

STUDY PROTOCOL

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Multimodal Imaging of Neuroplasticity in Upper Limb (UL) Recovery with Early Increased UL Practice after Stroke

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STUDY PROTOCOL

1. BACKGROUND AND RATIONALE

1.1. General Introduction

Stroke is a significant cause of disability and the overall fourth-highest cause of disease burden in Singapore. Crude incidence of stroke in Singapore has increased from 187.9 (2008) to 196.7 (2012) per 100 000 population¹. The prevalence of stroke events is expected to increase worldwide due to the increase in the population aged above 65 years old.

The mean annual direct medical cost of stroke in Singapore is S\$12473.7, of which 93.6% of the cost comes from inpatient hospitalization. This amount is substantially higher than other prevalent inpatient hospitalization and other Asian countries. It does not account for outpatient rehabilitation services and direct non-medical cost such as out-of-pocket expenses and loss of work. It is important to bear in mind that the economic impact of stroke goes beyond acute care; it extends to costly prolonged institutional chronic care and rehabilitation. Therefore, it is important to maximize recovery after stroke to reduce disability and burden of care on individuals, within Singapore and worldwide.

Upper limb (UL) recovery after stroke has been found to be limited. Singapore data has revealed that less than one-third of the stroke patients regained UL dexterity one year after their strokes. The remaining two-third of the stroke population had to live and cope with enormous losses associated with their UL impairments for the rest of their lives. In view of the importance of hand dexterity for the performance of daily functional tasks, rehabilitation efforts should be channelled into facilitating UL recovery after stroke.

The effects of rehabilitation on UL recovery after stroke appear to be more pronounced in the first six months post-stroke. It appears that there might be an optimal time window for gaining favourable rehabilitation outcome post-stroke.

During this optimal time window, the use and the practice of the affected UL influence neuroplasticity and facilitate UL recovery after stroke. On the other hand, the lack of affected UL use leads to the “learned non-use” phenomenon and the development of compensatory strategies (i.e. overuse of non-affected UL), which interfere and hinder the neuroplasticity process for recovery. Therefore, the use and practice of the affected UL at the early phase post-stroke is crucial and important.

This can also be seen from the results of early intervention studies. These studies have shown that additional early UL practice resulted in better UL functional outcomes when compared with conventional/ traditional therapy alone. For patients with less severe UL impairment, early increased UL practice appeared to have pushed them to a higher level of UL functional outcomes. This higher level of UL functional outcomes was maintained even up to 9 to 12months post-stroke.

Gap in the Knowledge of Optimal Effective Early UL Practice

For early increased UL practice, “more” might not necessary mean better as seen in the early constrained-induced movement therapy (CIMT) study results. In these studies, lower intensity CIMT intervention was found to be more beneficial when compared with higher intensity CIMT intervention during the early post-stroke phase. A possible explanation for this result was that the subjects in the higher intensity group might have experienced fatigue. In addition, the requirement of the higher intensity UL practice might have resulted in “blocked” practice schedule rather than the desired “distributed” practice schedule throughout the day. Distributed learning schedules have been found to lead to better motor task learning and performance. It appeared that early increased UL practice after stroke might be more effective if it is incorporated into affected UL practice and use distributed throughout the day rather than concentrating all the intensive UL practice within one therapy session in a day.

To date, there are still gaps in the knowledge of the optimal dosage intensity, optimal timeline to commence

UL practice and optimal effective mode of early UL practice after stroke.

Gap in the Knowledge of Neuroplasticity During Early Post-Stroke

To fill the gaps in knowledge of early UL practice, the understanding of the neuro-biological mechanism of recovery after stroke might be the key to answer these questions. In fact, it has been found that early increased UL practice does not necessarily mean better outcomes in neuroplasticity. In studies of rats, it was found that forced overuse of the affected limb 7 days (by casting the unaffected limb) after an induced stroke actually caused an expansion of the neural injury and interference in the restoration of affected limb function. Although it is always important to interpret results of animal studies with caution, the results show that there is a need for a greater understanding of the neuroplasticity mechanism behind early UL increased practice, otherwise we might possibly do more harm than good to stroke patients.

There is still a lack of studies investigating the neuroplastic reorganisation of increased UL practice during early post-stroke. Only one study has been found, thus far, on investigating the effect of early increased UL practice on neuroplasticity after stroke in humans. In this recent study by Hubbard et al, fMRI (functional magnetic resonance imaging) showed that stroke patients in the early increased training group showed increased activation in ipsilesional anterior cingulate and supplementary motor areas, and reduced activation in contralateral cerebellum, when compared to the standard care group. The increased activation of ipsilesional anterior cingulate and supplementary motor areas in the early increased training group was associated with early motor learning and attention, and the reduction in the activation of the contralateral cerebellum was interpreted as constituting better recovery in stroke patients.

Most studies investigated the effect of additional UL practice on neuroplasticity on patients of more than 3-months post-stroke and patients of chronic stroke (>6-months post-stroke). These studies did not include control groups of stroke patients without UL additional practice to compare their outcomes. If there was a control group in these studies, the controls were normal healthy subjects.

The results of these studies showed that for stroke patients who had undergone a period of increased UL practice, there was a trend towards a decreased activation of contralateral motor area and supplementary motor areas and an increased activation in the ipsilesional motor area. This trend was approaching towards the activation pattern of normal healthy controls in their studies. However for stroke patients who had lower UL baseline motor capability, the gain in UL recovery after UL practice corresponded to an increase in both the ipsilesional and contralateral motor areas and supplementary motor areas. The neural reorganisation after increased UL practice after stroke varied across different individuals.

The complexity of neural reorganisation and the lack of studies on early increased UL practice on the neuroplasticity mechanism after stroke cause this important scientific area to be poorly understood. The understanding of this scientific gap is important because most rehabilitations occur early post-stroke and it is during this period that UL training can interact to support spontaneous recovery for optimal functional outcomes for patients.

Importance of Identification of Potential Imaging Biomarkers for Recovery

Another key to understanding the neurobiological mechanism of recovery is to identify potential biomarkers for recovery at early post-stroke and its interaction with UL practice. In a study by Marshall et al, it showed a correlation between the results of early task-related fMRI with the motor recovery. Marshall et al suggested that the recovery related activation pattern justified the investigation on early intervention to enhance the recovery process. In a study by Yin et al, it was reported that a biomarker can potentially be identified for UL recovery after stroke using volume-based measurement and diffusion tensor imaging (DTI) based FA (Fractional Anisotropy) analysis.

In another study, Bylow used transcranial magnetic stimulation (TMS) and diffusion weighted image (DWI) and linear regression to predict the increase of UL Fugl-Meyer (FM) score with standardized and variable dosage of therapy in his study. The study showed that UL recovery could be predicted by presence of motor evoked potentials (MEP), FA analysis of the corticospinal tract but was insensitive to the dosage of UL practice. Deeper analysis of the study revealed that the variable therapy dosage which was defined as duration of time was not controlled in this study. To draw conclusion that therapy dose does not affect UL recovery might be premature. However, this showed that it is important to consider the interaction of both the neurobiology conditions and UL training in post-stroke recovery.

A potential biomarker to investigate in this study will be FA of the corticospinal tract and its interaction with increased UL practice during early post-stroke. FA of the corticospinal tract at early post-stroke has been found to correlate to later stage UL recovery. Interestingly, FA of the corticospinal tract has also been shown to increase significantly after training. Another potential biomarker to investigate in this study will be resting state fMRI, based on a previous A*STAR study which showed that individual gain in FM scores over 12 weeks could be predicted from the resting state fMRI.

Loss of Opportune Time During Early Post-Stroke To Influence Neuroplasticity

A recent systematic review of stroke patients in hospitals has revealed that the mean duration of UL activity training during a physiotherapy (PT) session was 4.1 minutes to 5.7 minutes and 17 minutes during an occupational therapy (OT) session during the acute phase. The duration and repetitions in each UL training session in clinical practice are far below what are required for to effect a cortical re-organization in motor skill learning. With further reduction in manpower in healthcare due to rising healthcare needs, implies that employing more therapist manpower to increase current UL practice after stroke will be even more challenging.

It has been found that stroke patients spent most of their time in the hospital ward being inactive. Studies have shown that the use and practice of affected UL was also minimal in the ward. Bernhardt et al (2007) found that stroke patients only used their affected UL 1% out of the activities of the day in the ward. The time during which the stroke patients spend in the ward is an opportune time for them to increase their affected UL practice and use. It also captures the optimal time window for neuroplasticity, and thus should be fully utilised and not be wasted.

The findings mentioned above showed that implementing therapy to increase UL practice in real clinical world is a challenge. The answers to the questions of neuroplasticity of early UL practice early post-stroke will only be meaningful if we can develop innovative ways to facilitate affected UL practice throughout the day in the ward to achieve the desired neuroplasticity for optimal recovery after stroke, even with reduced manpower. Hence, the purpose of this study is to develop and evaluate a new and innovative way of enhancing therapy to increase UL practice early post-stroke in the ward.

Background on Preliminary Data/ Results

Patients can be empowered to take ownership of their practice and recovery. This can be seen in a multi-site single blinded randomized controlled trial of GRASP (Graded Repetitive Arm Supplementary Program). GRASP is a self-administered graded program that incorporate additional complementary UL practice in the ward (on top of regular therapy sessions) by the patients without the direct involvement of therapists during practice. This is made feasible by giving stroke patients and their caregiver instruction booklets (based on the level of the affected UL function) and teaching them on the self-administered exercises found in the booklet. This is followed by regular and consistent reviews by therapists to check and progress the exercises. The result of this trial showed that patients in GRASP group achieved better UL outcomes post treatment than the control. In addition, the gains in the GRASP group were sustained 5 months after the program. The program has also utilized an important source of manpower – patient and their caregiver, which has often been underutilized or sometimes even neglected.

However, GRASP does not include the component of affected UL daily functional use. Affected UL daily functional use is important because from clinical experience, stroke patients often confine the practice of their affected UL to just during therapy sessions, as most of them do not know how to use their affected UL after stroke, especially for those with lower UL motor function. Reduced affected UL use perpetuates even to chronic phase as they have not learned how to incorporate the use of their affected UL in daily life, resulting in the phenomenon of “learned non-use”.

In this new proposed program, ***Self-empowered Upper Limb Repetitive Engagement (SURE)*** program, specific instructions and guidance according to the level of their affected UL motor function will be given to guide and increase affected UL daily functional use in stroke patients in the ward. In addition, prescribed specific exercise according to the level of their affected UL motor function will also be given and guided to facilitate patients to exercise by themselves in the ward. The activity of the affected UL will be objectively and accurately monitored, reviewed and feedback to the patients to facilitate affected UL use and practice using accelerometers. Accelerometry has been found to be valid and reliable in the measurement of UL use in

stroke patients.

To date, there is no therapy program that has specifically and systematically been introduced in Singapore to facilitate UL practice and use of the stroke patients in the ward. Taking into consideration the challenge of increased UL practice during early post-stroke in the real clinical world, it is, therefore, important to investigate the feasibility and effectiveness of **SURE** program and the potential imaging biomarkers to optimize the neuro-rehab

1.2. Rationale and justification for the Study

a. Rationale for the Study Purpose

The recovery of affected UL is important both functionally and psychologically for the stroke patients. Less than one-third of the stroke population has return of dexterity of affected UL after stroke. More can be and should be done to facilitate recovery of affected UL after stroke.

Early increase UL practice has been shown to improve UL recovery. However, there are still gaps in the knowledge of optimal dosage, optimal timeline of commencement and efficacious mode of UL practice. The understanding of neuroplasticity and identification of potential imaging biomarkers at early post-stroke stage might be the key to answer these questions in early UL practice.

Stroke patients can be empowered to take ownership of their practice and recovery. The practice of affected UL in the ward and the patients/ their caregivers are underutilized resources that can be tapped into to increase UL practice. **SURE** program is a new and innovative program that empower stroke patients to increase UL practice in the ward early post-stroke to influence neuroplasticity and improve functional outcomes.

Therefore, the aims of this study are:

- 1) To investigate the feasibility and effectiveness of **SURE** program to increase UL practice in the ward and improve UL functional outcomes after stroke. To correlate the imaging biomarkers with the neuro-recovery outcomes under **SURE** and controlled program.
- 2) To investigate the neuroplasticity in the brain through multiple neuro-imaging analysis (fMRI/MRI/DTI), with the goal to develop imaging biomarkers for image-guided neuro-rehabilitation framework so as to optimize early UL practice after stroke.

b. Rationale for Doses Selected

The subjects will perform the self-exercises for 60 minutes (3x 10-20minutes) and functional tasks (as and when is required in the daily function) per day for 6 days per week in the ward throughout the 4 weeks intervention period. These exercises are performed in addition to daily therapy. Additional period of self exercises and affected UL's functional use are performed in the ward to increase the dosage of affected UL activity as studies have found that amount of affected UL activity during therapy session are limited.

c. Rationale for Study Population

20 eligible stroke subjects with positive motor evoked potential (MEP +ve) and with severe to moderate UL paresis (ULFM score below or equal to 50) admitted to TTSRHC will be selected. Subjects with severe and moderate UL paresis are selected because based on clinical observation subjects with this level severity require intensive UL practice to improve their UL functions, while subjects with mild paresis do not require such high intensity of UL practice for significant improvement. Investigation of motor evoked potential on extensor carpi radialis on the affected hand will be performed on stroke patients at NUH using a single Magstim 200 to prognosticate recovery. Subjects with MEP +ve will be selected to ensure that all the subjects chosen in this study have intact ipsilesional cortical spinal tract function to make comparison of UL

recovery more consistent.

d. Rationale for Study Design

Randomized blinded controlled trial study design has been selected for this study to investigate whether if **SURE** program improves UL functional outcome and influences neuroplasticity positively to optimize UL recovery after stroke. This study design is chosen to prevent any bias in the assessment of outcomes.

2. HYPOTHESIS AND OBJECTIVES

2.1. Hypothesis

1. **SURE** program increases UL practice in the ward and improves UL functional outcomes post-stroke
2. Increased UL practice early post-stroke period influences neuroplasticity positively to optimize UL recovery
3. Imaging biomarkers are associated with rehab-methods and recovery of UL post-stroke

2.2. Primary Objectives

To investigate the effectiveness of SURE program to increase UL practice in the ward and improve UL functional outcomes after stroke. To correlate the imaging biomarkers with the neuro-recovery outcomes under SURE and controlled program.

2.3. Secondary Objectives

To investigate the neuroplasticity in the brain through multiple neuro-imaging analysis (fMRI/MRI/DTI), with the goal to develop imaging biomarkers for image-guided neuro-rehabilitation framework so as to optimize early UL practice after stroke.

2.4. Potential Risks and benefits:

a. End Points - Efficacy

SURE program improves UL functional outcome and influences neuroplasticity positively to optimize UL recovery after stroke.

b. End Points - Safety

SURE Program

Subjects might experience fatigue at the early part of the exercise program.

The CPI will tailor and review subjects' exercises and the use of stroke-affected hand in daily tasks according subjects' stroke-affected hand's capability.

The CPI will consult the treating rehabilitation team (including doctors, therapists, nurses) on the subjects' medical and neurological stability before the commencement of teaching the exercises and each review.

First session of teaching exercises

CPI will pause, observe and ask each subject after teaching each exercise if they are tired. If they are tired, the CPI will stop teaching and let the subjects rest until they are not tired and ready to learn the next exercise. If the subjects are observed or express tiredness, the CPI will stop teaching the exercises for that session. Subjects will be advised to stop doing their exercises if they feel tired or rest until they feel ready to continue to do the exercises. The caregivers who are assisting the subjects with self-exercises will be given the similar advice. Subjects will also be asked to record any pain or fatigue that they experience during performance of self-exercises in their Exercise Log.

The next day after the first session

CPI will check the subjects' performance of their exercises and their level of tiredness, and also to reinforce the advice to rest if they are feeling tired.

Subsequent reviews

CPI will check the subjects' performance of their exercises and their level of tiredness, and also to reinforce the advice to rest if they are feeling tired.

Doctors, therapists and nurses who are taking care of the subjects in TTSRHC will be informed of the subjects' participation in SURE program and will be kindly requested to keep a look out of any verbal and non-verbal expressions of fatigue by the subjects. They will be informed to give similar advice by CPI to stop exercise and rest before continually or stop the exercises entirely if the subjects are too tired on that particular day. CPI will be informed by the treating team of their findings and will continue to monitor the subjects' suitability to continue with SURE program if there are reports of persistent fatigue due to self-exercises in the ward. Additional monitoring of fatigue level will be performed at pre, 2 weeks of intervention, post-intervention assessment using the Stanford Fatigue Scale.

A possible minimal risk is the development of allergy to the straps of the sensors. However, many research studies had used the sensors and there have been no reports of any adverse allergic reactions or problems reported with wearing the sensors.

Transcranial Magnetic Stimulation (TMS) Screening

TMS safety parameters have been documented and stimulation guidelines have been established (Wasserman, 1998). Seizures are the most important potential adverse event but are extremely rare. Mild headache, neck pain and discomfort from facial twitches are the most common and resolve spontaneously shortly after stimulation.

Exclusion criteria include pregnancy, presence of cardiac pacemakers, orthodontics (braces), metal implant and intracranial implants such as ventriculoperitoneal shunts and clips.

Functional Magnetic Resonance Imaging Investigation

Magnetic Resonance Imaging use radio waves similar to those used in radio and TV transmission. These have a much lower energy than X rays and are considered biologically safe. We follow strict safety guidelines which are designed to prevent the possible hazards of MRI: burns and electric shocks. The main risk in MRI is injury or death arising from the action of the strong magnetic field on any metal objects on or within the body. This may result in flying metal objects or damage to tissue. Strict screening procedures to remove external metal objects and exclude unsuitable volunteers will be carried out.

Exclusion criteria include prior history of central nervous system disorders, significant head trauma, structural brain lesions and psychiatric disturbance. In addition, subjects shall be excluded if they have pacemakers, metallic foreign bodies in the brain or eye, claustrophobia, tattoo, work with metal, or are pregnant.

Other potential issues include (i) Excessive heating of tissue due to absorption of RF energy, (ii) peripheral nerve stimulation (PNS) that may cause an uncomfortable but not harmful tingling sensation in the limbs and (iii) acoustic noise generated during the scanning. Excess power deposition will be avoided by hardware and software controls that limit this to well within FDA limits of 3.2W/kg, and the scanner has in-built warnings that let the operator know when a given sequence may cause PNS, so that he/she may adjust the sequence accordingly. Such accidents have never occurred at CIRC and have only very rarely occurred elsewhere in the world.

3. STUDY POPULATION

3.1. List the number of subjects to be enrolled.

20 subjects will be randomly assigned to a control (N=10) and experimental group (N=10). 4 normal healthy subjects will also be recruited for fMRI investigations.

3.2. Criteria for Recruitment

CPI will identify patients that will be admitted to TTSHRC based on their medical records. CPI will then approach the patients' therapist to confirm their eligibility for the recruitment to the study.

3.3. Inclusion Criteria

All patient subjects have to fit in the following Inclusion criteria to be included in the study: diagnosis of stroke due to infarct resulting in UL hemiparesis (with confirmation from MRI and Computed axial tomography (CT) scans); and admitted within first 21days post-stroke in TTSHRC with Montreal Cognitive Assessment (MoCA) score ≥ 19 . In addition, stroke subjects with positive motor evoked potential (MEP +ve) and with severe to moderate UL paresis (Upper Limb Fugl Meyer (ULFM) score below or equal to 50) admitted to TTSHRC will be selected. Subjects with severe and moderate UL paresis are selected because based on clinical observation subjects with this level severity require intensive UL practice to improve their UL functions, while subjects with mild paresis do not require such high intensity of UL practice for significant improvement. Investigation of motor evoked potential on extensor carpi radialis on the affected hand will be performed in NUH on stroke patients using a single Magstim 200 to prognosticate recovery. Subjects with MEP +ve will be selected to ensure that all the subjects chosen in this study have intact ipsilesional cortical spinal tract function to make comparison of UL recovery more consistent.

All normal healthy subjects aged 21 years old and above who are able to give informed consent and follow commands will be recruited.

3.4. Exclusion Criteria

All subjects (includes the normal healthy subjects and patient subjects) meeting any of the following exclusion criteria at baseline will be excluded from participation.

Exclusion criteria for subjects are:

1. recurrent stroke or previous history of brain injury
2. bilateral stroke
3. cardiac disease that limit function by exertional dyspnea, angina or severe fatigue
4. hemiplegic shoulder pain – visual analogue scale (VAS) $>5/10$
5. previous orthopaedic conditions on affected UL such as frozen shoulder and fracture that interfere

with affected UL movement

6. existing peripheral nerve or orthopaedic conditions that interfere with affected UL movement
7. severe aphasia, neglect, agitation, or depression that can limit participation
8. Additional exclusion criteria for TMS screening (Refer to Section 2.4b Endpoints Safety- TMS screening).
9. Additional exclusion criteria for fMRI investigations are inability to follow the fMRI instructions, claustrophobia, and MRI safety concerns (Refer to Section 2.4b Endpoints Safety- fMRI investigations).

3.5. Withdrawal Criteria

Subject will be withdrawn from the study if the subject is unable to tolerate the SURE program

3.6. Subject Replacement

Subjects who drop out will be replaced

4. TRIAL SCHEDULE

	Clinical Assessments (TTSHRC)/ no. of visit	TMS Screening (NUH)/ no. of visit	fMRI Investigations (CIRC@NUS)/no. of visit
Pre-Intervention	1 (Inpatient)	1 (Inpatient)	1 (Inpatient)
Two weeks after start of Intervention	1 (Inpatient)	NIL	NIL
Post-Intervention	1 (In/ Outpatient)	NIL	1 (In/ Outpatient)
One Month Post-Intervention	1 (Outpatient)	NIL	NIL
Three Months Post-Intervention	1 (Outpatient)	NIL	1 (Outpatient)

SURE program intervention will start after pre-intervention TMS screening, clinical assessment and fMRI investigations. It consists of 4 weeks of additional 1 hour daily self-exercise and functional use of affected UL in the hospital ward 6 days a week. The subjects will require to wear an accelerometer on unaffected wrist for 3 weekdays of first week to establish baseline of use, and wear an accelerometer on affected wrist 3 weekdays (8am -8pm each day) per week so that the actual affected UL use can be feedback to subjects during review. Please see attached file: Patient's_Involvement_In_Study_15_April_2017 for summary of each subject's involvement in the study.

5. STUDY DESIGN

Experimental procedure

The subjects will be randomly assigned to a control (N=10) and experimental group (N=10).

Experimental Group

The experimental group will receive one **SURE** program booklet with specific instructions and pictorial illustrations of each exercise and functional use according to their affected ULFM score. There are 3 booklets available (Level 1A for patients with affected ULFM score from 0 to 10; Level 1B for patients with affected

ULFM score from 11 to 22; Level 2 for patients with affected ULM score from 23 to 50). Each **SURE** program booklet consists of stretching exercises, strengthening exercises and performance of simple motor tasks such as reaching, picking up cubes etc. Each exercise will be graded and progressed according to the subjects' affected UL capability by the Clinical Principal Investigator (CPI) who is an experienced principal physiotherapist. The **SURE** program booklet also consists of selected motor tasks to be performed by the subjects using their affected UL. Functional activities such as subjects will be using their affected UL to wipe their faces; wiping the table after eating; applying lotion by the affected hand along the unaffected arm and legs after shower; drinking water from cup/ bottle; lifting and placing affected hand on table top; etc. Each functional motor task will be selected, graded and progressed by the PI according to the subjects' affected UL motor capability during the reviews. The subjects will be taught how to perform the exercises and functional tasks during the first session. The performance of the exercises and functional motor tasks will be reviewed by the CPI three times per week for the first 2 weeks, two times for the third week and one time for the fourth week of intervention period.

The subjects will be asked to perform the exercises 60 minutes (3x 10- 20 minutes) distributed across morning, afternoon, and evening per day for 6 days per week during the 4-week intervention period. They will also be asked to perform the functional tasks as and when is required for their daily function for 6 days per week during the 4-week intervention period. The subjects will be given an Exercise Log to tick and track number of repetitions of the exercises and functional activities each day, the number of days the protocol is completed, and the level of fatigue and pain experienced. If the subject is discharged from the hospital before the 4 weeks of intervention period, he/ she has to carry on with the protocol till the intervention period ends. The CPI will continue to review exercises and the amount of affected UL use with the subjects at home if the subjects are discharged till the intervention period ends.

Control Group

The control group will receive an education booklet with 10 modules. The education booklet will contain information on stroke, recovery and management strategies after stroke. Subjects are to complete 2-3 modules per week and answer 1-2 simple questions after each module. Each module including answering questions takes approximately 5-10 minutes to complete. The CPI will review the information with the subjects 3 times per week for the first 2 weeks, two times for the third week and one time for the fourth week of intervention period.

Both groups will continue with their daily routine therapy sessions in the hospital. The treating therapists will record the duration of therapy of each subject per day.

UL functional outcome measures- ULM and ARAT will be assessed pre-intervention, 2 weeks after start of intervention, post-intervention, 1month and 3months post-intervention. Measures of **SURE** program's feasibility such as VAS pain score, fatigue level, and MAS of biceps and finger flexors will be measured pre-intervention, 2 weeks after start of intervention and post-intervention. A team member who is blinded to which group the subjects are in will perform the outcome measure assessments.

Multimodal imaging for the normal healthy control will be performed once to establish the activation pattern in normal. Multimodal imaging assessment for the patient subjects will be done pre-intervention, post-intervention, and 3months post-intervention.

5.1. Summary of Study Design

The subjects will be randomly assigned to a control (N=10) and experimental group (N=10). The control group will undergo an education program while the experimental group will undergo the **SURE** program. Clinical assessments for both groups will be performed by a blinded assessor at pre, two weeks after start of intervention, immediate post intervention, 1 and 3months post-intervention to determine if the **SURE** program improve affected UL functional outcomes compared with the control group. fMRI investigations will be performed at pre-intervention, post- intervention and 3months post-intervention to determine if the increased UL activity in the **SURE** program influences brain neuroplasticity positively early post-stroke.

6. METHODS AND ASSESSMENTS

Outcome Measures

UL functional outcome measures

UL Fugl Meyer Scale (ULFM)

UL Fugl Meyer Scale will be used to stratify subjects to their level of UL severity and assessment of UL impairment. ULFM score can be subdivided into 3 categories to define the different level of severity of paresis (severe= 0 to 22; moderate=23 to 50; mild>= 51). ULFM will be one of the primary outcome measures to determine UL recovery.

Action Research Arm Test (ARAT)

ARAT will be used as the primary outcome measure of UL functional outcome. ARAT will be one of the primary outcome measures to determine UL recovery.

Measure of UL practice and use

Rating of Everyday Arm-use in the Community and Home (REACH Scale)

The REACH scale is a self-report measure that captures how much an individual with stroke use affected UL outside clinical setting. It has one scale for subjects whose dominant UL is affected and another scale for subjects whose non-dominant UL is affected. It will be used to rate patients' affected UL use after discharge.

Measure of UL practice and use using accelerometry

One tri-axial accelerometer (ActiGraph GT3X+, ActiGraph LLC, Pensacola, Florida) will be placed on subject's unaffected wrist to establish the duration of unaffected UL use in the ward for 3 days in the first week. Another tri-axial accelerometer will be placed on subject's affected wrist for 3 weekdays each week of the 4-week intervention period to assess the amount of affected UL use. The accelerometers will be worn by all subjects (both experimental and control groups) from 8am to 8 pm on the day of donning.

Measures of feasibility of accelerometry in measuring affected UL use

The compliance rate of wearing the accelerometers during the 4 weeks of intervention period will be used to assess compliance. Subjects will also be asked to fill a questionnaire to rate the acceptance, ease, comfort, discomfort, benefits, and problems of wearing the accelerometers in the ward.

*Measures of **SURE** program's feasibility*

The key measure of **SURE** program's feasibility will be the additional affected UL practice and use per day as measured by the accelerometers.

Subjects' fatigue levels will be assessed using 0-10 point Stanford Fatigue Visual Numeric Scale (Stanford Patient Education Research Centre, n.d). Subjects' level of affected UL pain will be assessed using 0-10 rating Visual Analogue Scale (VAS). Subjects' UL spasticity level for biceps and finger flexors will be assessed using Modified Ashworth Scale (MAS). These assessments will be performed pre, 2 weeks in training, and post-training.

SURE program feasibility is also assessed by the compliance rate, dropout rate and any adverse events reported during the intervention period.

Subjects and their caregiver will be asked to fill up a questionnaire to rate on **SURE** program's ease, benefit and overall satisfaction based on VAS (rating 0 -10). In addition, 10 nurses in the ward who have taken care of the stroke patients undergoing the **SURE** program in the ward will also be asked to fill up a questionnaire to rate the ease, difficulty, advantages and disadvantages on **SURE** program implementation in the ward.

TMS Screening

A research staff will apply TMS to the subject. The subject will be seated comfortably and instructed to remain as still as he/ she can. A tight swim cap will be worn by the subject. The vertex will be marked on the cap. Single pulse TMS was delivered using a single Magstim 200 via the coil of figure of eight. The coil position was maintained manually by the assistant and the handle of the coil point to posterior with an angle of 45 degree to the sagittal plane. Surface electromyography (EMG) electrodes are attached to the extensor carpi radialis (ECR) for EMG recording. The "hot spot" of the motor evoked potential (MEP) from ECR is first identified and marked for affected side. This spot will be used for all recordings on that side. The subject will

be considered MEP positive if more than or equal 50 μ V amplitude can be elicited in 50% of 10 successive trials while the muscle is at rest. If this criterion is not met with stimuli delivered at maximal intensity, the subject will be considered MEP negative.

Multimodal MRI imaging and Analysis

MRI data acquisition

Multimodal MRI data (T1, T2-flair, PCASL, DTI, MRS, resting-state fMRI and task-based fMRI) will be collected immediately pre-, post-, and 3months post treatment using a 3T Siemens MR system at the Clinical Imaging Research Centre using a 32 channel receive array head coil. Measurements will be made to assess changes in brain structure, metabolic status and connectivity as follows:

For anatomical delineation of the stroke lesion, and for normalization of functional data, a T1 weighted MPRAGE image (1mm³ isotropic voxels, 176 sagittal slices, TI/TE/TR=900/3/1950ms) and a T2 weighted FLAIR image (1x0.5x2mm, TI/TE/TR= 2500/78/2500ms) will be acquired.

To investigate changes in metabolic status of the tissue, a pseudo-continuous arterial spin labelled acquisition (PCASL) will be used to assess brain perfusion (3mm isotropic voxels). In addition, magnetic resonance spectroscopy will be used to measure concentrations of gamma-amino butyric acid (GABA, the main inhibitory neurotransmitter) and Glx (glutamate plus glutamine concentrations, the main excitatory neurotransmitter and its cycling intermediate) from a MEGA-PRESS localized volume in primary motor cortex (2x2x2cm volume)

For assessment of changes in neuronal connectivity, Diffusion Tensor Images (68 directions, b=1000, TE/TR=98/9100ms, plus 6 b=0 scans, 2mm isotropic voxels) will be collected to assess changes in fractional anisotropy related to structural connectivity. To investigate changes in functional connectivity, resting state connectivity data will be collected, as well as assessing changes in activation induced fMRI BOLD responses during an elbow extension task.

fMRI Activation paradigm

Subjects will be asked to extend their elbow to push against a bulb distally in a self-directed comfortable pace on a wooden/plastic board secured on their abdomen during fMRI investigation. All subjects will practice outside the scanner to reduce the mirror movements from the unaffected UL. For the task based acquisition, 12s of active task will be followed by 16s of resting (to allow the hemodynamic response to return to baseline). This will be repeated 9 times.

Neuroplasticity Investigation

fMRI imaging and image Analysis

Imaging is performed as above. Blood oxygenation level dependent (BOLD) images will be acquired to study the dynamic changes in brain activation in subjects during the investigation. The semi-automatic segmentation of the brain and atlas registration using FSL with the standard human brain atlas will be used to map the fMRI to atlas. Group analysis used in FSL will be applied to identify the functional activation and changes.

Multi-modality longitudinal brain function analysis

The modalities include T1, T2-flair, event-based fMRI BOLD, resting-state fMRI BOLD and DTI, as shown in Fig. 1. T1 image provides the anatomical structural information of the subject's brain. Haemorrhagic lesion also can be observed in T1 image. T2-flair provides the ischemic stroke lesion information. Event-based fMRI BOLD images will be used to find out the cortical activities that relate to subject's movement. The difference between pre- and pro- retaliation will be analysed to verify the retaliation performance. The difference between resting-state fMRI BOLD and event-based fMRI BOLD imaging is that the subject is requested to keep conscious but don't do any activity. It is used to recover functional connectivity inside subject's brain. In this study, the change of functional connectivity between pre- and pro- retaliation will be analysed as well. Diffusion-tensor magnetic resonance imaging, or DTI, is to map white matter tractography in subject's brain. The longitudinal changes of patients' white matter connectivity will be analysed during rehabilitation.

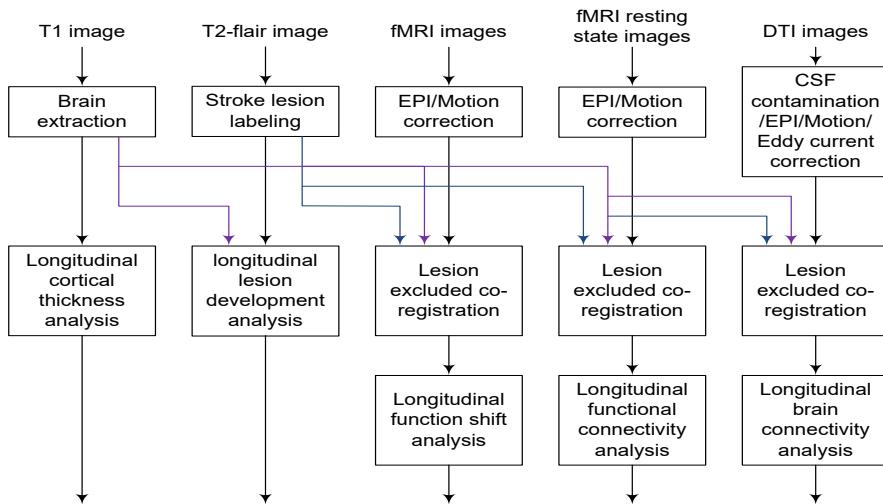


Figure 1. Multimodal MRI analysis diagram for early increased UL practice

The processing of the acquired multi-modality data is shown in Fig. 1. As the stroke may destroy part of patient's brain tissues, the stroke lesion(s) should be labelled using T2-flair image then convert to T1 image space. The brain structure should be accurately extracted from T1 structural image as it will be used as a middle point in fMRI-template, DTI-template co-registration and cortical thickness estimation as well. In the co-registration process, lesion areas should be excluded to prevent miss-alignment on the remaining healthy structures.

MRS data will be analysed using in-house based matlab scripts (GABA) and LCModel software (NAA, Cr, Cho, Glx). For LCModel an 18 metabolite basis dataset (TE appropriate) will be used. Concentrations will be corrected to absolute values using water referencing and, additionally, GABA concentrations will be provided relative to Cr. To account for tissue losses, concentrations will be corrected for tissue fraction based on the T1 weighted image (assuming concentrations are constant across tissue, no WM/GM differences). Concentrations of Lactate will not be tissue corrected as it exists in significant concentrations in CSF.

6.1. Randomisation and Blinding

Random assignment of subjects will be done using the randomizer found in the web (www.Randomizer.org/form.htm). A non-team member will generate the randomized allocation sequence, seal the group numbers in individual envelopes, and arrange them according to the randomized allocated sequence. These envelopes will be kept under lock and key in TTSRHC. The same non-team member will pass these sealed envelopes in accordance to the randomized allocated sequence one at a time to the CPI who will enrol and assign the eligible subjects to their groups. The unmasking of the randomization will be at the end of data collection and fMRI analysis. A blinded assessor will perform all the clinical assessments. The I2R who will be performing the fMRI analysis will be blinded to the group which the subjects are in.

6.2. Contraception and Pregnancy Testing

Although MRI is a non-invasive and non-radioactive imaging technology and is not known to affect baby's development, it is prudent to exclude pregnant women. If the subject become pregnant during this study, she must stop participating and call your doctor or the Principal Investigator immediately.

6.3. Study Visits and Procedures

a. *Screening Visits and Procedures*

CPI will identify patients that will be admitted to TTSHRC based on their medical records based on the study's patients' inclusion and exclusion criteria. Once the CPI check that the particular patient meets all the inclusion's criteria, she will then approach the patients' therapist to confirm that patient's eligibility for the recruitment to the study. In addition, the CPI will also check with the treating doctor to find out if that patient is medically fit and stable to travel to CIRC and back. Once the treating therapist confirms that that particular patient meets the study inclusion's criteria and the treating doctor confirms that the patient is medically fit and stable for the travel, the patient will then be approached to invite to participate in the study. If that patient agrees to participate in the study, he/ she will have to sign informed consent, and complete a pre-intervention clinical assessment and a fMRI investigation before the commencement of the study's intervention.

b. *Study Visits and Procedures*

	Clinical Assessments (TTSHRC)/ no. of visit	TMS Screening (NUH)/ no. of visit	fMRI Investigations (CIRC@NUS)/no. of visit
Pre-Intervention	1 (Inpatient)	1 (Inpatient)	1 (Inpatient)
Two weeks after start of Intervention	1 (Inpatient)	NIL	NIL
Post-Intervention	1 (In/ Outpatient)	NIL	1 (In/ Outpatient)
One Month Post-Intervention	1 (Outpatient)	NIL	NIL
Three Months Post-Intervention	1 (Outpatient)	NIL	1 (Outpatient)

4 weeks of **SURE** program intervention will start continuously after pre-intervention TMS screening, clinical assessment and fMRI investigation.

The CPI who has successfully completed all the prescribed requirements of the CPR + AED Course will accompany the inpatient subjects to NUH and CIRC at NUS for TMS screening and fMRI scanning in ambulance from TTSHRC and back to TTSHRC to ensure safety of the subjects during transit for TMS screening and fMRI scanning.

The CPI following the subjects to and back from NUH and CIRC will follow the routine inter-institution transportation protocol for patients with AWAS 0- 1 (see attached document: JCI-ACC-HAP-008.doc).

Before any TMS and fMRI appointment is made, the CPI will check with the treating doctor if the subject is clinical fit to travel, if the doctor deems the subject clinically unfit to travel, no TMS or fMRI appointment will be made.

On the day of travel, the CPI will check with the treating doctor if the subject is clinical fit to travel, if the doctor deems the subject clinically unfit to travel, the ambulance services and TMS/ fMRI will be cancelled for that day and the subject will remain in the ward and not travel. If the doctor deems the subject clinically stable to travel, the subject will be accompanied by CPI and paramedic in the ambulance service to NUH and CIRC.

In the process of transportation, if the subject suffers any adverse events or re-stroke, the paramedic accompanying the subject in the ambulance will manage the emergency situations and send the subject to the nearest hospital Emergency Department. The CPI will call the treating doctor in TTSHRC to inform and update on the emergency situation and subject's location. The CPI will stay with the subject until further instructions has been given by the treating doctor in TTSHRC.

c. *Final Study Visit:*

The final study visit will be either the three-months post-intervention clinical assessment or fMRI investigation.

d. *Post Study Follow up and Procedures*

CPI will check on all clinical assessments and TPI will check on all fMRI investigations to ensure that all the assessments and investigations are done accurately and the results obtained are accurately. CPI and TPI will also check and monitor for any adverse events occurring during **SURE** program and fMRI investigations.

e. *Discontinuation Visit and Procedures*

All clinical assessments and fMRI investigations should be done until the subject indicate to the CPI that he/she want to withdraw voluntarily from participation in the study at any time. Subjects may also withdraw voluntarily from receiving the **SURE** program for any reason. All the withdrawals and reasons for withdrawal will be evaluated to gain an insight to the feasibility of SURE program. As **SURE** program is a safe exercise program, no efforts will be made to follow-up if the subject chooses to withdraw from the program, unless subject has specifically requested for follow-up.

7. TRIAL MATERIALS

NIL

7.1. Trial Product (s)

NIL

7.2. Storage and Drug Accountability

NIL

8. TREATMENT

8.1. Rationale for Selection of Dose

Exercise Dosage of **SURE** Program

The subjects will perform the self-exercises for 60 minutes (3x 10-20 minutes) and functional tasks (as and when it is required in the subjects' daily function) per day for 6 days per week in the ward throughout the 4 weeks intervention period. These exercises are performed in addition to daily therapy. Additional period of self exercises and affected UL's functional use are performed in the ward to increase the dosage of affected UL activity as studies have found that amount of affected UL activity during therapy session are limited. Additional of 1 hour of affected UL activity (total of additional of 24 hours for intervention period) was chosen to enhance affected UL recovery

See Appendix 1 to see details of **SURE** program booklet

8.2. Study Drug Formulations

NIL.

8.3. Study Drug Administration

NIL.

8.4. Specific Restrictions / Requirements

NIL

8.5. Blinding

The therapists performing the clinical assessments, the I2R and CIRC scientists performing the fMRI investigations and analysis will be blinded to which group the subjects are in. Only the CPI and another team member who will be carrying out the **SURE** program when PI is unavailable will know which group the subjects are in. The subjects will not be told which group they are in as well. The **SURE** program and education program will be delivered to them without disclosing which group they are in. The unblinding is expected and will only occurs after all the clinical assessments and fMRI investigations are completed for individual subjects.

8.6. Concomitant therapy

Conventional therapy which occurs from Monday to Friday which includes approximately 45 minutes of physiotherapy and 45 minutes of occupational therapy daily in TTSHRC.

9. SAFETY MEASUREMENTS

9.1. Definitions

- UPIRTSO Events
Occurrence of epileptic seizures during fMRI investigations
- Serious Adverse Events
MRI related injury or death arising from the action of the strong magnetic field on any metal objects on or within the body
- Other adverse Events
MRI related burns and electric shocks
Development of allergy to the straps of the accelerometers

9.2. Collecting, Recording and Reporting of “Unanticipated Problems Involving Risk to Subjects or Others” – UPIRTSO events and “Serious Adverse Events” (SAEs) to the NHG Domain Specific Review Boards (DSRB)

Reporting Timeline for UPIRTSO Events and SAE to the NHG DSRB:

1. **Urgent Reporting:** All problems involving local deaths, whether related or not, should be reported immediately – within 24 hours after first knowledge by CPI.
2. **Expedited Reporting:** All other problems must be reported as soon as possible but not later than 7 calendar days after first knowledge by CPI.

9.3. Collecting, Recording and Reporting of "Serious Adverse Events" (SAEs) to the Health Science Authority (HSA)

NA

9.4. Safety Monitoring Plan

CPI will perform the clinical data and safety monitoring. TPI from I2R will perform the fMRI data and safety monitoring. Daily review of any possible adverse events such as medical adverse events, allergic reactions to the accelerometers' straps, adverse events of fMRI investigations.

Refer to Section 9 Safety Measurement to understand the plan in place to ensure the safety and well-being of the subjects.

Refer to 10.1 Data Quality Assurance to understand how integrity of data collected will be ensured.

9.5. Complaint Handling

CPI will handle complaints in consultation with TPI. If the data of complainants will still be used for analysis unless specified by the complainants not to be included in this study.

10. DATA ANALYSIS

10.1. Data Quality Assurance

CPI will check the data of all clinical assessments and TPI will check on the data of fMRI investigations to ensure that the data obtained from this research is accurate, complete and reliable. Reviews will be performed daily.

10.2. Data Entry and Storage

Data from clinical assessments will be entered on paper. Soft copy of the research data will be stored in research staff's computers, laptops and portable hard disks. All the research staff involved in the investigation will use password protected laptops and PCs for development and study. The subjects' identifiers will be coded and be stored separately from the research data. The original personal data will only be accessed by the CPI. The data can only be accessed with the permission from the CPI. The CPI's personal computer and laptop are protected by password. The hard disk and hard copy of the data will be kept in a secure cupboard in TTSHRC. The anonymized data will only be available to CO-Is and related research personnel involved in the study.

Data from fMRI investigations will be entered electronically. CPI, TPI, I2R scientists, CIRC scientists will have accessed to fMRI data. The data will be stored in a secured server (I2R), which is in a locked room and the data can only be accessed by password.

11. SAMPLE SIZE AND STATISTICAL METHODS

11.1. Determination of Sample Size

Since this study is a pilot exploratory trial, results of individual subjects from each group will be analysed in

detail to determine the difference in brain neuroplasticity between the control and experimental groups. N=10 from each group is chosen because it is deemed feasible to recruit stroke patients who will be willing to participate in this study clinically in the period of 2 years (the time period of the funding).

11.2. Statistical and Analytical Plans

a. Study Statistical Analysis

Statistical analysis will be performed using STATA version 10.0 for Windows (StataCorp, TX, USA). Descriptive statistics will be used to describe subjects' demographics in each group.

Analysis of UL Functional Outcome

Wilcoxon signed-ranked tests will be used at each time points (pre, 2 weeks after start of intervention, post, 1month post-intervention, 3months post-intervention) to compare UL functional outcomes between the experimental and control groups. The significance level for all statistical tests will be set at $p<0.05$ levels. All data will be analysed by intention to treat.

Analysis of feasibility of accelerometers to measure affected UL use

Analysis of the compliance rate and responses from the questionnaire will be used to determine the feasibility and acceptance of using accelerometers to measure affected UL use after stroke.

Analysis of SURE Program's feasibility

Wilcoxon signed-rank test will also be used to compare outcomes of pain, fatigue and spasticity pre-and post-intervention period. The differences in the duration of affected UL use per day and the ratio of affected UL use/ non-affected UL use between the experimental group and control group (from the accelerometer recordings), the rate of compliance, the proportion of subjects who successfully complete the whole training, and the dropout rate will be calculated to assess the feasibility of **SURE Program**.

Analysis of fMRI investigation

Comparison of activation in primary motor cortex, secondary motor cortex and cerebellar hemisphere for bilateral sides will be done by comparing mean percentage signal change (%SC), absolute voxel counts (VC) and changes in geometric centers of these areas on both sides¹⁷. In addition, a laterality index (LI) will also be calculated to determine the relative cortical activity. Asymmetry has been found to restore to normal (L=1.0) when the affected hand movement improved after rehabilitation⁴¹.

Analysis of resting state fMRI/DTI

Functional connectivity and tract integrity^{32,38} will be studied by FSL with the normalization method^{39,40}. The secondary degeneration of the brain in the remote region to the primary lesion will also be studied for the sub-groups of subjects with less motor recovery³⁰.

MRS analysis

A one-way ANOVA test will be performed to assess differences across the visits (pre, post, and 3months post intervention). Provided a significant difference is found, a paired t-test will be used. Due to an abundance of measurable metabolites, metabolite analysis will be limited to those defined by our hypothesis (changes in GABA and Glx) to minimise multiple comparisons.

b. Safety Analysis

Safety analysis will be done by monitoring the adverse events and complaints of the study daily.

c. Interim Analysis

Interim analysis of the data and study's safety and complaints will be performed every quarterly. If the analysis of study's safety shows that the subjects' safety is compromised because of the study procedures, the study will stop.

12. ETHICAL CONSIDERATIONS

12.1. Informed Consent

CPI will explain to TTSHRC therapists of the study and the subjects' inclusion and exclusion criteria. CPI will check through the admission list to check for patients who meet the study's inclusion criteria. When a patient is fit the study's inclusion criteria and does not have any of the exclusion criteria, the CPI will approach the treating therapists to verify. Once the TTSHRC treating therapists have confirmed the patient's suitability for the study, TTSHRC therapists will speak to that particular patient about the study. If that patient expresses interest in the study, the therapists will notify the CPI. The CPI will then approach the patient in the ward/ gym to explain the study in detail and give him/ her a Patient Information Sheet to consider. If the patient is willing to participate in the study, CPI will ask him/ her to sign the informed consent form.

The consent process will take place in the ward when the patients are resting or in the gym when the patients are waiting for their therapy sessions. At the time of approach, patients should be calm.

CPI who is currently doing her PhD study and not involve in direct patients' care will be the one doing informed consent for all the subjects. The CPI will speak in a soft and gentle voice so that the attention of the conversation by others will be minimal.

For patients who cannot speak English/ cannot read English/ cannot sign on the informed consent form, translation in Chinese will be provided by the CPI. A witness will be present to witness the translation and will sign on the informed consent form as a witness.

12.2. IRB review

This study will gain IRB / NHG DSRB approval before commencement.

12.3. Confidentiality of Data and Patient Records

Information collected for this study will be kept confidential. Subjects' records, to the extent of the applicable laws and regulations, will not be made publicly available.

However, NHG Domain-Specific Review Board and Ministry of Health will be granted direct access to the original medical records to check study procedures and data, without making any of subjects' information public.

Refer to Section 10.2 Data Entry and Storage to understand how data will be entered and stored to ensure confidentiality of data and patient records

13. PUBLICATIONS

The study findings will be published in established Scientific journals without revealing subjects' identities.

14. RETENTION OF TRIAL DOCUMENTS

Records for all participants, including CRFs, all source documentation (containing evidence to study eligibility, history and physical findings, laboratory data, results of consultations, etc.) as well as IRB records and other regulatory documentation will be retained by the CPI in a secure storage facility. Refer to Section 10.2 Data Entry and Storage to understand how data will be stored securely.

The records will be accessible for inspection and copying by authorized authorities. The data will be kept for minimum of 6 years in TTSHRC. I2R will use the anonymized data as specified by the research agreement.

List of Attachments

Appendix 1 Please see attached SURE Program Booklets in the DSRB Application:

SURE Program Booklet 1A

SURE Program Booklet 1B

SURE Program Booklet 2