tubes, 1 plank to place the washer, 2 tobacco tin lids, and a 37-cm-high shelf. Suggested standards for these materials appear in Table A2.

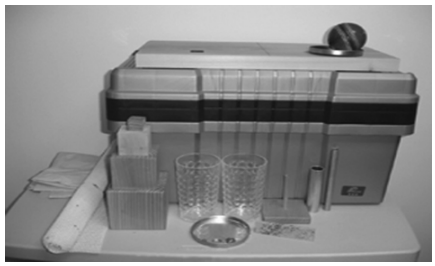


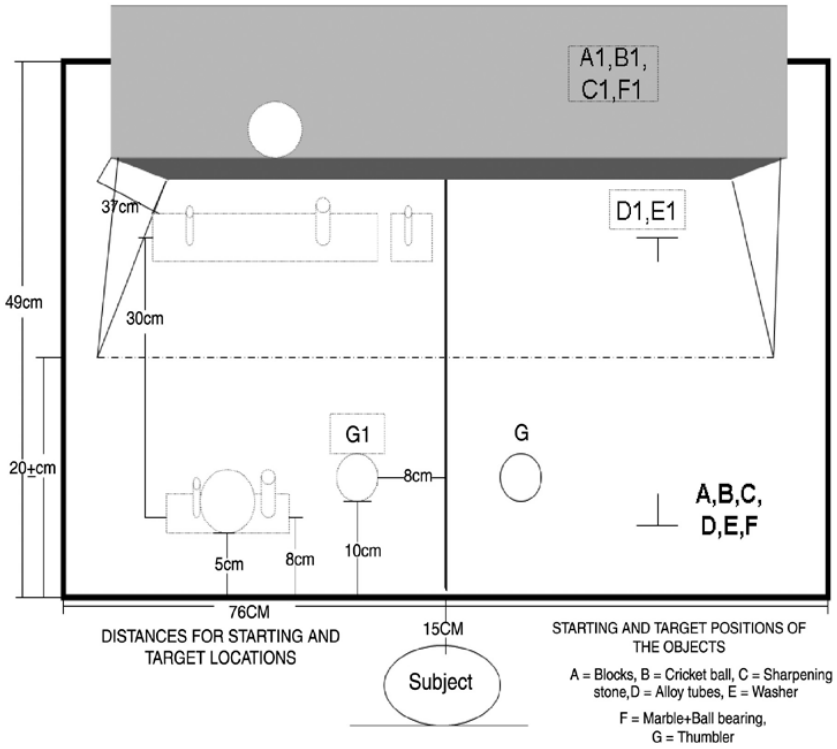
Figure 1. The complete ARAT kit is displayed.

Each material can be purchased at a large hardware store or together from vendors such as http://www.aratest.eu/. The wooden blocks are cut to appropriate sizes and are sanded and finished. We recommend fabricating these from pine, which is widely available, and has a consistent and light density. The cricket ball (The Pavilion, Dreamcricket, Hillsborough, NJ; www.dreamcricket.com), sharpening stone (Smith's Medium Arkansas Stone Knife Sharpener, Hot Springs, AR, CAT#MP4L; www.smithabrasives.com), marbles (widely available), ball bearings (made of steel, widely available), and plastic tumblers (widely available) are standard items that can be bought prefabricated. The alloy tubes are fabricated from aluminum tubing and are cut down to appropriate size with rough edges sanded down. A plastic toolbox (56 cm in length × 32 cm in width × 34 cm in height; Plano, Grab'n Go style, Part # 823-002, Plano, IL, http://www.planomolding.com) can be used for 2 purposes: first, to house/carry all materials, and second, as part of the 37-cm shelf employed during testing. To create the final shelf used in testing, a wood plank (3 cm in height) is placed on top of the box and is affixed with Velcro (Figure 1). If this plank is 23 cm in width × 46 cm in length, it will fit in the box with other materials during storage and affix to the top of the box snugly to create the needed 37-cm shelf. This system allows for ease of portability.

POSITIONING

Positioning of the Subject

Appropriate body posture for ARAT testing has the subject seated upright in a standard chair that has a firm back and no armrests. The assessor may provide foam padding to the back of the chair to ensure that upright position is maintained. The trunk must remain in contact with the back of the chair throughout testing. In this regard, the subject is instructed and regularly reminded not to lean forward, stand up, or move sideways, although we do not recommend that the subject's trunk be strapped to the chair. The head is held in a neutral upright position. The subject's legs are in front of the chair, with feet in contact with floor throughout testing.



All ARAT tasks are performed unilaterally. To promote this and keep the nontested hand in view, the subject is always asked to start with both hands in pronated position on the table, except for the "gross movement" subscale, which requires starting with both hands pronated on the lap. Suggested chair and testing-table dimensions are provided in Table A2. The testing-table level should approximate the subject's midabdomen, with the difference in chair-table height of about 30 cm considered optimal.

Positioning of the Materials for Each Task

The subject sits close to the table, with a 15-cm distance from the anterior torso to the front edge of table. In our experience, this distance allows enough upper-extremity mobility for the subject to be able to reach the top of the shelf, but maintains emphasis on the required body posture during testing. The use of a nonslip mat that is placed over the table is highly recommended. We have found it useful to draw prestated positions for each test object on this mat (Figure 2).

Further specifications for position of testing materials are specified under the instructions for each subscale.

SCORING

General Scoring Instructions

Instructions for each task are read aloud to the subject; however, if the subject has any difficulty understanding instructions, such as in the presence of aphasia, the assessor has the option of also providing a visual demonstration of the requested task. The subject is allowed to practice the task repeatedly to insure that instructions are fully understood.

Both upper extremities are separately assessed. For each of the 4 ARAT subscales, the subject starts with the nonaffected (or less affected) arm, and then the affected arm is assessed for that subscale. Thus, the order of testing is the nonaffected arm grasp subscale, the affected arm grasp subscale, the nonaffected arm grip subscale, the affected arm grip subscale, and so forth. The use of this order, combined with verbal and visual instruction, improves test instruction comprehension. We have found this method useful in patients with mild to moderate aphasia or neglect.

The 19 tests of the ARAT are distributed across 4 subscales, with 3 to 6 tasks each. Each task runs until the subject completes the task or until reaching a time limit that we have defined as 60 seconds. The quality of the task is rated on an ordinal 4 point-scale, that is, from 0 to 3. The maximum score for the ARAT is 57 for each arm, with a higher score indicating better arm motor status. A general scoring outline follows, with further specifics provided in each task's section.

A score of 3 is given when the task is performed normally. This requires the task be completed in less than 5 seconds, appropriate body posture, normal hand movement components, and normal arm movement components (see Table A3).

A score of 2 is given when the task is completed but either "with great difficulty or takes abnormally long." We define "great difficulty" as task completion in the setting of either (1) abnormal hand movement components (eg, use of wrong grasp), (2) abnormal arm movement components (eg, the elbow does not flex as required), or (3) abnormal body posture (eg, used as a substitute for impaired arm movements).

The amount of time used to distinguish a score of 2 versus 3 was not specified by Lyle.[6] A specific time limit was first suggested by Wagenaar et al,[11] who advocated using the mean ± 2 SDs, as determined from age-matched healthy control subjects. As an extension of this, we define "takes abnormally long" as 5 to 60 seconds.

A score of 1 is given when the subject only partially completes the task within the 60 seconds allotted for examining each task, regardless of the quality of hand and arm movement components or posture requirements. For grasp, grip, and pinch subscales, the subject cannot achieve a score of 1 for arm movements only. In order to attain a score of 1, the subject must initiate some form of hand movement, abnormal or normal, that achieves holding and lifting the object—simply pushing an object across the table with the dorsum of the hand does not constitute partial completion of the true task.

A score of 0 is given when the subject is unable to complete any part of the hand or arm movement components within the 60 seconds allotted for examining each task.

The score is based on the best performance. A subject is not penalized if a testing object is dropped and relifted. All performances must be performed with only 1 hand.

For subjects who have any finger amputations, scoring is as usual except for the pinch subscale. For any task that requires movement of an amputated body part, such as opposition of an amputated finger, the subject scores 0, and the assessor notes "task not done secondary to amputation."

Specific Scoring Instructions for the Grasp Subscale (ARAT Test Items 1 Through 6)

Object positioning. The nonslippery mat is placed over the table, and then the shelf and testing objects are placed in their predrawn positions (Figure 2). This approach has the shelf placed lengthwise, 20 ± 5 cm away from the proximal edge of the table on the mat; however, if the subject does not have sufficient range of motion for the fingertips to reach the top of shelf, such as due to contractures or increased tone, then the examiner can adjust this distance as needed.

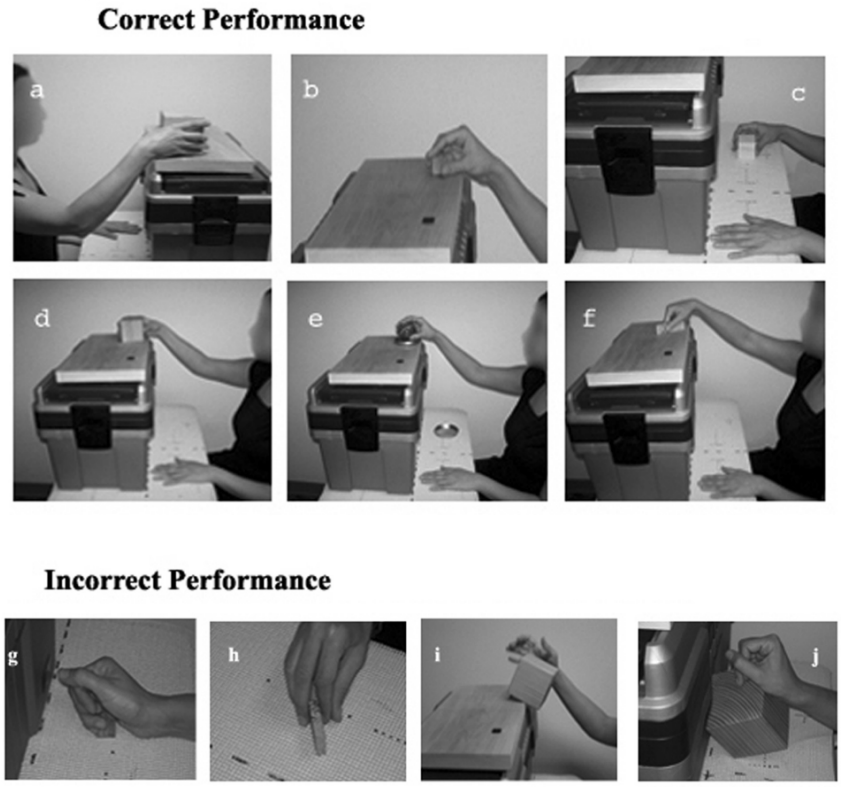
The items are placed, one at a time during the appropriate test, halfway between the subject's midsagittal line and the axillary line of the arm being tested. The hand being tested should be placed pronated, immediately lateral to the testing object, with the other hand also pronated atop the table. For all of the blocks, the assessor should not stabilize the object, nor can the subject stabilize the object with the nontested hand. For the sharpening-stone task, the stone has to be placed on its narrow long side in a slightly diagonal position (parallel to the axis of the palmar creases) for ease of grasping. If the sharpening stone falls to its side during grasping attempts, it can be repositioned onto its narrow long side by the examiner for up to 60 seconds. The 2 tin lids are used as the initial and final sites for the cricket ball. The distance between the proximal edge of the lower tin lid and the proximal edge of the table is 5 cm, whereas the proximal edge of the upper tin lid is the same as the proximal edge of the shelf. If desired, the upper tin lid can be attached to the top of the shelf using Velcro, in order to maintain stability, while the lower lid can be stabilized by the assessor as needed during task performance.

Instructions to subject. The subject is asked to grasp, lift vertically, place, and then release each object (block, ball, or stone) onto the top of the shelf. The instructions spoken to the subject are to "grasp the block [cricket ball, sharpening stone] that I have placed here, lift it up, and place then release it on top of that shelf."

Scoring. Start with the task of grasping the 10-cm block (the most difficult task in this subscale); if the score is 3, then the total score for this subscale is 18 for the arm being tested, and no further tasks need be tested for this arm on this subscale. If the score is 0 to 2, then continue to the task of grasping the 2.5-cm block (the easiest task in this subscale). If the score is 0, then the total score for this subscale is 0, and no further testing is required for this arm on this skip to subscale. If the score for the 2.5-cm block task is 1-3, however, continue with scoring all tasks in this subscale.

Score 3 indicates normal, complete, timely task completion. The subject must grasp the object, lift it up, and release it onto the shelf, all within 5 seconds, to obtain a score of 3. Appropriate hand movement components and arm movement components (Table A3) must be used, as well as posture requirements. The subject should not have the score reduced if the object falls off the shelf after successful task completion. The subject may release the object on any place on the shelf (Figure 3a-f).

Score 2 is given when the subject completes the task but does so "with great difficulty and/or takes abnormally long time." The subject can display great difficulty when (1) not using



appropriate hand movement components (Table A3), even if the task is otherwise completed (Figure 3g-h); (2) the subject displays abnormal arm movement components, such as abnormal object release when the object is brought to the shelf (Figure 3i); or (3) abnormal posture is evident (eg, if subject's trunk completely loses contact with the back of the chair). A score of 2 is also assigned if task completion takes 5 to 60 seconds.

For score 1, there are several possible means by which the subject can partially perform the task and thus receive a score of 1. For example, if the subject grasps and lifts the object, but does not reach the level of the shelf within the 60 seconds. A subject who can hold and lift the object—even with abnormal hand movement components and arm movement components —and lift it off the table any distance would score a 1 (Figure 3g and 3h). The subject must initiate some form of hand movement component to hold and lift the object, in order to attain a score of 1.

Score 0 indicates that the subject is unable to perform any part of the task within 60 seconds. A score of 0 would apply, for example, if the subject cannot open the hand to grasp the object, cannot extend and/or abduct the fingers or thumb to the size of object, at all within 60 seconds and/or the subject attempts to manipulate the object into the hand on the side being tested by stabilizing the object against the shelf or against the nontested hand, and/or moves the object across the table without any voluntary hand opening (Figure 3j). These are all permitted but provide no points and cannot be used to achieve a hold and lift hand movement component.

Specific Scoring Instructions for the Grip Subscale (ARAT Test Items 7-10)

Object positioning. The objects being tested are placed in their positions on the mat (Figure 2). For the pouring task, the cups are placed 8 cm apart on each side of the midline of the subject and 10 cm away from the proximal edge of the table. For alloy tube displacement, the starting plank is placed on the table so that the first peg is 8 cm away from the front edge of the table and the target plank is placed perpendicular to the proximal table edge so that the second peg is 30 cm distal to the first one. For washer displacement, the tin lid with the washer in it is placed 5 cm from the proximal edge of table and on the side being tested, whereas the washer's target peg is placed 30 cm distal to the middle of the tin lid. For the pouring task, the tumbler is filled with 4 ounces of water as indicated by a predrawn line on the cup. A water-resistant cover can be placed over the test subject's torso during task performance to protect from spills if desired.

Instructions to subject. The subject is asked to pour water from one cup to the other or to horizontally displace 2 different sized alloy tubes from a starting peg on a plank to a target peg on a plank and to horizontally displace a washer from a tin to a peg or bolt on a plank. The instructions spoken to the subject are to "pour the water from this cup to that other cup" or "grasp this tube [washer] and place it here [onto the peg on the plank]."

Scoring. Start with the task of pouring water from one glass to the other, which is the most difficult task in this subscale; if the score is 3, then the total score for the arm being tested on this subscale is 12, and no further testing on this subscale is required for that arm. If the score is 0 to 2 for the pouring task, then continue to the task of displacing the 2.25-cm alloy tube, which is the easiest task in this subscale. If the score on the 2.25-cm tube task is a 0, then the total score for this subscale is 0, and no further testing on this subscale is required for this arm. If the 2.25-cm tube task score is 1 to 3, continue with scoring all tasks in this subscale.

To score a 3, for the pouring task, the subject grasps the cup, lifts it, pours all of the water from 1 cup to the other without spilling, and releases the cup on the table. For the other 3 tasks, the subject must grasp the tube/washer, lift it off the plank/out of the tin, and displace it horizontally to the target plank peg and release. For all tasks, the effort must be completed within 5 seconds of starting the task (Figure 4a-d). The subject must complete the task with the appropriate hand movement components, arm movement components (Table A3), and posture.

A score of 2 is given when a subject completes the task (1) without the appropriate hand movement components, for example, uses alternative hand movement components as shown in Figures 4e-f; (2) with abnormal quality of arm movements, for example, for pouring task: subject grasps the cup, lifts it, pours water from 1 cup to the other with adequate forearm pronation, but spills some water; for tubes/washer: subject grasps the tube/washer, lifts it off the plank/out of the container, displaces it horizontally, places it in its target position, but is unable to release the object; or (3) without maintaining proper posture (eg, if subject's trunk completely loses contact with the back of the chair). A score of 2 is also given if task completion takes 5 to 60 seconds.

To score a 1, the subject partially completes the task and must initiate some type of hand movement that includes holding and lifting the object. For the pouring task, the subject might grasp the cup and lift it off the surface of the table but be unable to pour any water, or forearm pronation does not occur but is substituted, for example, by compensatory excessive lateral bending of the trunk (Figure 4g). For the other tasks, a score of 1 might be awarded if the subject extends the fingers sufficient to grasp the tube/ washer, lift it up off the plank/out of the tin, but is unable to make any horizontal movements or release the object within 60 seconds. As mentioned previously here, when scoring a 1, the subject must initiate some form of hand movement, abnormal or normal, that achieves holding and lifting the object; any type of hand movement is permitted (Figure 4e-f).

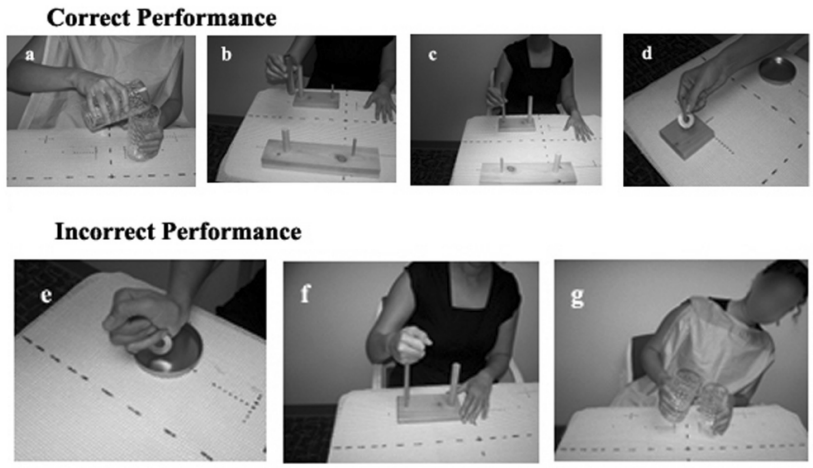
For a score of 0, the subject is unable to open the hand to grasp the cup/tube/washer (ie, extend and/or abduct the fingers or thumb to the size of the object) and/or takes greater than 60 seconds. A score of 0 is also given if the subject stabilizes the object in order to manipulate it into the hand and/or moves the object without any voluntary hand opening.

Specific Scoring Instructions for the Pinch Subscale (ARAT Test Items 11 Through 16)

Object positioning. The mat is placed over the table, with testing objects placed in their predrawn positions. The 2 tin lids are placed in the same positions as stated in the grasp subscale. Each marble or ball bearing is placed within the lower tin lid, and the subject is asked to grasp the object with the appropriate fingers, lift it up to the shelf, and release it into the target lid. Notes can be recorded in relationship to fingernail length as desired, but this does not change scoring.

Instructions to subject. The subject is asked to grasp a ball bearing or a marble from a tin lid, lift it up vertically, then place and release it into a target tin lid placed on the shelf. This requires that the subject independently move the fingers in opposition to the thumb with accompanying distal mobility and stabilization. The instructions spoken to the subject are to "grasp the ball bearing [marble] using these fingers, lift it up, and place it in the tin on top of the shelf."

Scoring. This subscale starts with the task of lifting the 6-mm ball bearing, the most difficult task; if score is 3, then the total score for the arm being tested on this subscale is 18, and no further testing is needed for this arm on this subscale. If the score is 0 to 2, then next is the task of lifting the marble with the first finger and thumb, that is, the easiest task in this subscale. If the score is a 0, then the total score for this arm on this subscale is 0, and no further testing is required for this arm on this subscale. If the score is 1 to 3, continue with scoring all tasks in this subscale.



An important note specific to pinch subscale tasks is that correct hand movement components (finger opposition; see Figure 5g) must be present to score more than 0. Thus, regardless of arm movement components, posture, and time used, the score can only be 0 if an incorrect finger opposition is employed, for example, holding the object in the palm with all 4 fingers flexed and thumb adducted/flexed (Figure 5h). As an extension of this note, task completion, necessary for a score of 2 or 3, is only deemed to be present if correct hand movement components are used. In addition, a score of 3 can only be generated if the finger opposition specifically uses the pads of the fingers.

A score of 3 is awarded for normal, complete, timely task completion. The subject grasps the marble or ball bearing from the tin, lifts the object up to the shelf, and releases it into the target tin, all within 5 seconds (Figure 5a-f). The task is completed using correct arm movement components, as well as hand movement components, including finger pads (Table A3), while maintaining proper posture. The score is not reduced if the object bounces off the shelf after successful task completion.

A score of 2 is awarded if (1) the quality of the arm movement component or the hand movement component is abnormal, as might occur for example with inability to release the object from the fingers into the target tin, or if the object falls out of the tin/off the shelf when attempting to release, or if the subject is unable to use the pads of the fingers to grasp the object (Figure 5g); (2) abnormal posture is displayed (eg, if subject's trunk completely loses contact with the back of the chair); or (3) performance takes 5 to 60 seconds.

A score of 1 is awarded if the subject partially completes the task, for example, grasps the object, lifts it up, but drops the object or is unable to reach the height of the shelf. The task must be completed within 60 seconds.

With a score of 0, the subject is unable to initiate the task within 60 seconds or, again for this subscale only, does not display the correct hand movement components, that is, finger opposition. The subject (1) is unable to open the hand to grasp the test object, that is, to extend and/or abduct the fingers or thumb to at least the size of the object; (2) attempts to manipulate the object into the fingers by stabilizing it with the nontested hand or some other object; (3) moves the object in the tin lid without any voluntary finger/thumb extension; or (4) attempts take greater than 60 seconds.

Specific Scoring Instructions for the Gross Movement Subscale (ARAT Test Items 17 Through 19)

Object positioning. The subject starts with both pronated hands on the lap. The assessor reminds the subject to keep the head still and in a neutral upright position. For item 17, the subject must touch the back of the head with the palmar side of the hand being tested; for 18, the subject must touch the top of the head, with the palmar side of the hand being tested, and for 19, the subject must touch the mouth with the palmar side of the hand being tested. The subject's hand can be in flexed posture if full finger extension/abduction cannot be maintained.

Instructions to subject. These tasks require the subject to move the shoulder and elbow across a wide range of motion, with

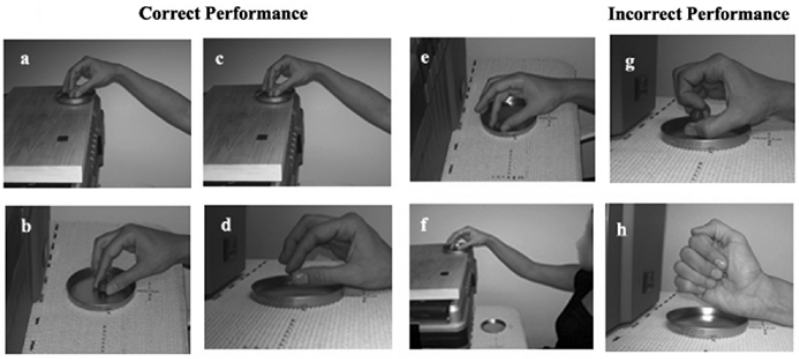


Figure 5. Pinch subscale. Correct performances are shown (a-f). Examples of incorrect performance: (g) subject is unable to use the pads of the appropriate fingers to grasp the marble, (h) uses palm to hold the ball bearing without any finger/thumb opposition.

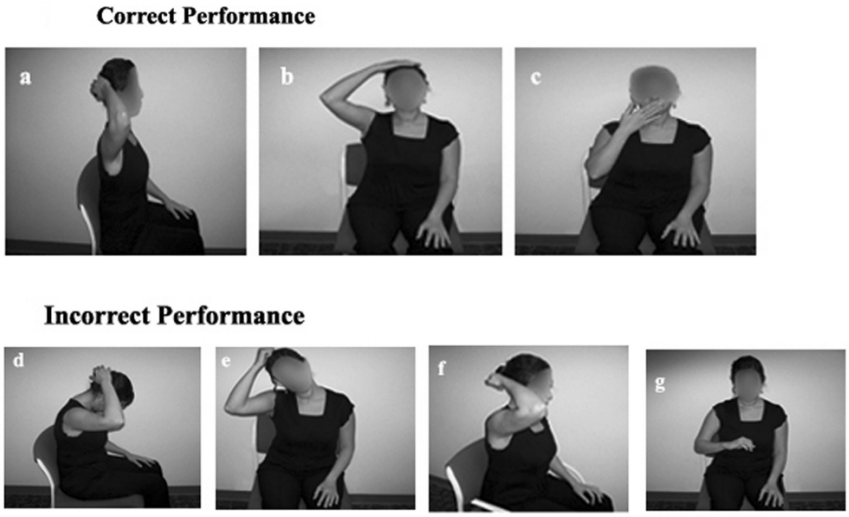


Figure 6. Gross movement subscale. Correct performances are shown (a-c). Examples of incorrect performance are as follows: compensation occurs via (d) neck flexion, (e) neck lateral flexion, (f) task completed with forearm in pronation, and (g) subject only partially completes the task.

accompanying forearm movement. The instructions spoken to the subject are to "touch the back of your head [top of your head, mouth] with the palm of your hand."

Scoring. Start with the task of placing the hand behind the head; if the score is 3, then the total score for this subscale is 9 for the arm being tested, and ARAT testing is completed. If the score is a 0, then the total score for the arm being tested is 0 on this subscale, and ARAT testing is completed. In this regard, the gross movement subscale is an exception in that the hardest and the easiest task have effectively been collapsed into a single task. If the score is 1 or 2, the arm being examined is then tested for the other tasks in this subscale.

For a score of 3, the subject places the hand behind the head

(not the neck), on top of the head (not the forehead), or to the mouth (not the chin) with the palmar side of the hand while maintaining the head in an upright, neutral position (Table A3), and the task is completed within 5 seconds (Figure 6a-c).

A subject scores 2 if the movement is completed abnormally (eg, the subject completes the task by flexing the neck [Figure 6d-f], or the trunk loses contact with the back of the chair, or the task takes 5 to 60 seconds to complete).

For a score of 1, the subject only partially completes the task (eg, starts shoulder/elbow flexion but the hand does not reach the target position within 60 seconds) (Figure 6g).

For a score of 0, the subject is unable to initiate any part of the task within 60 seconds.

Table A1. Action Research Arm Test Scoring Sheet

Test Number	Item	Score	
	Grasp subscale	Left	Right
1	Block, 10 cm^3	0 1 2 3	0 1 2 3
2	Block, 2.5 cm^3	0 1 2 3	0 1 2 3
3	Block, 5 cm^3	0 1 2 3	0 1 2 3
4	Block, 7.5 cm^3	0 1 2 3	0 1 2 3
5	Cricket ball	0 1 2 3	0 1 2 3
6	Sharpening stone	0 1 2 3	0 1 2 3
		Subtotal ____/18	____/18
	Grip subscale		
7	Pour water from one glass to another	0 1 2 3	0 1 2 3
8	Displace 2.25-cm alloy tube from one side of table to the other	0 1 2 3	0 1 2 3
9	Displace 1-cm alloy tube from one side of table to the other	0 1 2 3	0 1 2 3
10	Put washer over bolt	0 1 2 3	0 1 2 3
		Subtotal ____/12	____/12
	Pinch subscale		
11	Ball bearing, held between ring finger and thumb	0 1 2 3	0 1 2 3
12	Marble, held between index finger and thumb	0 1 2 3	0 1 2 3
13	Ball bearing, held between middle finger and thumb	0 1 2 3	0 1 2 3
14	Ball bearing, held between index finger and thumb	0 1 2 3	0 1 2 3
15	Marble, held between ring finger and thumb	0 1 2 3	0 1 2 3
16	Marble, held between middle finger and thumb	0 1 2 3	0 1 2 3
		Subtotal ____/18	____/18
	Gross movement subscale		
17	Hand to behind the head	0 1 2 3	0 1 2 3
18	Hand to top of head	0 1 2 3	0 1 2 3
19	Hand to mouth	0 1 2 3	0 1 2 3
		Subtotal ____/18	____/9
		Total ____/57	____/57

There are 4 subscales. The tests in each are ordered so that if subject scores 3 on the first test, no more tests need to be administered in that subscale, and the subject automatically scores top marks (all 3s) for all tests in that subscale. If subject fails the first test (score 0) and fails the second test (score 0) of the subscale, the subject automatically scores zero for all tests in that subscale, and again no more tests needed to be performed in that subscale; and (3) otherwise the subject needs to complete all tasks within the subtest
Score: 3 = subject performed the test normally within 5 seconds; 2 = subject could complete the test but took abnormally long (5 to 60 seconds) or had great difficulty; 1 = subject could only partially perform the test within 60 seconds; and 0 = subject could not perform any part of the test within 60 seconds.

Table A2. Suggested Test Materials Used in Performing the Action Research Arm Test

Task Material	Dimensions	Weight of Test Items Lifted During Testing (g)
Table	Height, 75 cm; width, 76 cm; depth, 49 cm	
Chair	Height of seat 46 cm from floor; no arm rests	
Shelf (or box on the table)	37 cm above level of table	
Four wooden blocks	10.0, 7.5, 5, and 2.5 cm^3, respectively	492, 196, 55, and 6.5, respectively
Large alloy tube	Diameter, 2.5 cm; length, 11.5 cm	38.5
Small alloy tube	Diameter, 1 cm; length, 16 cm	14.2
Cricket ball	Diameter, 7.1 cm	159
Marble	Diameter, 1.6 cm	5.4
Sharpening stone	10.0 × 2.5 × 1 cm	60.3
Ball bearing	6-mm diameter	1.1
Two plastic tumblers	Upper diameter, 7 to 8 cm; lower diameter, 6 to 7 cm; height, 12 to 15 cm	125.4 (empty)
Washer	Outer diameter, 3.5 cm; inner diameter, 1.5 cm	16
Plank for the tubes		
Starting point	1.5 × 8.5 × 8.5 cm	
Target point	3.5 × 8.5 × 34 cm	
Bolt for the large alloy tube		
Starting position	Round wooden peg; diameter, 2.0 cm; height, 13.5 cm	
Target position	Round wooden peg; diameter, 2.0 cm; height, 8.0 cm	
Bolt for the small alloy tube		
Starting position	Round wooden peg; diameter, 0.8 cm; height, 6.0 cm	
Target position	Round wooden peg; diameter, 0.8 cm; height, 6.0 cm	
Plank for the washer	1.5 × 8.5 × 8.5 cm	
Bolt for the washer	Round wooden peg; diameter, 0.8 cm; height, 8.5 cm	
Tin lid	Diameter, 9 cm; rim height, 1 cm	

Table A3. Specific Details for Action Research Arm Test Tasks

Task Number	Task Materials and Details	Hand Movement Components	Arm Movement Components
1-4	Blocks: displace vertically to shelf	Hand voluntarily opens to the size of the block. Any type of grasp involving the thumb and fingers in opposition is acceptable.	a. Forearm is between midposition and pronation. b. Elbow flexed when first grasping object and then extends to reach top of shelf. c. Shoulder flexion to reach top of the shelf, and shoulder stabilization to maintain position as object is released onto shelf. d. Thumb and finger extension to release the object.
5	Cricket ball: displace vertically to shelf	Spherical grasp; fingers and thumb slightly flexed and abducted to the size of the ball.	
6	Sharpening stone: displace vertically to shelf	Lateral grip; sharpening stone is between the pad of thumb and the radial side of the index finger at or near interphalangeal joints.	
7	2 cups: pour water from one cup to another	Cylindrical grasp around cup	a. Forearm pronation to pour, then forearm supination to return cup to table. b. Thumb and finger extension to release the cup.
8-9	Alloy tubes: displace from starting plank to target plank	Any type of grasp, such as 3 jaw-chuck pinch, involving the pads of the thumb opposed with pads of any number of fingers in order to grasp the alloy tube	a. Forearm is between midposition and pronation. b. Elbow is sufficiently extended to reach the distal target plank. c. Shoulder movement and stabilization to maintain position

10	Washer: displace distally from tin to target plank	Pincer or 3 jaw-chuck grasp, with pads of the thumb and fingers in opposition, in order to grasp the washer	as object is released. d. Thumb and finger extension to release tube/washer.
11, 13, 14	Ball bearing, from tin on table, vertically displaced to tin on shelf	Opposition of pads of ring finger and thumb, middle finger and thumb, and index finger and thumb, respectively	a. Forearm is between midposition and pronation. b. Elbow flexed when first grasping object, then extends to reach top of shelf.
12, 15,16	Marble, from tin on table, displace vertically to tin on shelf	Opposition of pads of index finger and thumb, ring finger and thumb and middle finger and thumb, respectively	c. Shoulder flexion to reach top of shelf and shoulder stabilization to maintain position as object is released.
17-19	Hand from lap to various pericranial positions	Palmer side of hand (hand does not need to be open) reaches to back side of head, to top of head, and to mouth, respectively	a. Forearm pronation and supination. b. Full elbow flexion c. Shoulder abduction, flexion, and external rotation.

Appendix 3: The National Institutes of Health Stroke Score

N I H
STROKE
SCALE

Patient Identification. ___ ___-___ ___ ___-___ ___ ___

Pt. Date of Birth ___ ___/___ ___/___ ___

Hospital _____(___ ___-___ ___)

Date of Exam ___ ___/___ ___/___ ___

Interval: [ ] Baseline [ ] 2 hours post treatment [ ] 24 hours post onset of symptoms ±20 minutes [ ] 7-10 days
[ ] 3 months [ ] Other _____(___ ___)

Time: ___ ___:___ ___ [ ]am [ ]pm

Person Administering Scale _____

Administer stroke scale items in the order listed. Record performance in each category after each subscale exam. Do not go back and change scores. Follow directions provided for each exam technique. Scores should reflect what the patient does, not what the clinician thinks the patient can do. The clinician should record answers while administering the exam and work quickly. Except where indicated, the patient should not be coached (i.e., repeated requests to patient to make a special effort).

Instructions	Scale Definition	Score
1a. Level of Consciousness: The investigator must choose a response if a full evaluation is prevented by such obstacles as an endotracheal tube, language barrier, orotracheal trauma/bandages. A 3 is scored only if the patient makes no movement (other than reflexive posturing) in response to noxious stimulation.	0 = **Alert;** keenly responsive. 1 = **Not alert;** but arousable by minor stimulation to obey, answer, or respond. 2 = **Not alert;** requires repeated stimulation to attend, or is obtunded and requires strong or painful stimulation to make movements (not stereotyped). 3 = Responds only with reflex motor or autonomic effects or totally unresponsive, flaccid, and areflexic.	_____
1b. LOC Questions: The patient is asked the month and his/her age. The answer must be correct - there is no partial credit for being close. Aphasic and stuporous patients who do not comprehend the questions will score 2. Patients unable to speak because of endotracheal intubation, orotracheal trauma, severe dysarthria from any cause, language barrier, or any other problem not secondary to aphasia are given a 1. It is important that only the initial answer be graded and that the examiner not "help" the patient with verbal or non-verbal cues.	0 = **Answers** both questions correctly. 1 = **Answers** one question correctly. 2 = **Answers** neither question correctly.	_____
1c. LOC Commands: The patient is asked to open and close the eyes and then to grip and release the non-paretic hand. Substitute another one step command if the hands cannot be used. Credit is given if an unequivocal attempt is made but not completed due to weakness. If the patient does not respond to command, the task should be demonstrated to him or her (pantomime), and the result scored (i.e., follows none, one or two commands). Patients with trauma, amputation, or other physical impediments should be given suitable one-step commands. Only the first attempt is scored.	0 = **Performs** both tasks correctly. 1 = **Performs** one task correctly. 2 = **Performs** neither task correctly.	_____
2. Best Gaze: Only horizontal eye movements will be tested. Voluntary or reflexive (oculocephalic) eye movements will be scored, but caloric testing is not done. If the patient has a conjugate deviation of the eyes that can be overcome by voluntary or reflexive activity, the score will be 1. If a patient has an isolated peripheral nerve paresis (CN III, IV or VI), score a 1. Gaze is testable in all aphasic patients. Patients with ocular trauma, bandages, pre-existing blindness, or other disorder of visual acuity or fields should be tested with reflexive movements, and a choice made by the investigator. Establishing eye contact and then moving about the patient from side to side will occasionally clarify the presence of a partial gaze palsy.	0 = **Normal.** 1 = **Partial gaze palsy;** gaze is abnormal in one or both eyes, but forced deviation or total gaze paresis is not present. 2 = **Forced deviation,** or total gaze paresis not overcome by the oculocephalic maneuver.	_____

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NIH STROKE SCALE

Patient Identification. ___ ___-___ ___ ___-___ ___ ___

Pt. Date of Birth ___ ___/___ ___/___ ___

Hospital _____(___ ___-___ ___)

Date of Exam ___ ___/___ ___/___ ___

Interval: [ ] Baseline [ ] 2 hours post treatment [ ] 24 hours post onset of symptoms ±20 minutes [ ] 7-10 days
[ ] 3 months [ ] Other _____(___ ___)

3. Visual: Visual fields (upper and lower quadrants) are tested by confrontation, using finger counting or visual threat, as appropriate. Patients may be encouraged, but if they look at the side of the moving fingers appropriately, this can be scored as normal. If there is unilateral blindness or enucleation, visual fields in the remaining eye are scored. Score 1 only if a clear-cut asymmetry, including quadrantanopia, is found. If patient is blind from any cause, score 3. Double simultaneous stimulation is performed at this point. If there is extinction, patient receives a 1, and the results are used to respond to item 11.	0 = **No visual loss.** 1 = **Partial hemianopia.** 2 = **Complete hemianopia.** 3 = **Bilateral hemianopia** (blind including cortical blindness).	_____
4. Facial Palsy: Ask – or use pantomime to encourage – the patient to show teeth or raise eyebrows and close eyes. Score symmetry of grimace in response to noxious stimuli in the poorly responsive or non-comprehending patient. If facial trauma/bandages, orotracheal tube, tape or other physical barriers obscure the face, these should be removed to the extent possible.	0 = **Normal** symmetrical movements. 1 = **Minor paralysis** (flattened nasolabial fold, asymmetry on smiling). 2 = **Partial paralysis** (total or near-total paralysis of lower face). 3 = **Complete paralysis** of one or both sides (absence of facial movement in the upper and lower face).	_____
5. Motor Arm: The limb is placed in the appropriate position: extend the arms (palms down) 90 degrees (if sitting) or 45 degrees (if supine). Drift is scored if the arm falls before 10 seconds. The aphasic patient is encouraged using urgency in the voice and pantomime, but not noxious stimulation. Each limb is tested in turn, beginning with the non-paretic arm. Only in the case of amputation or joint fusion at the shoulder, the examiner should record the score as untestable (UN), and clearly write the explanation for this choice.	0 = **No drift;** limb holds 90 (or 45) degrees for full 10 seconds. 1 = **Drift;** limb holds 90 (or 45) degrees, but drifts down before full 10 seconds; does not hit bed or other support. 2 = **Some effort against gravity;** limb cannot get to or maintain (if cued) 90 (or 45) degrees, drifts down to bed, but has some effort against gravity. 3 = **No effort against gravity;** limb falls. 4 = **No movement.** UN = **Amputation** or joint fusion, explain: _____ **5a. Left Arm** **5b. Right Arm**	_____ _____
6. Motor Leg: The limb is placed in the appropriate position: hold the leg at 30 degrees (always tested supine). Drift is scored if the leg falls before 5 seconds. The aphasic patient is encouraged using urgency in the voice and pantomime, but not noxious stimulation. Each limb is tested in turn, beginning with the non-paretic leg. Only in the case of amputation or joint fusion at the hip, the examiner should record the score as untestable (UN), and clearly write the explanation for this choice.	0 = **No drift;** leg holds 30-degree position for full 5 seconds. 1 = **Drift;** leg falls by the end of the 5-second period but does not hit bed. 2 = **Some effort against gravity;** leg falls to bed by 5 seconds, but has some effort against gravity. 3 = **No effort against gravity;** leg falls to bed immediately. 4 = **No movement.** UN = **Amputation** or joint fusion, explain: _____ **6a. Left Leg** **6b. Right Leg**	_____

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NIH STROKE SCALE

Patient Identification. ___ ___-___ ___ ___-___ ___ ___

Pt. Date of Birth ___ ___/___ ___/___ ___

Hospital _____(___ ___-___ ___)

Date of Exam ___ ___/___ ___/___ ___

Interval: [ ] Baseline [ ] 2 hours post treatment [ ] 24 hours post onset of symptoms ±20 minutes [ ] 7-10 days
[ ] 3 months [ ] Other _____(___ ___)

7. Limb Ataxia: This item is aimed at finding evidence of a unilateral cerebellar lesion. Test with eyes open. In case of visual defect, ensure testing is done in intact visual field. The finger-nose-finger and heel-shin tests are performed on both sides, and ataxia is scored only if present out of proportion to weakness. Ataxia is absent in the patient who cannot understand or is paralyzed. Only in the case of amputation or joint fusion, the examiner should record the score as untestable (UN), and clearly write the explanation for this choice. In case of blindness, test by having the patient touch nose from extended arm position.	0 = **Absent.** 1 = **Present in one limb.** 2 = **Present in two limbs.** UN = **Amputation** or joint fusion, explain: _____	____
8. Sensory: Sensation or grimace to pinprick when tested, or withdrawal from noxious stimulus in the obtunded or aphasic patient. Only sensory loss attributed to stroke is scored as abnormal and the examiner should test as many body areas (arms [not hands], legs, trunk, face) as needed to accurately check for hemisensory loss. A score of 2, "severe or total sensory loss," should only be given when a severe or total loss of sensation can be clearly demonstrated. Stuporous and aphasic patients will, therefore, probably score 1 or 0. The patient with brainstem stroke who has bilateral loss of sensation is scored 2. If the patient does not respond and is quadriplegic, score 2. Patients in a coma (item 1a=3) are automatically given a 2 on this item.	0 = **Normal;** no sensory loss. 1 = **Mild-to-moderate sensory loss;** patient feels pinprick is less sharp or is dull on the affected side; or there is a loss of superficial pain with pinprick, but patient is aware of being touched. 2 = **Severe to total sensory loss;** patient is not aware of being touched in the face, arm, and leg.	____
9. Best Language: A great deal of information about comprehension will be obtained during the preceding sections of the examination. For this scale item, the patient is asked to describe what is happening in the attached picture, to name the items on the attached naming sheet and to read from the attached list of sentences. Comprehension is judged from responses here, as well as to all of the commands in the preceding general neurological exam. If visual loss interferes with the tests, ask the patient to identify objects placed in the hand, repeat, and produce speech. The intubated patient should be asked to write. The patient in a coma (item 1a=3) will automatically score 3 on this item. The examiner must choose a score for the patient with stupor or limited cooperation, but a score of 3 should be used only if the patient is mute and follows no one-step commands.	0 = **No aphasia;** normal. 1 = **Mild-to-moderate aphasia;** some obvious loss of fluency or facility of comprehension, without significant limitation on ideas expressed or form of expression. Reduction of speech and/or comprehension, however, makes conversation about provided materials difficult or impossible. For example, in conversation about provided materials, examiner can identify picture or naming card content from patient's response. 2 = **Severe aphasia;** all communication is through fragmentary expression; great need for inference, questioning, and guessing by the listener. Range of information that can be exchanged is limited; listener carries burden of communication. Examiner cannot identify materials provided from patient response. 3 = **Mute, global aphasia;** no usable speech or auditory comprehension.	____
10. Dysarthria: If patient is thought to be normal, an adequate sample of speech must be obtained by asking patient to read or repeat words from the attached list. If the patient has severe aphasia, the clarity of articulation of spontaneous speech can be rated. Only if the patient is intubated or has other physical barriers to producing speech, the examiner should record the score as untestable (UN), and clearly write an explanation for this choice. Do not tell the patient why he or she is being tested.	0 = **Normal.** 1 = **Mild-to-moderate dysarthria;** patient slurs at least some words and, at worst, can be understood with some difficulty. 2 = **Severe dysarthria;** patient's speech is so slurred as to be unintelligible in the absence of or out of proportion to any dysphasia, or is mute/anarthric. UN = **Intubated** or other physical barrier, explain: _____	____

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NIH STROKE SCALE

Patient Identification. ___ ___-___ ___ ___-___ ___ ___

Pt. Date of Birth ___ ___/___ ___/___ ___

Hospital _____(___ ___-___ ___)

Date of Exam ___ ___/___ ___/___ ___

Interval: [ ] Baseline [ ] 2 hours post treatment [ ] 24 hours post onset of symptoms ±20 minutes [ ] 7-10 days
[ ] 3 months [ ] Other _____(___ ___)

11. Extinction and Inattention (formerly Neglect): Sufficient information to identify neglect may be obtained during the prior testing. If the patient has a severe visual loss preventing visual double simultaneous stimulation, and the cutaneous stimuli are normal, the score is normal. If the patient has aphasia but does appear to attend to both sides, the score is normal. The presence of visual spatial neglect or anosagnosia may also be taken as evidence of abnormality. Since the abnormality is scored only if present, the item is never untestable.	0 = **No abnormality.** 1 = **Visual, tactile, auditory, spatial, or personal inattention** or extinction to bilateral simultaneous stimulation in one of the sensory modalities. 2 = **Profound hemi-inattention or extinction to more than one modality;** does not recognize own hand or orients to only one side of space.	____

____

____

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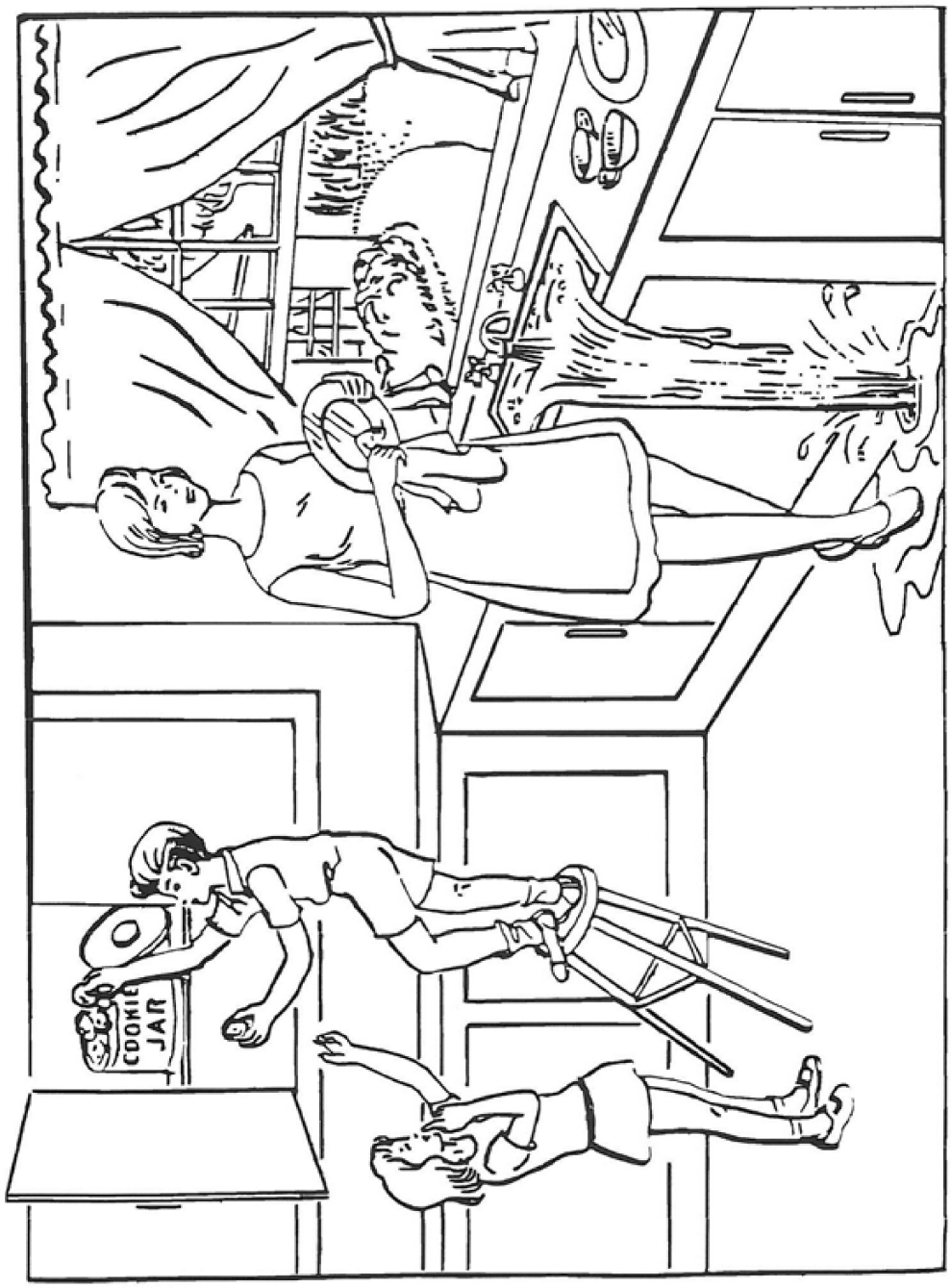

COOKIE
JAR

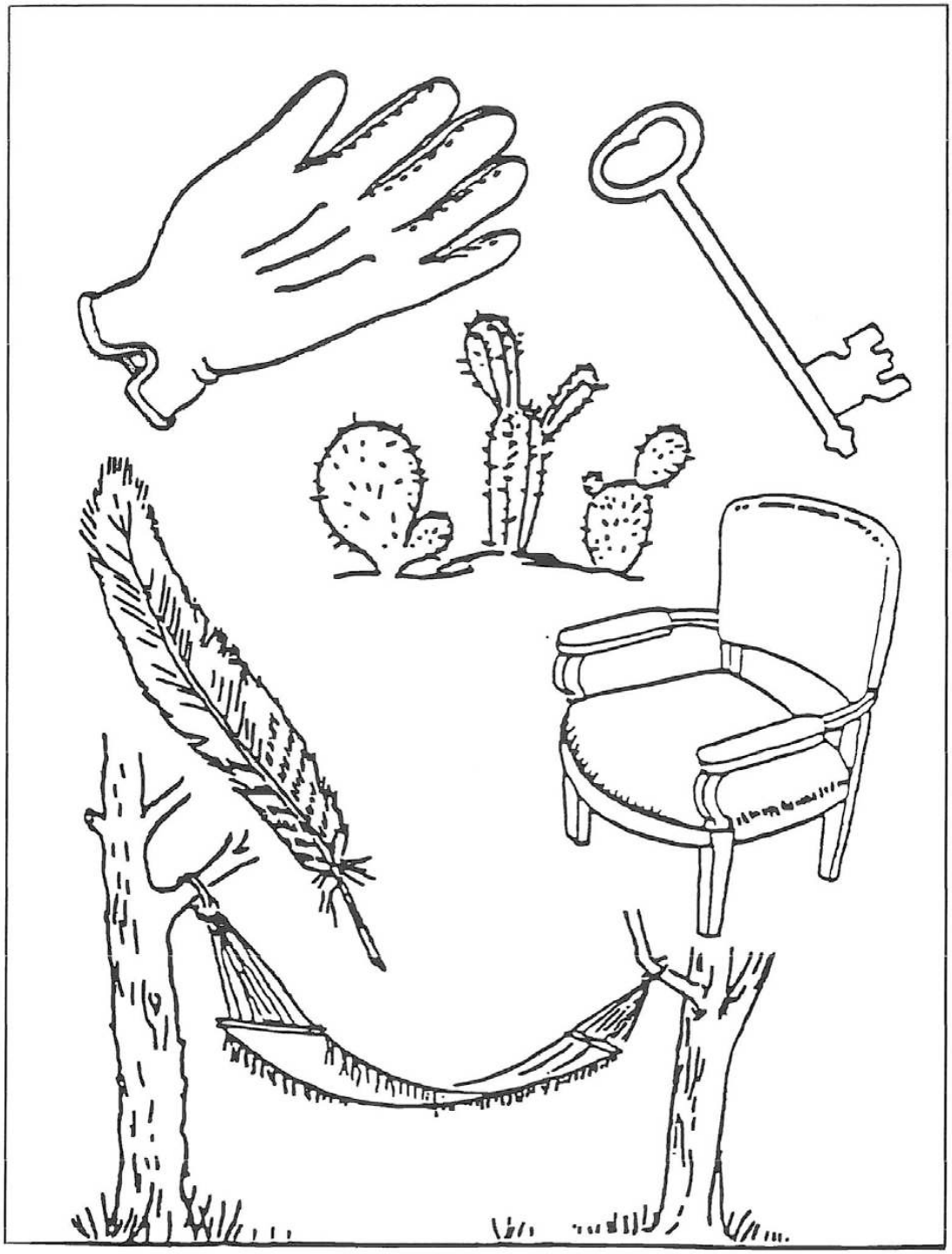
You know how.

Down to earth.

I got home from work.

Near the table in the dining room.

They heard him speak on the radio last night.



MAMA

TIP – TOP

FIFTY – FIFTY

THANKS

HUCKLEBERRY

BASEBALL PLAYER

Appendix 4: Measurement of Rankin Focused Assessment

Instructions
Rankin Focused Assessment (RFA)

Introduction

The Modified Rankin Scale (MRS) is widely used as a functional outcome measure in stroke:

Modified Rankin Scale	
6	**Dead**
5	**Severe disability**: bedridden, incontinent and requiring constant nursing care and attention.
4	**Moderately severe disability**: unable to walk without assistance, and unable to attend to own bodily needs without assistance.
3	**Moderate disability**: requiring some help, but able to walk without assistance.
2	**Slight disability**: unable to carry out all routine activities but able to look after own affairs without assistance.
1	**No significant disability:** despite symptoms: able to carry out all usual duties and activities.
0	**No disability**

The purpose of the Rankin Focused Assessment (RFA) is to assign patients to MRS grades in a systematic way. The assessment consists of five sections corresponding to the levels of disability among stroke survivors on the MRS.

General Instructions

Timing

This assessment is intended for use after stabilisation in hospital or after discharge from the hospital.

Sources of Information

Use the best sources of information available. Information should be obtained from the patient and/or family, friends, nursing staff, physical and occupational therapists, any person who is familiar with the daily routine of the patient, and from medical records. Interview both the patient and a close family member/friend or caregiver whenever possible. If the patient lacks insight into some difficulties, or responses are inconsistent, it is often helpful to interview a caregiver or relative independently.

Procedure
The responses to the separate sections should generally be hierarchical (for example if a person indicates that they require assistance to attend to bodily needs, then it is inconsistent if they then say that they go out alone for social and leisure activities). Thus, responses to later questions may suggest revisions to earlier responses. Check for consistency as you proceed. Ask all questions and go back to clarify, if necessary.

When performed serially over time in the same patient, responses in one interview should be consistent with responses in prior interviews. If an improvement or a decrement on an interview item is noted from a prior response, confirm the change with the informant and document the reason for the change (e.g. new interval recurrent stroke to explain decrement; or new interval functional gain in the course of rehabilitation therapy to explain improvement).

Rater Judgment
Your judgment determines the final rating for assessment items. Some sources of information may be conflicting or unreliable. For example, patients with denial of hemiplegia (anosognosia) may report they are fully functional and have no symptoms when in fact they are severely disabled. In contrast, an overprotective family member may report that a patient is incapable of independent shopping or managing their finances when in fact the patient could perform these activities if he/she absolutely had to. After collecting information from all key sources available, you should complete each item using your best judgment of the patient's actual functional capacity. Finally, if after review of all information, you have substantial doubt which of two alternatives on the scale would be most appropriate, the worse option should be chosen.

Specific Section Instructions

SECTIONS 5: BEDRIDDEN

5. BEDRIDDEN

Patients cannot walk even with assistance and cannot self-propel if placed in a wheelchair: patients may not actually remain in bed all the time, but moving them from the bed to sitting will require major assistance. Patients will also need assistance with other activities.

SECTIONS 4 AND 3: ASSISTANCE FOR ACTIVITIES OF DAILY LIVING

Assistance may be considered essential when there is the need for physical help (by another person) with an activity or there is a need for supervision, or the person needs prompting or reminding to do a task.

Mark responses based on the ability of the patient to perform the activity and not whether the patient actually performs the activity currently. Please probe using the specific questions given in the sections below. Please use your judgment to decide whether the person can actually do something before recording a response. The need for supervision for safety reasons should be due to objective danger that is posed, rather than 'just in case'. People may feel that a person who has had stroke should not be left on their own, but that does not make the person with stroke dependent. A general need for companionship, care, or protection should not be considered assistance.

4. ASSISTANCE FOR WALKING (OR WHEELCHAIR)

Specific question to ask: "If absolutely necessary, could you walk across the room, even if your caregiver was not present?"
(For patients who use wheelchairs, determine if they can propel themselves effectively throughout the house.

3. ASSISTANCE TO LOOK AFTER OWN AFFAIRS

(Could the patient live alone if he/she had to?)

3.1 Preparing a simple meal. Specific questions to ask: "If the person were on their own: Would they go hungry?

Might they be at risk of burning the house down if they tried to cook?"

3.2 Performing basic household chores. Specific questions to ask: "Are they *able to do* chores, if necessary, even if

they do not normally do them." Men may report that they need assistance more often than women. Please clarify by probing about the person's ability to perform the chores.

3.3 Looking after household expenses. Specific questions to ask: "Do you look after your own pension/income? If you absolutely had to, would you be able to? Do you arrange to pay bills? If you absolutely had to, would you be able to?" Look for a change from previous level of responsibility. Note: the person may be reluctant to admit a problem. The question is NOT about financial needs (e.g. assistance from benefit agencies). It refers to whether or not patients are able to take responsibility for the money that they have.

3.4 Local travel. Specific questions to ask: "If you need to get somewhere can you manage to call a taxi or bus?" The patient should be able to at least order and take a taxi or bus alone. This question is NOT about being able to afford a taxi, but about the tasks involved. The question refers to whether or not the patients can get around locally by themselves.

3.5 Local shopping. Specific Questions to ask: "If your life depended on it – could you get out and buy even single items?" "Can the person go to a local shop to buy milk or a loaf of bread?" Could also include going to a local coffee house or mini-mart, ordering and paying for a drink by themselves.

2. USUAL DUTIES AND ACTIVITIES

The set of questions in Section 2 are about how the patient usually spends his/her day. In this section, questions concerning status before stroke are asked first, to establish which areas are relevant. If an activity is not relevant (e.g. the person was not working before stroke), then it is assumed that there is no change, and the interviewer proceeds to ask about the next area. Concentrate on key areas relevant to the particular person. Not all will apply, but almost everyone will have some regular pre-stroke social & leisure activities.

Problems should come from impairment (not social circumstances). For example, change in financial circumstances may produce a change in social activities but this is not relevant. However, for section 2, unlike sections 3 and 4, answers are based on what activities a patient is actually no longer doing due to stroke deficits, whether or not he/she could do those activities if he/she absolutely had to.

Possible improvement in the future is not relevant (e.g. "I plan to go back to work next month"). The relevant time period is within the previous week or so.

2.1 Work

2.1.1 Work refers to paid employment, and does not include voluntary work (which can be included under 'social and leisure activities'). Many elderly patients will have retired and this section will not be relevant.

2.2 Family responsibilities

Refers to the patient's ability to look after others. Probe using specific examples such as "babysitting, looking after your partner, your parents, your grandchildren or dependent others".

2.3 Social & leisure activities

This refers to any specific free-time activities which the person did for pleasure. It is useful to first establish the person's main activities before stroke, and then ask about change in participation since the stroke. Probe with specific questions: "How did you spend your day before the stroke? How often did you get out? What activities did you do in your free time at home? Do you think your level of activity has changed?"

2.4 Other physical/medical condition

If the patient received an mRS of 2 on the prestroke assessment due to pre-existing physical/medical condition, and that condition continues to substantially restrict work, family responsibility, or social/leisure activities, check yes. If that condition has improved and no longer substantially restricts work, family responsibility, check no and explain.

If the patient since the trial-qualifying stroke has developed an additional physical/medical condition (e.g. recurrent stroke after the trial-qualifying stroke, new motor vehicle accident causing quadriplegia, new chronic, severe heart failure) that substantially restricts work, family responsibility, or social/leisure activities, check yes and explain.

1. SYMPTOMS AS A RESULT OF THE STROKE

1.1 This question is used to establish a spontaneous report of symptoms due to stroke, before going through the checklist.

1.2 SYMPTOM CHECKLIST

These can be any symptoms or problems reported by the patient. It is important to exclude common problems and complaints not due to stroke.

Assigning an overall grade on the Modified Rankin Scale

1. Rankin categories are given in brackets beside specific responses.

2. The overall rating is simply the lowest disability category indicated by the person's answers. Rankin 5 is the worst category among stroke survivors, and Rankin 0 is the best.

3. If the person has no limitations or symptoms, then the Rankin grade is 0.

Study Number: _____ PatientPatient Initials: __ __ __ Date of Visit: ___ / ___ /____

Rating Form
Rankin Focused Assessment (RFA)

Name of rater performing assessment: _____

Information for completing this form was obtained from (check all that apply):

[ ] Patient
[ ] Spouse
[ ] Son
[ ] Daughter
[ ] Father
[ ] Mother
[ ] Physical therapist
[ ] Speech therapist
[ ] Medical record
[ ] Other individual, specify role: _____
[ ] Sister
[ ] Brother
[ ] Other relative, specify relationship: _____
[ ] Friend
[ ] Nurse
[ ] Home health aide
[ ] Occupational therapist
[ ] Physician

Please mark (X) in the appropriate box. Please record responses to all questions (unless otherwise indicated in the text). Please see instruction sheets for further information.

5	**BEDRIDDEN**	
5.1	**Is the person bedridden?** The patient is unable to walk even with another person's assistance. (If placed in a wheelchair, unable to self-propel effectively). May frequently be incontinent. Will usually require nearly constant care - someone needs to be available at nearly all times. Care may be provided by either a trained or untrained caregiver.	☐ Yes ☐ No (5)

If yes, explain:

_____
_____
_____
_____
_____
_____

4	**ASSISTANCE TO WALK**	
4.1	**Is another person's assistance essential for walking?** Requiring another person's assistance means needing another person to be always present when walking, including indoors around house or ward, to provide physical help, verbal instruction, or supervision. (Patients who use physical aids to walk, e.g. stick/cane, walking frame/walker, but do not require another person's help, are NOT rated as requiring assistance to walk). (For patients who use wheelchairs, patient needs another person's assistance to transfer into and out of chair, but can self-propel effectively without assistance.)	☐ Yes ☐ No (4)

If yes, explain:

_____
_____
_____
_____
_____
_____

Study Number: _____ PatientPatient Initials: __ __ __ Date of Visit: ___ / ___ /____

3	**ASSISTANCE TO LOOK AFTER OWN AFFAIRS**		
	Assistance includes physical assistance, or verbal instruction, or supervision by another person. Central issue--Could the patient live alone for 1 week if he/she absolutely had to?		
3.1	**Is assistance ABSOLUTELY essential for preparing a simple meal?** (For example, able to prepare breakfast or a snack)	☐ Yes (3)	☐ No
3.2	**Is assistance ABSOLUTELY essential for basic household chores?** (For example, finding and putting away clothes, clearing up after a meal. Exclude chores that do not need to be done every day, such as using a vacuum cleaner.)	☐ Yes (3)	☐ No
3.3	**Is assistance ABSOLUTELY essential for looking after household expenses?**	☐ Yes (3)	☐ No
3.4	**Is assistance ABSOLUTELY essential for local travel?** (Patients may drive or use public transport to get around. Ability to use a taxi is sufficient, provided the person can phone for it themselves and instruct the driver.)	☐ Yes (3)	☐ No
3.5	**Is assistance ABSOLUTELY essential for local shopping?** (Local shopping: at least able to buy a single item)	☐ Yes (3)	☐ No

If yes to any of the above, explain:

_____
_____
_____
_____
_____
_____

Study Number: _____ PatientPatient Initials: __ __ __ Date of Visit: ___ / ___ /____

2. USUAL DUTIES AND ACTIVITIES. The next sets of questions are about how the patient usually spends his/her day.

2.1 Work

2.1	**Has the new stroke substantially reduced (compared to prestroke status) the person's ability to work (or, for a student, study)?** e.g. change from full-time to part-time, change in level of responsibility, or unable to work at all.	☐ Yes ☐ No (2)

If yes, explain:

_____
_____
_____
_____
_____
_____

2.2 Family responsibilities

2.2	**Has the new stroke substantially reduced (compared to prestroke status) the person's ability to look after family at home?**	☐ Yes ☐ No (2)

If yes, explain:

_____
_____
_____
_____
_____
_____

2.3 Social & leisure activities

(Social and leisure activities include hobbies and interests. Includes activities outside the home or at home. Activities outside the home: going to the coffee shop, bar, restaurant, club, church, cinema, visiting friends, going for walks. Activities at home: involving "active" participation including knitting, sewing, painting, games, reading books, home improvements).

2.3	**Has the new stroke reduced (compared to prestroke status) the person's regular free-time activities by more than one half as often?**	☐ Yes ☐ No (2)

If yes, explain:

_____
_____
_____
_____
_____
_____

2.4 Other physical/medical condition

2.4	**Are the patient's work, family, and/or social/leisure activities substantially reduced by a physical/medical condition other than the stroke that led to trial enrolment?**	☐ Yes ☐ No (2)

Provide explanation if 1) answer is yes, but prestroke assessment section 2 answers were all no, or 2) answer is no, but any prestroke assessment 2 section answer was yes:

_____
_____
_____
_____
_____
_____

Study Number: __ __ __ - __ __ __ __ Initials: __ __ __ Date of Visit: ___ / ___ /____

1. SYMPTOMS AS A RESULT OF THE STROKE

(Can be any symptoms or problems reported by the patient).

1.1 SPONTANEOUSLY REPORTED SYMPTOMS

1.1	**Does the patient have any symptoms resulting from the new stroke?**	□ Yes (1) □ No

If yes, record symptoms here:

_____

_____

1.2. SYMPTOM CHECKLIST

1.2.1	**Does the person have difficulty reading or writing as a result of the new stroke?**	□ Yes (1) □ No
1.2.2	**Does the person have difficulty speaking or finding the right word as a result of the new stroke?**	□ Yes (1) □ No
1.2.3	**Does the person have problems with balance or coordination as a result of the new stroke?**	□ Yes (1) □ No
1.2.4	**Does the person have visual problems as a result of stroke?**	□ Yes (1) □ No
1.2.5	**Does the person have numbness (face, arms, legs, hands, feet) as a result of the new stroke?**	□ Yes (1) □ No
1.2.6	**Does the person have weakness or loss of movement (face, arms, legs, hands, feet) as a result of the new stroke?**	□ Yes (1) □ No
1.2.7	**Does the person have difficulty with swallowing as a result of the new stroke?**	□ Yes (1) □ No
1.2.8	**Does the person have any other symptoms related to the new stroke?**	□ Yes (1) □ No

Details supporting any "Yes" checked boxes in Section 1:

_____

_____

_____

_____

Rankin Grade = ☐

Is this Rankin Grade score lower (better) than the prestroke Rankin Grade? □ Yes □ No

If yes, explain why:

_____
_____
_____
_____

Appendix 5: The Barthel Index

THE BARTHEL INDEX

Patient Name: _____
Rater Name: _____
Date: _____

Activity	Score
FEEDING 0 = unable 5 = needs help cutting, spreading butter, etc., or requires modified diet 10 = independent	_____
BATHING 0 = dependent 5 = independent (or in shower)	_____
GROOMING 0 = needs to help with personal care 5 = independent face/hair/teeth/shaving (implements provided)	_____
DRESSING 0 = dependent 5 = needs help but can do about half unaided 10 = independent (including buttons, zips, laces, etc.)	_____
BOWELS 0 = incontinent (or needs to be given enemas) 5 = occasional accident 10 = continent	_____
BLADDER 0 = incontinent, or catheterized and unable to manage alone 5 = occasional accident 10 = continent	_____
TOILET USE 0 = dependent 5 = needs some help, but can do something alone 10 = independent (on and off, dressing, wiping)	_____
TRANSFERS (BED TO CHAIR AND BACK) 0 = unable, no sitting balance 5 = major help (one or two people, physical), can sit 10 = minor help (verbal or physical) 15 = independent	_____
MOBILITY (ON LEVEL SURFACES) 0 = immobile or < 50 yards 5 = wheelchair independent, including corners, > 50 yards 10 = walks with help of one person (verbal or physical) > 50 yards 15 = independent (but may use any aid; for example, stick) > 50 yards	_____
STAIRS 0 = unable 5 = needs help (verbal, physical, carrying aid) 10 = independent	_____
TOTAL (0–100):	_____

Provided by the Internet Stroke Center — www.strokecenter.org

The Barthel ADL Index: Guidelines

1. The index should be used as a record of what a patient does, not as a record of what a patient could do.
2. The main aim is to establish degree of independence from any help, physical or verbal, however minor and for whatever reason.
3. The need for supervision renders the patient not independent.
4. A patient's performance should be established using the best available evidence. Asking the patient, friends/relatives and nurses are the usual sources, but direct observation and common sense are also important. However direct testing is not needed.
5. Usually the patient's performance over the preceding 24-48 hours is important, but occasionally longer periods will be relevant.
6. Middle categories imply that the patient supplies over 50 per cent of the effort.
7. Use of aids to be independent is allowed.

Copyright Information

Appendix 6: Fugl-Meyer Assessment

APPENDIX A

FUGL- MEYER ASSESSMENT OF PHYSICAL PERFORMANCE

General Procedure and Rules

PROCEDURE

Description: This assessment is a measure of upper extremity (UE) and lower extremity (LE) motor and sensory impairment.

Equipment: A chair, bedside table, reflex hammer, cotton ball, pencil, small piece of cardboard or paper, small can, tennis ball, stop watch, and blindfold.

Administration: The complete assessment usually requires 45 minutes.

GENERAL RULES

Perform the assessment in a quiet area when the patient is maximally alert.

Volitional movement assessment: This includes flexor synergy, extensor synergy, movement combining synergies, movement out of synergy, wrist, hand, and coordination/speed. For all tests of volitional motion, these guidelines are to be followed:

1. Give clear and concise instructions. Mime as well as verbal instructions permissible.
2. Have patient perform the movement with non-affected extremity first. On affected side, check for available passive range of motion (PROM) prior to asking patient to perform the movement.
3. Repeat each movement 3x on the affected side and score best performance. If full score is attained on trials 1 or 2, do not have to repeat 3 times. Only test Coordination/speed, one time.
4. Do not assist patient, however verbal encouragement is permitted.
5. Test the wrist and hand function independently of the arm. **During the wrist tests (items 7a-e), support under the elbow may be provided to decrease demand at the shoulder; however, the patient should be activating the elbow flexors during the elbow at 90 degree tests and activating the elbow extensors during the elbow at 0 degree tests. In contrast, assistance can be provided to the arm at the elbow and just proximal to the wrist in order to position the arm during the hand tests (items 8a-g)**.

Fugl-Meyer Motor Assessment

Lower Extremity

Item	Procedure	Scoring

I. Reflex activity	• Patient is supine or sitting.
	• Attempt to elicit the Achilles and patellar reflexes.
	• Assess the unaffected side first.
	• Test affected side.
		• *Scoring* (Maximum possible score = 4):
		  • (0) - No reflex activity can be elicited;
		  • (2) - Reflex activity can be elicited. Items to be scored are Achilles and patellar reflexes.

IIA. Flexor synergy	• Patient is supine.
	• Have patient perform movement with unaffected side first.
	• On the affected side, check patient's available PROM at each joint to be tested.
	• Start with leg fully extended at hip, knee, and ankle. Instruct the patient to "bring your knee to your chest and
		• *Scoring* (Maximum possible score = 6):
		  • (0) - Cannot be performed at all
		  • (1) – Partial motion
		  • (2) – Full motion

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	pull up your toes" (therapist is observing for evidence of hip, knee, ankle flexion in order to assess the presence of all components of the flexor synergy). Therapist can cue the patient to move any missing component. • Test 3x on the affected side and score best movement at each joint.	• Items to be scored are: Hip flexion, knee flexion, ankle dorsiflexion.
IIB. <u>Extensor synergy</u>	• Patient is sidelying. • Have patient perform movement with unaffected side first. • On the affected side, check patient's available PROM at each joint to be tested. • Start in 90 degrees hip flexion, 90 degrees knee flexion and ankle dorsiflexion. • Instruct the patient to "push your foot down and kick down and back". (Ankle plantarflexion, knee extension, hip adduction and hip extension.) • Slight resistance should be applied in adduction which is gravity-assisted in this position to ensure patient is actively adducting. • Test 3x on the affected side and score best movement at each joint.	• *Scoring* (Maximum possible score = 8): • (0) – No motion • (1) – Partial motion • (2) – Full motion • Items to be scored are: Hip extension, hip adduction, knee extension, ankle plantarflexion.
III. <u>Movement combining synergies (in sitting)</u>	<u>3a. Knee flexion beyond 90°:</u> • Patient is sitting, feet on floor, with knees free of chair. Knee to be tested is slightly extended beyond 90° knee flexion. Calf muscles should not be on stretch. To decrease friction, patient's shoes can be removed, but socks should remain on. • Have patient perform movement with unaffected side first. • Check patient's available PROM on the affected side for this motion. • Patient is instructed to "pull your heel back and under the chair." • Test 3x on the affected side and score best movement.	• *Scoring* (Maximum possible score = 2)*:* • (0) – No active motion • (1) – From slightly extended position, knee can be flexed but not beyond 90° or hip flexes while attempting to flex knee • (2) – Knee flexion beyond 90°
	<u>3b. Ankle Dorsiflexion:</u> • Patient is sitting, feet on floor, with knees free of chair. Calf muscles should not be on stretch. • Have patient perform movement with unaffected side first. • On the affected side, check patient's available PROM at the ankle joint. • Patient is instructed to "keeping your heel on the floor, lift your foot." • Test 3x on the affected side and score best movement.	• *Scoring* (Maximum possible score = 2): • (0) – No active motion • (1) – Incomplete active flexion (heel must remain on floor with medial and lateral borders of the forefoot clearing the floor during dorsiflexion) • (2) – Normal dorsiflexion (full within available ROM, heel remains on the floor)

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IV. Movement out of synergy (Standing, hip at 0 degrees)	4a. Knee Flexion: • Patient is standing, hip at 0 degrees (or full available ROM up to 0 degrees). On leg that is being tested, hip is at 0 degrees (or full available ROM up to 0 degrees), but the knee is flexed, and the patient's toes are touching the floor slightly behind. Evaluator can provide assistance to maintain balance and patient can rest hands on a table. • Have patient perform movement with unaffected side first. • Check patient's available PROM on the affected side for this motion. • Patient is instructed to "keeping your hip back, kick your bottom with your heel." • Test 3x on the affected side and score best movement.	• *Scoring* (Maximum possible score = 2): • (0) – Knee cannot flex without hip flexion • (1) – Knee flexion begins without hip flexion but does not reach to 90° or hip begins to flex in later phase of motion • (2) – Knee flexion beyond 90° (Knee flexion beyond 90 degrees with hip maintained in extension)
IV. Movement out of synergy (Standing, hip at 0 degrees)	4b. Ankle Dorsiflexion: • Patient is standing, hip at 0 degrees. If patient's calf muscle length is limiting active dorsiflexion in this starting position, then leg that is being tested can be positioned forward, so the hip is at approximately 5 degrees of flexion, and calf muscles are in lengthened position. Knee must stay fully extended. Evaluator can provide assistance to maintain balance and patient can rest hands on a table. • Have patient perform movement with unaffected side first. • On the affected side, check patient's available dorsiflexion PROM. • Patient is instructed to "keeping your knee extended and your heel on the floor, lift your foot." • Test 3x on the affected side and score best movement	• *Scoring* (Maximum possible score = 2): • (0) – No active motion • (1) – Partial motion (less than full available range with knee extended; heel must remain on floor with medial and lateral borders of the forefoot clearing the floor during dorsiflexion, or hip and/or knee flexes during motion while attempting dorsiflexion) • (2) – Full motion (within available dorsiflexion range with knee extended and heel on the floor)
V. Normal Reflexes (sitting)	• This item is only included if the patient achieves a maximum score on all previous lower extremity items, otherwise score 0 . • The examiner shall elicit patellar and Achilles phasic reflexes with a reflex hammer and knee flexors with quick stretch of the affected leg and note if the reflexes are hyperactive or not.	• *Scoring* (Maximum possible score = 2): • (0) - At least 2 of the 3 phasic reflexes are markedly hyperactive • (1) – One reflex is markedly hyperactive or at least 2 reflexes are lively • (2) - No more than one reflex is lively and none are hyperactive

VI. <u>Coordination/speed - Sitting: Heel to opposite knee repetitions in rapid succession</u>	• Patient positioned in sitting with eyes open.
	• Starting position is with heel to be tested resting on opposite ankle.
	• Have patient perform movement with unaffected side first.
	• Check available PROM on the affected side.
	• Patient is instructed to "Bring your heel from your opposite ankle to your opposite knee, keeping your heel on your shin bone, move as fast as possible."
	• Use a stopwatch to time how long it takes the patient to do 5 full (ankle to knee to ankle) repetitions.
	• Use the full achieved active ROM on the unaffected limb as the comparison for the affected limb. If active ROM of affected limb is significantly less than that of unaffected limb, patient should be scored "0" for speed.
	• Repeat the same movement with the affected leg. Record the time for both the unaffected and affected sides. Observe for evidence of tremor or dysmetria during the movement
	• NOTE: This item attempts to discriminate between basal ganglia, thalamic, or cerebellar strokes in which tremor or dysmetria may result as a direct result of lesion to these areas. The majority of stroke cases are in the middle cerebral artery or basilar artery distributions where we expect to observe paralysis that affects movement speed but does not cause tremor or dysmetria. In cases of complete paralysis, observe for any indication of tremor or dysmetria that may be evident in face, voice, arms or legs. If there are no indicators of tremor or dysmetria, then score these items 2 and score speed 0.
		• *Scoring Tremor* (Maximum possible score = 2):
		  • (0) - Marked tremor
		  • (1) – Slight tremor
		  • (2) – No tremor
		• *Scoring Dysmetria* (Maximum possible score = 2):
		  • (0) - Pronounced or unsystematic dysmetria
		  • (1) – Slight or systematic dysmetria
		  • (2) – No dysmetria
		• *Scoring Speed* (Maximum possible score = 2):
		  • (0) - Activity is more than 6 seconds longer than unaffected leg
		  • (1) – 2-5.9 seconds longer than unaffected leg
		  • (2) - less than 2 seconds difference

Muir KW, *et al. J Neurol Neurosurg Psychiatry* 2020; 91:1–6. doi: 10.1136/jnnp-2019-322515

Upper Extremity		
Item	**Instructions**	**Scoring**

I. <u>Reflex activity</u>	• Patient is sitting.
	• Attempt to elicit the biceps and triceps reflexes.
	• Test reflexes on unaffected side first.
	• Test affected side.
		• *Scoring* (Maximum possible score = 4):
		  • (0) - No reflex activity can be elicited
		  • (2) - Reflex activity can be elicited

II. <u>Flexor synergy</u>	• Patient is sitting.
	• Have patient perform movement with unaffected side first.
	• On the affected side, check patient's available PROM at each joint to be tested.
	• The starting position should be that of full extensor synergy. If the patient cannot actively achieve the starting position, the limb may be passively placed extended towards opposite knee in shoulder adduction/internal rotation, elbow extension, and forearm pronation.
	• Instruct the patient to fully supinate his/her forearm, flex the elbow, and bring the hand to the ear of the affected side. The shoulder should be abducted at least 90 degrees.
	• Test 3x on the affected side and score best movement at each joint
		• *Scoring* (Maximum possible score = 12):
		  • (0) - Cannot be performed at all
		  • (1) - Performed partly
		  • (2) - Performed faultlessly
		• Items to be scored are: Elevation (scapular), shoulder retraction (scapular), shoulder abduction (at least 90 degrees) and external rotation, elbow flexion, and forearm supination.
		  •

III. <u>Extensor synergy</u>	• Patient is sitting.
	• Have patient perform movement with unaffected side first.
	• On the affected side, check patient's available PROM at each joint to be tested.
	• The starting position should be that the limb is passively placed at patient's side in elbow flexion and supination. The examiner must ensure that the patient does not rotate and flex the trunk forward, thereby allowing gravity to assist with the movement. The pectoralis major and triceps brachii tendons may be palpated to assess active movement.
	• Instruct the patient to adduct & internally rotate the shoulder, extend his arm towards the unaffected knee with the forearm pronated.
	• Test 3x on the affected side and score best movement at each joint.
		• *Scoring* (Maximum possible score = 6):
		  • (0) - Cannot be performed at all
		  • (1) - Performed partly
		  • (2) - Performed faultlessly
		• Items to be scored are: Shoulder adduction/internal rotation, elbow extension, and forearm pronation.

IV. <u>Movement combining synergies</u> **The patient is asked to perform**	4a. Hand to lumbar spine: • Patient is sitting with arm at side, shoulder at 0°, elbow at 0°. • Have patient perform movement with unaffected side first.	• *Scoring* (Maximum possible score = 2): • (0) – No specific action is performed (or patient moves but does not reach

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three separate movements (4a, 4b, 4c).	• Check patient's available PROM on the affected side for this motion. • Patient is instructed to actively position the affected hand on the lumbar spine by asking them to "put your hand behind your back". • Test 3x on the affected side and score best movement.	ASIS) • (1) - Hand must pass anterior superior iliac spine (performed partly) • (2) - Performed faultlessly (patient clears ASIS and can extend arm behind back towards sacrum; full elbow extension is not required to score a 2)
	4b. Shoulder flexion to 90°, elbow at 0°: • Patient is sitting with hand resting on lap. • Have patient perform movement with unaffected side first. • On the affected side, check patient's available PROM for shoulder flexion to 90° and full elbow extension. • Patient is instructed to flex the shoulder to 90°, keeping the elbow extended. The elbow must be fully extended throughout the shoulder flexor movement; the forearm can be in pronation or in a mid-position between pronation and supination. • Test 3x on the affected side and score best movement.	• *Scoring* (Maximum possible score = 2): • (0) – Arm is immediately abducted, or elbow flexes at start of motion • (1) - Abduction or elbow flexion occurs in later phase of motion • (2) - Performed faultlessly (patient can flex shoulder keeping elbow extended)
	4c. Pronation/supination of forearm, elbow at 90°, shoulder at 0°: • Patient is sitting with arm at side, elbow flexed, and forearm in supination. • Have patient perform movement with unaffected side first. • On the affected side, check patient's available PROM for end range of pronation and supination. • Patient is instructed to actively flex the elbow to 90° and pronate/supinate the forearm through the full available ROM. • Test 3x on the affected side and score best movement.	• *Scoring* (Maximum possible score = 2): • (0) – Correct position of shoulder held in adduction at side of body and elbow flexion, and/or pronation or supination cannot be performed. • (1) – Active pronation or supination can be performed even within a limited range of motion, with elbow flexed at 90° and arm at side. • (2) - Complete pronation and supination with with elbow flexed at 90° and arm at side.
<u>V. Movement out of synergy</u> **The patient is asked to perform three separate movements (5a, 5b, 5c).**	5a. Shoulder abduction to 90°, elbow at 0°, and forearm pronated: • Patient is sitting with arm and hand resting at side. • Have patient perform movement with unaffected side first. • Check patient's available PROM on the affected side for this motion. • Patient is instructed to abduct the shoulder to 90°, in a pure abduction motion, with the elbow fully extended	• *Scoring* (Maximum possible score = 2): • (0) – Initial elbow flexion occurs, or any deviation from pronated forearm occurs • (1) - Motion can be performed partly, or, if during motion, elbow is

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	and the forearm pronated. • Test 3x on the affected side and score best movement.	flexed, or forearm cannot be kept in pronation; • (2) - Performed faultlessly (patient can fully abduct shoulder, keeping forearm pronated with no elbow flexion)
	5b. Shoulder flexion from 90°-180°, elbow at 0°, and forearm in mid-position: • Patient is sitting with elbow extended, hand resting on knee. • Have patient perform movement with unaffected side first. • Check patient's available PROM on the affected side for this motion. • Patient is instructed to flex the shoulder above 90°, with the elbow fully extended and the forearm in the mid-position between pronation and supination. • Test 3x on the affected side and score best movement.	• *Scoring* (Maximum possible score = 2): • (0) – Initial flexion of elbow or shoulder abduction occurs (arm is immediately abducted, or elbow flexes at start of motion) • (1) – Elbow flexion or shoulder abduction occurs during shoulder flexion (in later phases of motion) • (2) - Performed faultlessly (patient can flex shoulder above, with forearm in mid-position and no elbow flexion)
	5c. Pronation/supination of forearm, elbow at 0°, and shoulder at 30°-90° of flexion: • Patient is sitting with elbow extended, shoulder between 30°-90° of flexion. • Have patient perform movement with unaffected side first. • Check patient's available PROM on the affected side for this motion. • Patient is instructed to pronate and supinate the forearm as the shoulder remains flexed between 30-90° and the elbow is fully extended. • Test 3x on the affected side and score best movement.	• *Scoring* (Maximum possible score = 2): • (0) – Supination and pronation cannot be performed at all, or elbow and shoulder positions cannot be attained • (1) – Elbow and shoulder properly positioned and supination performed in a limited range • (2) - Performed faultlessly (complete pronation and supination with correct positions at elbow and shoulder)
VI. <u>Normal Reflexes</u> (sitting)	• This item is only included if the patient achieves a maximum score on all previous upper extremity items, otherwise score 0. • The examiner shall elicit biceps and triceps phasic reflexes with a reflex hammer and finger flexors with quick stretch and note if the reflexes are hyperactive or not.	• *Scoring* (Maximum possible score = 2): • (0) - At least 2 of the 3 phasic reflexes are markedly hyperactive • (1) – One reflex is markedly hyperactive or at least 2 reflexes are lively • (2) - No more than one

		reflex is lively, and none are hyperactive
VII. Wrist During the wrist tests, support under the elbow to may be provided to decrease demand at the shoulder; however, the patient should be activating the elbow flexors during the elbow at 90 degree tests and activating the elbow extensors during the elbow at 0 degree tests. The patient is asked to perform five separate movements (7a, 7b, 7c, 7d, 7e).	7a. Stability, elbow at 90°, and shoulder at 0°: • Patient is sitting with arm and hand resting at side. • Have patient perform movement with unaffected side first. • Check patient's available PROM on the affected side for this motion. • Patient is instructed to dorsiflex (extend) the wrist to the full range of 15° (or full available range) with the elbow at 90° flexion and the shoulder at 0°. If full range of dorsiflexion is attained, slight resistance is given. • Test 3x on the affected side and score best movement.	• *Scoring* (Maximum possible score = 2): • (0) - Patient cannot dorsiflex wrist to required 15° • (1) – Dorsiflexion is accomplished, but no resistance is taken • (2) - Position can be maintained with some (slight) resistance
	7b. Flexion/extension, elbow at 90°, and shoulder at 0°: • Patient is sitting with arm and hand resting at side. • Have patient perform movement with unaffected side first. • Patient is instructed to perform repeated smooth alternating movements from 15 degrees of flexion (wrist extension) to 15 degrees of extension. • Test 3x on the affected side and score best movement	• *Scoring* (Maximum possible score = 2): • (0) - Volitional movement does not occur • (1) – Patient cannot actively move through the wrist joint throughout the total range of motion • (2) – Faultless, smooth movement (repetitive through full available ROM)
	7c. Stability, elbow at 0°, and shoulder at 30° flexion: • Patient is sitting with elbow extended, hand resting on knee and forearm pronated. • Have patient perform movement with unaffected side first. • Check patient's available PROM on the affected side for this motion. • Patient is instructed to dorsiflex (extend) the wrist to the full range of 15° (or full available range) with the elbow fully extended and the shoulder at 30° flexion. If full range of dorsiflexion is attained, slight resistance is given. • Test 3x on the affected side and score best movement.	• *Scoring* (Maximum possible score = 2): • (0) - Patient cannot dorsiflex wrist to required 15° • (1) – Dorsiflexion is accomplished, but no resistance is taken • (2) - Position can be maintained with some (slight) resistance
	7d. Flexion/extension, elbow at 0°, and shoulder at 30° flexion: • Patient is sitting with elbow extended, hand resting on knee and forearm pronated. • Have patient perform movement with unaffected side first. • Patient is instructed to perform repeated smooth alternating movements from maximum dorsiflexion to maximum volar flexion with the fingers somewhat flexed to the full range of 15° (or full available range)	• *Scoring* (Maximum possible score = 2): • (0) - Volitional movement does not occur • (1) – Patient cannot actively move throughout the total range of motion; • (2) – Faultlessly, smooth movement (repetitive through full ROM)

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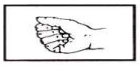
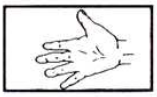
	with the elbow fully extended and the shoulder at 30° flex. • Test 3x on the affected side and score best movement.	
	7e. Circumduction: • Patient is sitting with arm at side elbow flexed to 90°, and forearm pronated. • Have patient perform movement with unaffected side first. • Check patient's available PROM on the affected side for this motion. • Patient is instructed to circumduct the wrist with smooth alternating movements throughout the full range of circumduction. • Test 3x on the affected side and score best movement.	• *Scoring* (Maximum possible score = 2): • (0) – Cannot be performed (volitional movement does not occur) • (1) – Jerky motion or incomplete circumduction • (2) – Complete motion with smoothness (performs faultlessly, smooth, repetitive movement through full ROM)
VIII. Hand During the hand tests, assistance can be provided to the arm at the elbow and just proximal to the wrist in order to position the arm for the grasp tasks. The patient is asked to perform seven separate movements (8a, 8b, 8c, 8d, 8e, 8f, 8g). The object is not placed in the hand but presented to the patient so that it requires sufficient opening to grasp test object, closure on object, ability to hold against a slight tug.	8a. Finger mass flexion:  • Patient is sitting with arm on bedside table or lap. • Have patient perform movement with unaffected side first. • Check patient's available PROM on the affected side for this motion. • Starting from the position of finger extension (this may be attained passively if necessary), instruct the patient to fully flex all fingers. • Test 3x on the affected side and score best movement	• *Scoring* (Maximum possible score = 2): • (0) – No flexion occurs • (1) – Some flexion, but not full motion • (2) – Completed active flexion (compared to unaffected hand)
	8b. Finger mass extension:  • Patient is sitting with arm on bedside table or lap. • Have patient perform movement with unaffected side first. • Check patient's available PROM on the affected side for this motion. • Starting from the position of finger flexion (this may be attained passively if necessary), instruct the patient to fully extend all fingers. • Test 3x on the affected side and score best movement.	• *Scoring* (Maximum possible score = 2): • (0) – No extension occurs • (1) – Patient can release an active mass flexion grasp • (2) – Full active extension (compared to unaffected side)
	8c. Grasp I: • Patient is sitting with arm on bedside table. • Have patient perform movement with unaffected side first. • Check patient's available PROM on the affected side for	• *Scoring* (Maximum possible score = 2): • (0) – Required position cannot be attained • (1) – Grasp is weak

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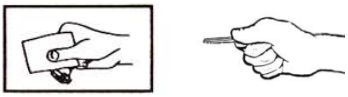
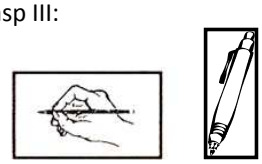
	this motion. • Instruct the patient to extend the metacarpophalangeal joints of digits II-V and flex the proximal & distal interphalangeal joints. Test this grip against resistance. You can tell the patient "pretend you are holding the handle of a briefcase." • Test 3x on the affected side and score best movement.	• (2) – Grasp can be maintained against relatively great resistance
	8d. Grasp II:  • Patient is sitting with arm on bedside table. • Have patient perform movement with unaffected side first. • Instruct the patient to abduct the thumb to grasp a piece of paper. Then ask the patient to perform pure thumb adduction with the scrap of paper interposed between the thumb and first digit (as in figure). Test this grip against resistance by asking the patient to hold as you attempt to pull the paper out with a slight tug. • Test 3x on the affected side and score best movement.	• *Scoring* (Maximum possible score = 2): • (0) – Function cannot be performed • (1) – Scrap of paper interposed between the thumb and index finger can be kept in place, but not against a slight tug • (2) – Paper is held firmly against a tug
	8e. Grasp III:  • Patient is sitting with arm on bedside table. • Have patient perform movement with unaffected side first. • Instruct the patient to grasp a pen or pencil by opposing the thumb and index finger pads around the pen. The tester may support the patient's arm but may not assist with the hand function required for the retrieval task. The pen may not be stabilized by the therapist or the patient's other hand. To minimize excessive movement, however, a pen with a 'pocket clip' that prevents rolling more than 180° may be used. • Once the pencil is retrieved, instruct the patient to oppose the thumb pad against the pad of the index finger with a pencil interposed. Test this grip against resistance by asking the patient to hold as you attempt to pull the pencil out with a slight tug upwards. • Test 3x on the affected side and score best movement.	• *Scoring* (Maximum possible score = 2): • (0) – Function cannot be performed • (1) – A pencil interposed between the thumb pad and the pad of the index finger can be kept in place, but not against a slight tug • (2) – Pencil is held firmly against a tug
	8f. Grasp IV:  • Patient is sitting with arm on bedside table.	• *Scoring* (Maximum possible score = 2): • (0) Function cannot be performed • (1) – A can interposed between the thumb and

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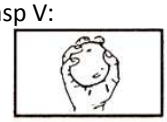
	• Have patient perform movement with unaffected side first. • Instruct the patient to grasp a small can (placed upright on a table without stabilization) by opening the fingers and opposing the volar surfaces of the thumb and digits. The arm may be supported but the tester may not assist with hand function. • Once the can is grasped, test this grip against resistance by asking the patient to hold as you attempt to pull the can out with a slight tug. Test 3x on the affected side and score best movement.	index finger can be kept in place, but not against a slight tug • (2) – Can is held firmly against a tug • NOTE: the hand must open and close on the can; it is not acceptable to have the patient grasp can by coming down from the top of the can.
	8g. Grasp V:   • Patient is sitting with arm on bedside table. • Have patient perform movement with unaffected side first. • Instruct the patient to perform a spherical grasp by grasping a tennis ball The tester may support the patient's arm but may not assist with the hand function required for the retrieval task. The ball may not be stabilized by the therapist or the patient's other hand. To minimize excessive movement, the ball can be placed on an object that reduces rolling. An inverted medium-sized bottle cap placed under the ball to prevent rolling is acceptable. Once the tennis ball is grasped, test this grip against resistance by asking the patient to hold as you attempt to pull the ball out with a slight tug. • Test 3x on the affected side and score best movement.	• *Scoring (*Maximum possible score = 2): • (0) Function cannot be performed • (1) – A tennis ball can be kept in place with a spherical grasp, but not against a slight tug • (2) – Tennis ball is held firmly against a tug
IX. Coordination and speed - Sitting: Finger to nose (5 repetitions in rapid succession)	• Patient positioned in sitting with eyes open. • Starting position is with hand on lap. • Have patient perform movement with unaffected side first. • Check patient's available PROM on the affected side for this motion. • Patient is instructed to "bring your finger from your knee to your nose, as fast as possible." • Use a stopwatch to time how long it takes the patient to do 5 repetitions. • Repeat the same movement with the affected arm. Record the time for both the unaffected and affected sides. Observe for evidence of tremor or dysmetria during the movement. • NOTE: This item attempts to discriminate between basal ganglia, thalamic, or cerebellar strokes in which tremor or dysmetria may result as a direct result of lesion to these areas. The majority of stroke cases are	• *Scoring Tremor* (Maximum possible score = 2): • (0) - Marked tremor • (1) – Slight tremor • (2) – No tremor • *Scoring Dysmetria* (Maximum possible score = 2): • (0)- Pronounced or unsystematic dysmetria • (1) – Slight or systematic dysmetria • (2) – No dysmetria • *Scoring Speed* (Maximum possible score = 2): • (0) – Activity is more than 6 seconds longer than unaffected hand • (1) – (2-5.9) seconds longer

	in the middle cerebral artery or basilar artery where we expect to observe paralysis that affects movement speed but does not cause tremor or dysmetria. In cases of complete paralysis, observe for any indication of tremor or dysmetria that may be evident in face, voice, arms or legs. If there are no indicators of tremor or dysmetria, then score these items 2 and score speed 0. If active ROM of affected limb is significantly less than that of affected limb, patient should be scored "0" for speed.	than unaffected side • (2) – less than 2 seconds difference

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Fugl Meyer Sensory Assessment		

Light Touch	**Procedure:**
	• For light touch assessment, area of skin to be touched, should be free of clothing and exposed.
	• The procedure can be tested in the sitting or supine positions. Explain to the patient with their eyes open, "I am going to touch you with this cotton ball and I would like you to tell me if you can feel that you are being touched." Lightly touch patient with cotton ball over the unaffected muscle belly. Ask them, "Can you feel that you are being touched?" This part of the procedure confirms that the patient understands the test.
	• Explain to the patient, "I am going to ask you to close your eyes. Then I am going to touch you with the cotton ball on your right/left (unaffected) side followed by your right/left (affected) side. When I ask you, tell me if you can feel the touch." Ask the patient to close their eyes. Lightly touch unaffected area with cotton ball and ask, "Do you feel this?" Lightly touch affected area with cotton ball and ask "Do you feel this?" If the patient says they feel the touch on both sides, then repeat the procedure by touching first the unaffected side immediately followed by the affected side and ask the following question. "Does 'this' (unaffected area touch) feel the same as 'this' (affected area touch)?" The intent is to determine if there are differences in the characteristics of the touch between the two sides.
	• If the tester is not confident that the patient understands this procedure or that the response is inconsistent, the tester may confirm their impression by using the following procedure. With the eyes closed, touch the patient on the affected side and ask them to point to where they were touched with the unaffected side. If the patient does not recognize that they are being touched, the score would be absent. If they recognize the touch but are not accurate on the localization, the score will be impaired. If they recognize the touch and are accurate on the localization, the score will be intact.
	
	Upper Extremity
	• <u>Upper arm:</u> Follow above procedure by touching patient over the unaffected and affected biceps muscle belly.
	• <u>Palmar surface of the hand</u>: Follow above procedure by touching patient over the unaffected and affected palmar surface of the hand.
		• *Scoring :*
		  • (0) – Absent - If the patient states that he does not feel the touch on the affected side, the score is absent.
		  • (1) – Impaired - If the patient states that he feels the touch on the affected side and the touch does not feel the same between affected and unaffected sides or the response is delayed or unsure, the score is impaired.
		  • (2) – Intact - If the patient states that he feels the touch on the affected side and the touch feels the same between affected and unaffected sides, the score is intact.

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	Lower Extremity • Thigh: Follow above procedure by touching patient over the unaffected and affected thigh of the leg. • Sole of foot: Follow above procedure by touching patient over the unaffected and affected sole of the foot.	

Proprioception
The objective of this test is to determine a consistent response that is accurate and timely. If unsure, the tester can add additional repetitions to determine if a missed response is true sensory loss or an error by the patient due to test length not sensory loss.
	Procedure:
	• Proprioception can be tested in the sitting or supine positions for the upper extremity and in supine for the lower extremity. Start with the unaffected limb. Explain to the patient with their eyes open, "I am going to move your arm. This is up; this is down (demonstrate test). I want you to close your eyes and tell me if I am moving you up or down." Use the hand positions described below for each joint movement.
	• Move the joint through a small range of motion (approximately 10 degrees for the limb joints and 5 degrees for the digit joints of the hand and foot). Move the limb at least 3 times in random directions. If the patient is wrong on any direction, then add several more repetitions to determine if the accuracy is great than 75% (score 2) or 75% or less (score 1).
	• Start with the most proximal limb joint on the unaffected side. Move to the same joint on the affected side. The intent is to determine if there are differences in the perception of proprioception between the two sides. For example, if the patient identifies the movement stimulus with the same accuracy and responsiveness of the unaffected side then the score would be 2. However, if the patient is accurate but responses are delayed or unsure then the score would be 1. (At this point, you could ask the patient if the movement on this side feels the same as the other side). No perception of joint movement is scored 0.
	
	Upper Extremity
	• Shoulder: Therapist supports patient's arm by the medial and lateral epicondyles of the humerus and at the distal ulnar and radius. Have patient look at arm. Move shoulder, saying "This is up. This is down." I am now going to have you close your eyes and I'm going to move your shoulder in either direction. I want you to tell me "up" or "down." Randomly move arm approximately 10 degrees, 4 times (more if needed), keeping track of correct responses.
	• Elbow: Therapist supports patient's arm by the medial and lateral epicondyles and the distal ulnar and radius. Have patient look at elbow. Move elbow, saying "This is up. This is down." I am now going to have you close
		• *Scoring:*
		  • (0) – Absent (no sensation)
		  • (1) – Impaired (inconsistent response or three quarters of answers are correct, but considerable difference in sensation compared with unaffected side)
		  • (2) – Intact (all answers are correct, little or no difference).

	your eyes and I'm going to move your elbow in either direction. I want you to tell me "up" or "down." Randomly move elbow approximately 10 degrees, 4 times (more if needed) keeping track of correct responses. • Wrist: Therapist supports patient's wrist at the distal ulna and radius and the heads of the 2nd and 5th metacarpal. Have patient look at wrist. Move wrist, saying "This is up. This is down." I am now going to have you close your eyes and I'm going to move your wrist in either direction. I want you to tell me "up" or "down." Randomly move wrist approximately 10 degrees, 4 times (more if needed), keeping track of correct responses. • Thumb: Therapist supports patient's thumb proximal to the interphalangeal joint and either side of the most distal aspect of the thumb. Have patient look at thumb. Move thumb at interphalangeal joint, saying "This is up. This is down." I am now going to have you close your eyes and I'm going to move your thumb in either direction. I want you to tell me "up" or "down." Randomly move thumb approximately 10 degrees, 4 times (more if needed), keeping track of correct responses. **Lower Extremity** • The hip and knee should be tested in the supine position. The ankle and toe can be tested in the supine or sitting position. • Hip: Therapist supports patient's leg at the femoral condyles and the medial and lateral malleolus. Have patient look at leg. Move hip, saying "This is up. This is down." I am now going to have you close your eyes and I'm going to move your hip in either direction. I want you to tell me "up" or "down." Randomly move hip approximately 10 degrees, 4 times (more if needed), keeping track of correct responses. • Knee: Therapist supports patient's leg at the femoral condyles and the medial and lateral malleolus. Have patient look at knee. Move knee, saying "This is up. This is down." I am now going to have you close your eyes and I'm going to move your knee in either direction. I want you to tell me "up" or "down." Randomly move knee approximately 10 degrees, 4 times (more if needed), keeping track of correct responses. • Ankle: Therapist supports patient's leg at the medial and lateral malleoli and the heads of the 1st and 5th metatarsal. Have patient look at ankle. Move ankle, saying "This is up. This is down." I am now going to	

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	have you close your eyes and I'm going to move your ankle in either direction. I want you to tell me "up" or "down." Randomly move ankle approximately 10 degrees, 4 times (more if needed), keeping track of correct responses. • Toe: Therapist supports patient's toe at the interphalangeal joint and either side of the most distal aspect of the great toe. Have patient look at great toe. Move interphalangeal joint, saying "This is up. This is down." I am now going to have you close your eyes and I'm going to move your big toe in either direction. I want you to tell me "up" or "down." Randomly move great toe approximately 10 degrees, 4 times (more if needed), keeping track of correct responses.	

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APPENDIX B
FUGL-MEYER ASSESSMENT OF PHYSICAL PERFORMANCE

Motor Function Upper Extremity				
TEST	ITEM	SCORE		SCORING CRITERIA
		Pre	Post	
I. Reflexes	Biceps			0-No reflex activity can be elicited
	Triceps			2-Reflex activity can be elicited
II. Flexor Synergy	Elevation			0-Cannot be performed at all
	Shoulder retraction			1-Performed partly
	Abduction (at least 90^0)			2-Performed faultlessly
	External rotation			
	Elbow flexion			
	Forearm supination			
III. Extensor Synergy	Shoulder add./int. rot.			0-Cannot be performed at all
	Elbow extension			1-Performed partly
	Forearm pronation			2-Performed faultlessly
IV. Movement combining synergies	Hand to lumbar spine			0-No specific action performed 1-Hand must pass anterior superior iliac spine 2-Performed faultlessly
	Shoulder flexion to 90^0, elbow at 0^0			0-Arm is immediately abducted, or elbow flexes at start of motion 1-Abduction or elbow flexion occurs in later phase of motion 2-Performed faultlessly
	Pronation/supination of forearm with elbow at 90^0 & shoulder at 0^0			0-Correct position of shoulder and elbow cannot be attained, and/or pronation or supination cannot be performed at all 1-Active pronation or supination can be performed even within a limited range of motion, and at the same time the shoulder and elbow are correctly positioned 2-Complete pronation and supination with correct positions at elbow and shoulder
V. Movement out of synergy	Shoulder abduction to 90^0, elbow at 0^0, and forearm pronated			0-Initial elbow flexion occurs, or any deviation from pronated forearm occurs 1-Motion can be performed partly, or, if during motion, elbow is flexed, or forearm cannot be kept in pronation 2-Performed faultlessly
	Shoulder flexion 90-180^0, elbow at 0^0, and forearm in mid-position			0-*Initial* flexion of elbow or shoulder abduction occurs 1-Elbow flexion or shoulder abduction occurs during shoulder flexion 2- Performed faultlessly
	Pronation/supination of forearm, elbow at 0^0 and shoulder between 30-90^0 of flexion			0-Supination and pronation cannot be performed at all, or elbow and shoulder positions cannot be attained 1-Elbow and shoulder properly positioned and pronation and supination performed in a limited range 2-Performed faultlessly
VI. Normal reflex activity	Biceps and/or finger flexors and triceps (This item is only included if the patient achieves a maximum score on all previous items, otherwise score 0)			0-At least 2 of the 3 phasic reflexes are markedly hyperactive 1-One reflex is markedly hyperactive, or at least 2 reflexes are lively 2-No more than one reflex is lively and none are hyperactive

TEST	ITEM	SCORE		SCORING CRITERIA
VII. Wrist	Stability, elbow at 90^0, shoulder at 0^0			0-Patient cannot dorsiflex wrist to required 15^0 1-Dorsiflexion is accomplished, but no resistance is taken 2-Position can be maintained with some (slight) resistance
	Flexion/extension, elbow at 90^0, shoulder at 0^0			0-Volitional movement does not occur 1-Patient cannot actively move the wrist joint throughout the total ROM 2-Faultless, smooth movement
	Stability, elbow at 0^0, shoulder at 30^0			0-Patient cannot dorsiflex wrist to required 15^0 1-Dorsiflexion is accomplished, but no resistance is taken 2-Position can be maintained with some (slight) resistance
	Flexion/extension, elbow at 0^0, shoulder at 30^0			0-Volitional movement does not occur 1-Patient cannot actively move the wrist joint throughout the total ROM 2-Faultless, smooth movement
	Circumduction			0-Cannot be performed 1-Jerky motion or incomplete circumduction 2-Complete motion with smoothness
VIII. Hand	Finger mass flexion			0-No flexion occurs 1-Some flexion, but not full motion 2-Complete active flexion (compared with unaffected hand)
	Finger mass extension			0-No extension occurs 1-Patient can release an active mass flexion grasp 2-Full active extension
	Grasp I - MCP joints extended and proximal & distal IP joints are flexed; grasp is tested against resistance			0-Required position cannot be acquired 1-Grasp is weak 2-Grasp can be maintained against relatively great resistance
	Grasp II - Patient is instructed to adduct thumb, with a scrap of paper inter-posed			0-Function cannot be performed 1-Scrap of paper interposed between the thumb and index finger can be kept in place, but not against a slight tug 2-Paper is held firmly against a tug
	Grasp III - Patient opposes thumb pad against the pad of index finger, with a pencil interposed			0-Function cannot be performed 1-Pencil interposed between the thumb and index finger can be kept in place, but not against a slight tug 2-Pencil is held firmly against a tug
	Grasp IV - The patient should grasp a can by oppos-ing the volar surfaces of the 1st and 2nd digits.			0-Function cannot be performed 1-A can interposed between the thumb and index finger can be kept in place, but not against a slight tug 2-Can is held firmly against a tug
	Grasp V - The patient grasps a tennis ball with a spherical grip or is instructed to place his/her fingers in a position with abduction position of the thumb and abduction flexion of the 2nd, 3rd, 4th & 5th fingers			0-Function cannot be performed 1-A tennis ball can be kept in place with a spherical grasp but not against a slight tug 2-Tennis ball is held firmly against a tug
IX.Coordination/ Speed- Finger from knee to nose (5 repetitions in rapid succession)	Tremor			0-Marked tremor 1-Slight tremor 2-No tremor
	Dysmetria			0-Pronounced or unsystematic dysmetria 1-Slight or systematic dysmetria 2-No dysmetria
	Speed			0-Activity is more than 6 seconds longer than unaffected hand 1-(2-5.9) seconds longer than unaffected hand 2-Less than 2 seconds difference
Upper Extremity Total				**Maximum = 66**

Motor Function - Lower Extremity				
TEST	ITEM	SCORE		SCORING CRITERIA
		Pre	Post	
I. Reflex Activity	Achilles			0-No reflex activity can be elicited 2-Reflex activity can be elicited
	Patellar			
II. A. Flexor Synergy (in supine)	Hip flexion			0-Cannot be performed at all 1-Partial motion 2-Full motion
	Knee flexion			
	Ankle dorsiflexion			
II. B. Extensor Synergy (in side lying)	Hip extension			0-Cannot be performed at all 1-Partial motion 2-Full motion
	Adduction			
	Knee extension			
	Ankle plantar flexion			
III. Movement combining synergies (sitting: knees free of chair)	A. Knee flexion beyond 90°			0-No active motion 1-From slightly extended position, knee can be flexed, but not beyond 90° 2- Knee flexion beyond 90°
	B. Ankle dorsiflexion			0-No active flexion 1-Incomplete active flexion 2-Normal dorsiflexion
IV. Movement out of synergy (standing, hip at 0°)	A. Knee flexion			0-Knee cannot flex without hip flexion 1-Knee begins flexion without hip flexion, but does not reach to 90°, or hip flexes during motion 2-Full motion as described
	B. Ankle dorsiflexion			0-No active motion 1-Partial motion 2-Full motion
V. Normal Reflexes (sitting)	Knee flexors Patellar Achilles (This item is only included if the patient achieves a maximum score on all previous items, otherwise score 0)			0-At least 2 of the 3 phasic reflexes are markedly hyperactive 1-One reflex is markedly hyperactive, or at least 2 reflexes are lively 2-No more than one reflex is lively and none are hyperactive
VI. Coordination/speed - Sitting: Heel to opposite knee (5 repetitions in rapid succession)	A. Tremor			0-Marked tremor 1-Slight tremor 2-No tremor
	B. Dysmetria			0-Pronounced or unsystematic dysmetria 1-Slight or systematic dysmetria 2- No dysmetria
	C. Speed			0-Activity is more than 6 seconds longer than unaffected side 1-(2-5.9) seconds longer than unaffected side 2-Less than 2 seconds difference
Lower Extremity Total				Max = 34
Total Motor Score (UE + LE)				Max = 100

Sensation				
TYPE OF SENSATION	AREA	SCORE		SCORING CRITERIA
		Pre	Post	
I. Light Touch	Upper Arm			0-Anesthesia 1-Hyperesthesia / dysesthesia 2-Normal
	Palm of Hand			
	Thigh			
	Sole of Foot			
II. Proprioception	Shoulder			0-No Sensation 1-75% of answers are correct, but considerable difference in sensation relative to unaffected side 2- All answers are correct, little or no difference
	Elbow			
	Wrist			
	Thumb			
	Hip			
	Knee			
	Ankle			
	Toe			
Total Sensation Score				Maximum = 24
Total Motor and Sensory Score				Maximum = 124
Comments	**Pre:**			
	Post:			