# TRANScranial direct current stimulation for POst-stroke motor Recovery - a phase II sTudy (TRANSPORT2)

**cIRB Protocol Number: 2018-4092**

**National Clinical Trial (NCT) Identified Number: NCT03826030**

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**IDE Sponsor: N/A**

**Funded by: NINDS**

**Version Number: v5.0**

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## STATEMENT OF COMPLIANCE

The trial will be conducted in accordance with International Conference on Harmonization Good Clinical Practice (ICH GCP), applicable United States (US) Code of Federal Regulations (CFR), and the NIH/NINDS Terms and Conditions of Award. The Principal Investigators will assure that no deviation from, or changes to the protocol will take place without prior agreement from the sponsor and documented approval from the Central Institutional Review Board (cIRB), except where necessary to eliminate an immediate hazard(s) to the trial subjects. All personnel involved in the conduct of this study have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all subject materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any subject is enrolled. Any amendment to the protocol will require review and approval by the cIRB before the changes are implemented to the study. All changes to the consent form will be cIRB approved; a determination will be made regarding whether a new consent needs to be obtained from subjects who provided consent, using a previously approved consent form.

## 1 PROTOCOL SUMMARY

### 1.1 SYNOPSIS

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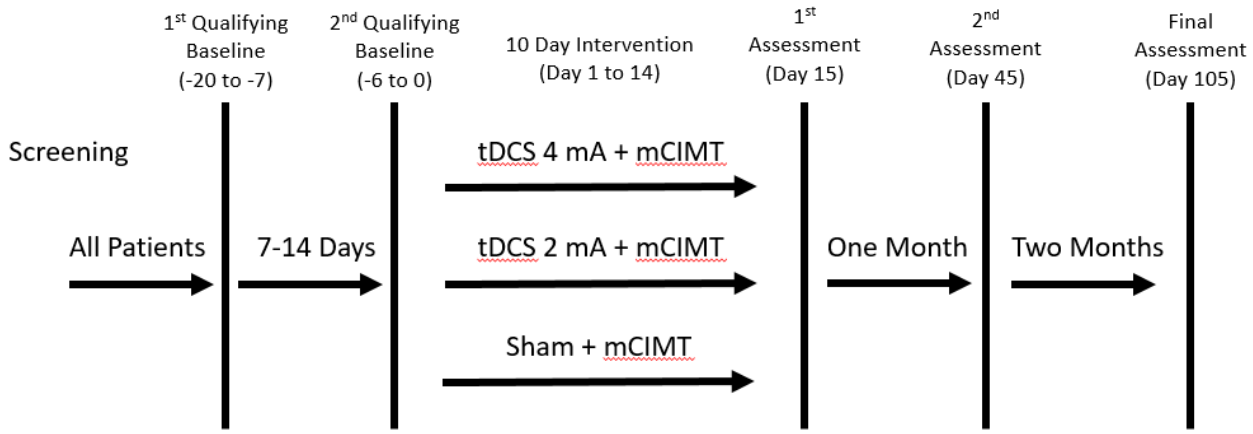
|---|---|
| **Title:** | TRANScranial direct current stimulation for POst-stroke motor Recovery- a phase II sTudy (TRANSPORT2) |
| **Study Description:** | TRANSPORT2 is a phase II multi-center transcranial direct current stimulation (tDCS) dosing selection study based on the preliminary efficacy, safety, tolerability and feasibility. |
| **Objectives:** | **Primary Objective:** To determine whether there is an overall treatment effect among 3 dosing groups (sham+mCIMT, 2 mA+mCIMT, and 4 mA+mCIMT) on day 15 (± 2 days) after the start of the intervention in the Fugl-Meyer Upper-Extremity (FM-UE) scale, a measure of motor impairment. Additional outcome measures include the Wolf-Motor-Function-Test (WMFT), a measure of functional motor activity, and the Stroke-Impact-Scale (SIS), a measure of quality of life. Sustained benefit will be assessed at day 45 (± 5 days) and day 105 (± 10 days).<br><br>**Secondary Objectives:** To confirm that the proposed intervention is safe (no significant differences in rate of adverse events), tolerable (no significant differences in discomfort as measured by Visual-Analog-Scale), and feasible to administer in a multi-site trial (>80% of subjects complete the treatment protocol and no unexplained/unresolved variability by site).<br><br>**Exploratory Objectives:** To examine whether wCST-LL (structural assessment of integrity of descending motor tract) or MEPs (functional assessment of integrity of descending motor tract) or combination of both are correlated with changes in FM-UE scale, and evaluate the utility of these measures as biomarkers for patient selection criteria in the future confirmatory Phase III study. We also |

| | |
|---|---|
| | aim to examine whether structural and functional changes within descending motor tracts correlate with changes in motor impairment and functional motor activity. |
| **Endpoints:** | **Primary Endpoint:** Fugl-Meyer Upper-Extremity (FM-UE) scale a measure of motor impairment;<br>**Secondary Endpoints:** Wolf Motor Function Test (WMFT) time score, a measure of functional motor activity; Stroke-Impact-Scale hand subscale as an assessment of patient-centered quality of life. |
| **Study Population:** | Subject who is 18-80 years of age of any gender or ethnicity, who had first-ever unihemispheric ischemic stroke in past 30-180 days resulting in unilateral limb weakness with FM-UE ≤ 54 (out of 66) that is stable across two baseline visits at the time of randomization and an mRS ≤ 2 pre-stroke. We are planning to enroll 129 subjects across three arms. |
| **Phase:** | 2 |
| **Description of Sites/Facilities Enrolling Subjects:** | Subject recruitment will occur at approximately 15 enrolling sites in the USA. |
| **Description of Study Intervention:** | There are 3 intervention arms: sham, 2 mA, and 4 mA tDCS combined with modified Constrained-Induced Movement Therapy (mCIMT). The tDCS session is 30 minutes, and mCIMT is 120 minutes per session, 10 intervention sessions are completed over a 2-week (14 days) period. |
| **Study Duration:** | 48 months |
| **Subject Duration:** | Approximately 4 months |

## 1.2 SCHEMA

Please Refer to **Section 1.3, Schedule of Activities**, for details



## 1.3 SCHEDULE OF ACTIVITIES (SOA)

| Event | Baseline 1 | Baseline 2[3] – | Treatment Sessions (1-10) | Follow Up 1 | Follow Up 2 | Follow Up 3 | End of Study |
|---|---|---|---|---|---|---|---|
| | Day -20 to -7 | Day -6 to 1 | | Day 15 ±2 | Day 45 ±5 | Day 105 ±10 | |
| Informed Consent | X | | | | | | |
| Inclusion/Exclusion[1] | X | | | | | | |
| Demographics | X | | | | | | |
| Montreal Cognitive Assessment (MOCA) | X | | | | | | |
| Medical and Social History (Including Pre-Stroke Modified Rankin Scale (mRS) | X | | | | | | |
| Medication Checklist | X | X | | | | | |
| Vital signs | X | | | | | | |
| Fugl-Meyer Assessment– Upper Extremity Scale | X | X | | X | X | X | |
| NIH Stroke Scale (NIHSS) | | X | | | | | |
| Transcranial Magnetic Stimulation (TMS) | | X[1] | | X | | | |
| Randomization | | | X[2] | | | | |
| tDCS Treatment Activation Code Request (Retrieved at beginning of every Treatment Session) | | | X | | | | |
| tDCS Questionnaires (Pre-Stimulation, Post-Stimulation, Post mCIMT) | | | X | | | | |
| tDCS | | | X | | | | |
| Modified Constraint Induced Movement Therapy (mCIMT) | | | X | | | | |
| Wolf Motor Function Test (WMFT) | | X | | X | X | X | |
| Stroke Impact Scale (SIS) | | X | | X | X | X | |
| MRI | | X[1] | | X | | | |
| Concomitant Therapy | | | | X | X | X | |
| Blindedness Assessment | | | X[4] | | | | |
| Visual Analog Scale | | | X[4] | | | | |
| End of Study | | | | | | | X |

1. A urine pregnancy test for all women of child-bearing potential will be given at this visit.

2. Randomization ONLY occurs at 1st Treatment Session

3. This visit can be broken up over several days. All baseline activities need to take place prior to Day 1 treatment activities. Please see MOP and FAQs for more details on this visit.

4. End of Treatment ONLY occurs at Final Treatment Session

# 2 INTRODUCTION

## 2.1 STUDY BACKGROUND

There are 795,000 new strokes each year in the US[1], and stroke is a leading cause of long-term disability leaving more than half of stroke survivors with residual impairments, with motor deficits being the most common deficits after stroke[2-6]. The costs associated with health services, lost wages and productivity are estimated to be several billion dollars per year. The development of effective approaches that can enhance motor recovery and reduce disability remains an important priority for healthcare professionals, patients and caregivers, and society in general.

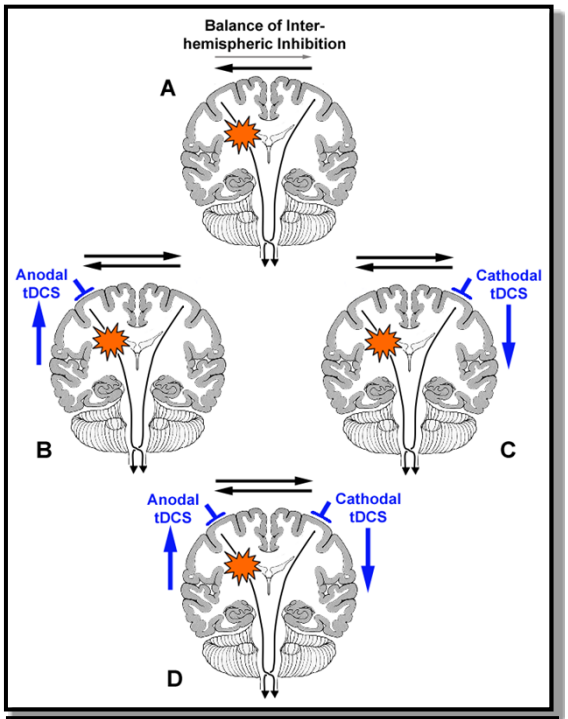



**Fig. 1A-D:** Inter-hemispheric Imbalance and Electrode Montages to rectify this imbalance.

Despite the growing interest in improving motor recovery after a stroke with invasive[7, 8] and non-invasive[9] brain stimulation such as tDCS, new equipment to increase motor activities[10, 11], pharmacological approaches[12], and stem cell therapies[13], several key questions remain unanswered: Can any therapy improve neurologic function beyond the brain's spontaneous recovery ability[14-17]? What is the adequate dose and intensity of an intervention[18]? What is the optimal period of an intervention[14, 19]? Which brain regions and physiological processes must be relatively intact and can be targeted to improve functional outcome[20]? Can the combination of a peripheral rehabilitation activity with a centrally acting technique/tool (such as tDCS) lead to an enhancement of brain plasticity?

## 2.2 STUDY RATIONALE

### A.1. The role of non-invasive brain stimulation in facilitating stroke recovery and the effects of different electrode montages

Non-invasive brain stimulation (NIBS) might have two potentially synergistic ways of modulating brain function that in turn can enhance recovery from a stroke. **First**, it has been shown in well-designed studies that non-invasive brain stimulation can enhance brain plasticity[21, 22] and remodeling of motor tracts[23]; simultaneously, NIBS can interfere in a stroke-induced abnormal inter-hemispheric imbalance in transcallosal inhibition[24-26]. This imbalance model implies that there is decreased activity in the ipsi-lesional motor regions, excessive (uninhibited) activity in the contralesional motor regions, and that excessive (i.e., uninhibited) activity in the contralesional motor area will, in turn, lead to increased transcallosal inhibition of motor regions on the lesional hemisphere (**Fig. 1A)**. Neurophysiological studies in chronic stroke patients strongly support this model by showing that *disinhibition* of *contralesional* motor regions coexists with *increased inhibition* of *ipsilesional* motor regions resulting in an imbalance of inter-hemispheric interactions[25, 27, 28]. Second, Imaging studies in well-recovered patients have shown that brain reorganization during the recovery phase is associated with re-activation or over-activation of motor and premotor networks in the lesional hemisphere and that transient activation of the ipsilateral

(contralesional) motor cortex occurs but does not seem to be associated with good outcome[29-31]. Three different electrode montages have provided empirical support that NIBS can modulate the inter-hemispheric imbalance in inhibition. Anodal stimulation of the lesional hemisphere[32, 33] can up-regulate its cortical excitability (**Fig. 1B**); cathodal stimulation of the contra-lesional hemisphere can down-regulate its excessive cortical excitability[34, 35] which in turn decreases the transcallosal inhibition of the lesional hemisphere (**Fig. 1C**); Bi-hemispheric stimulation with anodal stimulation of the lesional hemisphere and cathodal stimulation of the non-lesional hemisphere[36-38] can do both physiological processes at the same time (Fig. 1D). All three montages demonstrated improvements of motor functions in proof-of-concept studies, although a meta-analysis found that a **bihemispheric montage might have the strongest effect**[39]. Similarly, two studies[40, 41] in healthy subjects showed stronger motor learning effects after bi-hemispheric stimulation than after uni-hemispheric stimulation. A recent meta-analysis revealed that a bihemispheric montage up to 2 mA in chronic stroke patients (**Fig 2**) has higher odds of demonstrating tDCS efficacy with a summary effect of 1.30 (-0.14, 2.75)[39]. This includes two tDCS studies by Co-PI Dr. Schlaug: a bi-hemispheric study[36] with a larger effect than an independently conducted uni-hemispheric study (cathodal stimulation of the contralesional hemisphere) showing a smaller effect[42].

### A.2. Rigorous experimental studies have proven that tDCS modulates brain activity and affects behavior that draws on targeted brain regions.

tDCS includes the use of an electrode that is typically large enough to have an impact on a network of motor, premotor and sensory cortices (all of them have been shown to play a role in the recovery of sensorimotor function). The use of a battery-operated device allows tDCS to be combined with a peripheral rehabilitation technique in real-time (e.g., simultaneous constraint-induced movement therapy while tDCS is delivered).

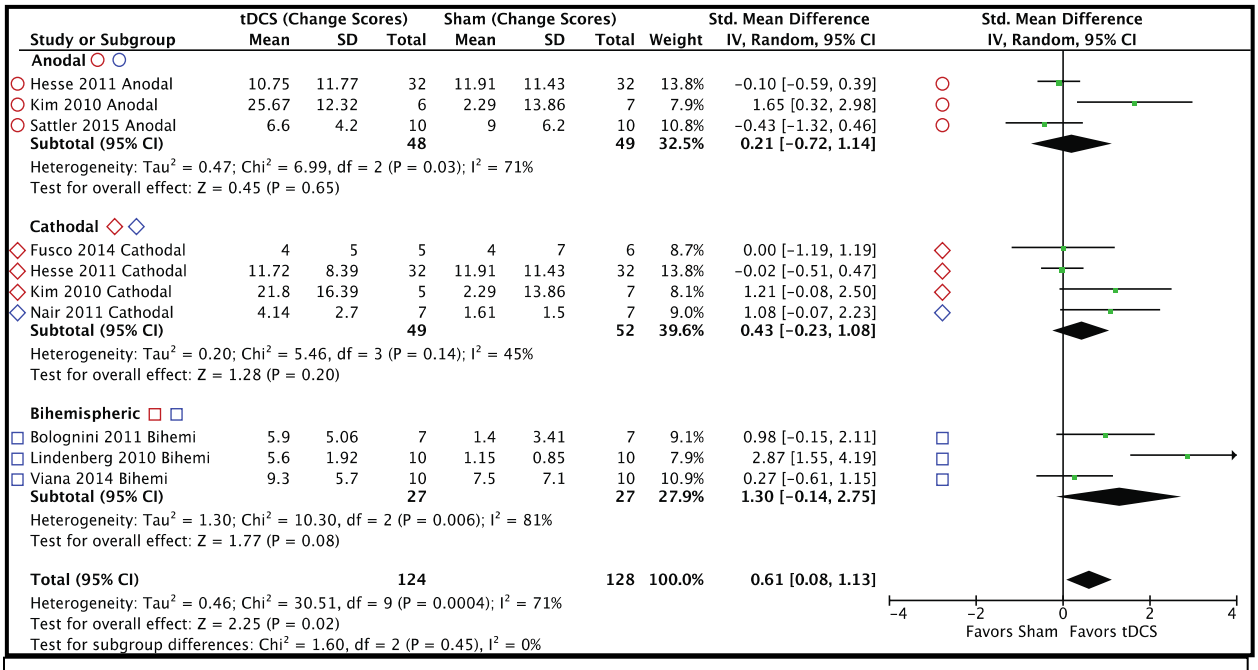



**Fig 2: Meta-analysis indicating the bihemispheric montage demonstrates higher FM-UE change scores than anodal or cathodal montage. Chronic studies highlighted in blue.**

The concept of therapeutically applying electricity on excitable tissues such as the brain is not new. Effects of direct currents on animal and human brain tissue prompted a novel approach of non-invasive brain stimulation with weak direct currents, which came to be known as tDCS[43]. tDCS provides a sub-threshold stimulus that modulates the likelihood that neurons will fire by decreasing (cathodal stimulation) or increasing excitability (anodal stimulation) without inducing any action potential[43, 44]. The prolonged sensory, motor and cognitive effects of tDCS have been attributed to a persistent bidirectional modification of post-synaptic connections similar to long-term potentiation (LTP) and long-term depression (LTD)-like effects[45]. Dextromethorphan, an N-methyl-D-aspartate (NMDA) antagonist suppressed both anodal and cathodal tDCS effects, strongly suggesting the involvement of NMDA receptors in both types of DC-induced neuroplasticity[46]. In contrast, Carbamazepine selectively eliminated anodal effects[47]. Since Carbamazepine stabilizes the membrane potential through voltage-gated sodium channels (stabilizing the inactivated state of sodium channels), the results were interpreted as indicative that after-effects of anodal tDCS require a depolarization of membrane potentials. Ardolino and colleagues[48] also proposed a non-synaptic mechanism involving changes in membrane excitability and ionic shifts. Some studies have already examined changes in blood flow directly under the electrode as well as in remote regions as surrogate markers of the biological activity exerted by tDCS[49-52]. Other studies have examined tDCS modulation of indirect measures of blood flow using the BOLD effect either by itself or in combination with sensorimotor or cognitive tasks[53-64]. MR spectroscopy has been used to examine the modulation of certain neurotransmitter-receptor systems to better understand the excitatory and inhibitory effects of tDCS[57, 65-67]. We showed in a combined tDCS-MRI experiment that an 8-minute stimulation (anodal and cathodal) to the right motor cortex repeated 3 times with 8-minute gaps between stimulations modulates regional blood flow response (as measured by arterial spin labeling) in the underlying motor region and modality-specific effects were seen in the after-stimulation phase (**Fig. 3, above**). Regional CBF changes under the anodal and to a slightly lesser degree under the cathodal electrode showed a significant correlation with applied current levels[52]. Modulating activity in the motor cortex via tDCS has been demonstrated to affect implicit and explicit finger sequence learning in healthy subjects[40, 68-74]. Some studies have shown substantial evidence that effects lasted for up to a week while one study even found motor learning effects of multisession tDCS in healthy subjects to last for at least 3 months[72].

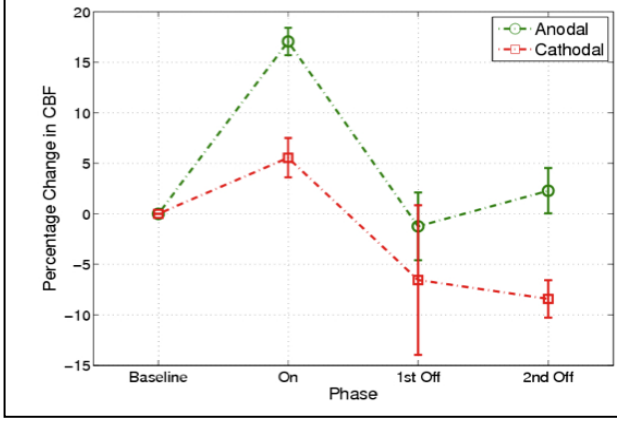



**Fig 3.** *Average CBF changes (normalized to zero) for the anodal and cathodal condition across 4 assessments.* Comparing the changes between the anodal and cathodal conditions, we found significant differences in the relative CBF increases ($p<0.001$) when the stimulation was turned ON and when the stimulation was switched OFF ($p<0.05$), and relative CBF differences in the changes between first and second OFF time points ($p<0.05$)

### A.3. The need for higher current dose while maintaining safety

Theoretical knowledge regarding safety and tolerability of tDCS is based on physical aspects of this technique and data from both animal and human studies, suggesting “no significant risk for human subjects at currents ranging from 0.5 to 2.0 mA for up to 40 minutes[75-81]. Commonly reported side effects occur at the site where the electrodes are placed and are mostly minor issues[75, 76] such as local scalp itching, tingling or burning sensations, transient mild headaches, contact dermatitis[82] or rarely stimulation-induced skin lesions[83, 84], which were mainly due to inadequately applying contact media.

The size of the electrode pad also matters in term of safety and tolerability. Electrode size determines current density which is defined as current/electrode contact area. Current density at 4 mA is approximately 0.11 mA/cm$^2$ with a pad size of 5×7cm². The charge density even at 4 mA for 30 minutes **(Fig. 4, right)** is considerably below the safety threshold determined by invasive stimulation techniques[80] and is ~ 4% of the safety limit established in an animal study[79].

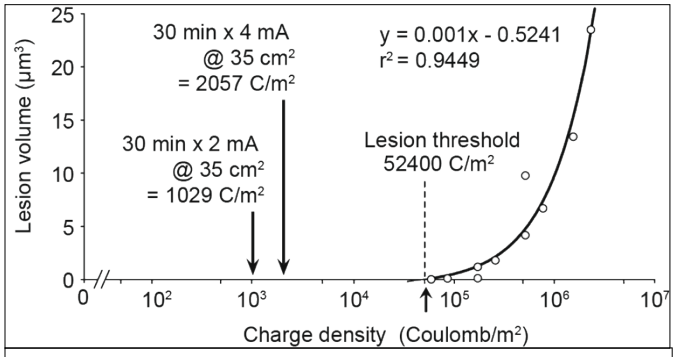



**Fig. 4: Safety limits of tDCS in animal studies and comparison to human dosage**

While underdosing is possible, lack of published data at higher currents and theoretical concerns of safety has led researchers to avoid higher tDCS current than 2 mA in stroke patients. A meta-analysis indeed showed a dose-response relationship with motor improvement and current up to 2 mA[39]. **The Phase I current escalation study of tDCS (PI Feng, NINDS P20 GM109040) found that doses up to 4 mA are safe and tolerable to ischemic stroke patients (*manuscript is highlighted in the Brain Stimulation Journal cover page and accompanied by an editorial*)**[85, 86]. There were no second-degree skin burns, clinical seizures, or DWI abnormalities. Furthermore, no subjects discontinued from the trial. Thus, while this phase I study suggests that doses up to 4 mA are safe and tolerable, it is not clear whether a dose-response relationship in treatment effect would extend beyond 2 mA. It is a logical next step to test a dose effect in a phase II multicenter study while we continue to collect data on safety and tolerability.

### A.4. The choice of a peripheral rehabilitation therapy

Several studies have shown that the combined effects of tDCS or other non-invasive brain stimulation technique and a peripheral rehabilitation therapy typically elicit behavioral changes and subsequent improvement of motor functions greater than when either intervention is done by itself[35-37, 42, 87]. If the variance and effect (or the duration and intensity) from peripheral rehabilitation therapy is not well controlled and balanced across the groups, the real effect from tDCS is likely contaminated and attenuated. For example, various peripheral rehabilitation therapies have been tested in combination with tDCS such as customary occupational/physical therapy[36, 88], robot-assisted therapy[89] and virtual reality therapy[38]. Although we do know from experimental studies that combining peripheral stimulation with central stimulation will increase synaptic plasticity, it is harder to determine whether tDCS in combination with untested peripheral rehabilitation is efficacious as the efficacy of these peripheral rehabilitation therapies by themselves have not been proven[21].

One peripheral therapy, namely constraint-induced movement therapy (CIMT), has been tested in an NIH- funded multi-center trial and demonstrated to be effective in improving motor function in patients between 3-9 months after stroke.[19] CIMT focuses on peripheral sensorimotor stimulation targeting the known phenomenon of “learned nonuse” of the affected hand[14, 19]. CIMT has two principal components[90]: 1) *Constraint* - restraining of the unaffected upper extremity (UE), typically in a mitten, and 2) *Inducement* - training of the hemiparetic UE using a shaping and repetitive exercise paradigm. Shaping is defined as the development of a new behavior by the reinforcement of successive approximations of closer approximation and the extinguishing of preceding approximation of the behavior. More recently, The VECTOR study which enrolled subjects in the subacute phase demonstrated that stroke patients responded better to a modified CIMT (2 hours of shaping and at least 6 hours on constraints for 10 sessions over a 14 day period) rather than the high-intensity CIMT[14]. There is often a concern that patients eligible for CIMT might have only limited impairments, and that too few patients would be eligible. The mean score

of FM-UE scale[91] from patients included in the EXCITE trial was 42.5 (SD 11.7) for the CIMT group and 41.1 (SD 12.9) for the usual care group. Thus, even a patient with an FM-UE score of around 30 was eligible for the EXCITE trial[19]. Furthermore, we are planning to include patients that correspond to both the higher and lower functioning groups in the EXCITE trial to have as many eligible subjects as possible. However, the baseline disability will be adjusted for in both the randomization and analysis to ensure that the treatment groups are balanced. The lower-functioning subjects in the EXCITE trial had at least 10° of active wrist extension, at least 10° of thumb abduction/extension, and at least 10° of extension in at least 2 additional digits. Higher-functioning subjects demonstrated at least 20° of wrist extension and at least 10° of active extension of each metacarpophalangeal and interphalangeal joint of all digits[19]. The assembled TRANSPORT2 team include several key personnel from the EXCITE and VECTOR trial to ensure that mCIMT, the peripheral rehabilitation therapy in our TRANSPORT2 trial, is done with the highest level of consistency across sites. Using a standardized intervention and providing experienced trainers and oversight for other sites, will minimize the variance that a peripheral rehabilitation therapy could introduce, and it will increase the odds to detect efficacy from tDCS.

### A.5. Timing of the proposed intervention

Determining the best timing for a proposed tDCS intervention is complicated. It is well acknowledged that there are many confounders and uncertainties for conducting stroke recovery trials in the subacute phase, such as, ongoing challenging medical issues; robust spontaneous recovery; lack of validated patient selection tool in the acute or subacute phase, etc. It is also true that our prior meta-analysis demonstrated that tDCS trial is likely to be successful in the chronic phase than in the subacute phase based on the data from existing studies[39]. However, there are several study design deficits in the existing tDCS trials that have been carried out in the subacute stage which may explain the failures. Arguing for earlier intervention is that the natural biological recovery process early after a stroke can be robust[15-17] and has not been well harnessed by the stroke rehabilitation trials. We are not discouraged by challenges of early intervention. Instead, we followed the reviewers' recommendation to extend the trial to the subacute phase (i.e. as early as one month after stroke). We developed several procedures and processes to mitigate these risks. For example, we will require a stable deficit (≤2 points change in FM-UE scale in two screening assessments) for eligibility. We will also balance these patients during the randomization process by stratifying randomization on time from stroke onset (≤ 90 days or > 90 days) to ensure a good balance of subjects in the three arms, further mitigating expected variability. In addition, we will include 'time from stroke onset to randomization' as a covariate in the model and record additional rehabilitation therapy outside of the 14 day intervention. We will carefully conduct both intent-to-treat and per-protocol analysis to delineate these challenges.

### A.6. The need of a multi-center Phase II study for examining tDCS efficacy

Table 2 below provides a sense of the variability of reported mean changes (and standard deviations) of FM-UE scale from 3 different studies with bihemispheric stimulation montage in chronic stroke patients. Several possible explanations for this variability in effect size include the type of peripheral rehabilitation therapy, the severity of functional motor impairment at baseline, the number of sessions, small sample size, and the lack of a central adjudication of the outcome measure. A large, multicenter, double-blinded, sham-controlled Phase II trial with a centrally adjudicated assessment of the preliminary effect of several doses of tDCS will yield the necessary rigorous, reproducible results necessary before a definitive Phase III trial is justified.

**Table 2: Characteristics of tDCS and peripheral rehabilitation therapies in three studies using a bihemispheric tDCS montage.**

| | Study Characteristics | | | | Baseline | | Change | |
|---|---|---|---|---|---|---|---|---|
| | Sample size *1:1 allocation* | Current *per session* | Length of intervention | Peripheral therapy | Active Stim. | Sham Stim. | Active Stim. | Sham Stim. |
| [36] | 20 | 1.5 mA × 30 min. | 5 sessions (1 hour per session) | Customized Occupational therapy | 38.2 ± 13.3 | 39.8 ± 11.5 | 5.60 ± 1. 93 | 1.15 ± 0.85 |
| [37] | 14 | 2.0 mA × 40 min. | 10 sessions for up to 4 hours per session | Variant of mCIMT | 25.4 ± 10.8 | 27.6 ± 18.2 | 5.90 ± 5.06 | 1.40 ± 3.41 |
| [38] | 20 | 2.0 mA × 13 min. | 15 sessions over 5 weeks (1 hour per session) | Virtual reality training | 41.3 ± 16.2 | 39.2 ± 17.6 | 9.30 ± 5.70 | 7.50 ± 7.10 |

## A.7. Structural and functional biomarkers for patient selection and therapy response

Recently, novel neuroimaging and/or neurophysiological measures have been investigated to provide a quantitative assessment of the injury to descending motor tract after a stroke and to reveal a patient's motor recovery potential. Both Motor-evoked Potentials (MEP) by transcranial magnetic stimulation (TMS) and weighted corticospinal tract lesion load (wCST-LL; **Fig 5**) are methods to assess the impact of a stroke lesion onto the corticospinal tract. Two studies suggested that the presence of MEPs could aid subject selection for both noninvasive[89] and invasive brain stimulation study[92] – **subjects with MEPs are more likely to show improvements than subjects without MEPs**. Similarly, a two-center study led by Drs. Feng and Schlaug demonstrated that wCST-LL could effectively predict motor recovery from the acute phase to 3 months (Fig 6A) and wCST-LL of ≥7.0cc in the acute phase would lead to poor motor recovery (FM-UE ≤25 out of 66) at 3 months[16]. Overall, wCST-LL provided a more graded and sensitive measure of CST injury than the clinical assessment and

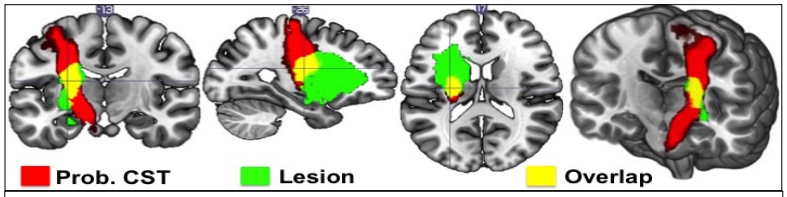



**Fig. 5:** Weighted CST-Lesion Load using a canonical probabilistic tract and lesion overlap.

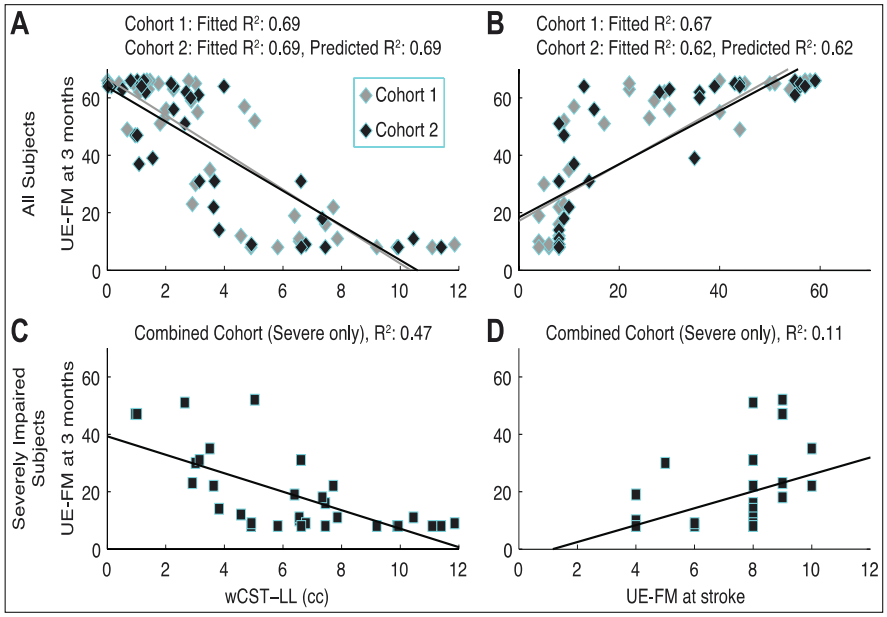



**Fig. 6:** Scatter plot and correlation for wCST-LL vs. Initial motor impairment. Both FM-UE (7B) and wCST-LL (7A) predict outcome in two different cohorts, but wCST-LL offers superior prediction of 3-month outcome ($R^2$=0.47) than FM-UE ($R^2$=0.11) in the subgroup with severe initial impairment (7C+D).

correlated better with motor outcomes, especially in the severely impaired subgroup (Fig 6C and D). wCST-LL in the chronic phase is also correlated with the motor impairment measured by FM-UE scale[93]. Furthermore, a slight variation of a CST-lesion load variable was found to be a predictor of improvement in an experimental trial in chronic stroke patients[20]. It has not been formally investigated whether MEPs or wCST-LL or a combination of both can be used as both a predictive and prognostic biomarker in the same study.

**A8. Choice of outcome measures for tDCS stroke motor recovery.**

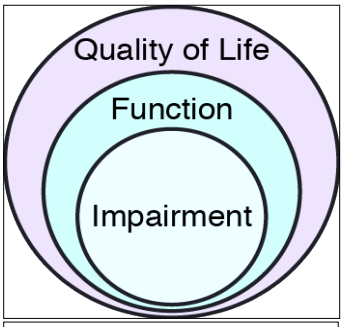



**Fig. 7: Hierarchies of Recovery**

The comparison among the three groups requires a highly standardized measure of outcome that is relevant and suitable for the clinical question, valid for the population studied, and meaningful for the patients. As stroke represents a leading global cause of adult disability, important considerations for any study of stroke rehabilitation are impairment reduction, recovery of functional skills, and quality of life improvement **(Fig. 7)**. These three aspects represent a hierarchy with the quality of life improvements predicated on functional improvements, and similarly, functional improvements first require a reduction in impairment. As such, this TRANSPORT2 trial is designed using a primary outcome of FM-UE, a commonly recommended primary outcome measure[94]. However, in accordance with regulatory authorities and expert panels, who now recommend addressing all of these issues above, the Wolf-Motor Function Test *(to illustrate a functional motor improvement)* and the Stroke Impact Scale-Hand *(to show quality of life enhancement)* will be measured as secondary outcome measures to comprehensively assess the efficacy of tDCS for stroke motor recovery[94]. In a prior tDCS study by PI Schlaug, it has been shown that the change in the FM-UE scale is highly correlated with the change in the WFMT (Pearson correlation coefficient=-0.62, p=0.004). All these three scales have excellent psychometric property[94]. Nevertheless, our phase II study would provide the necessary data to assess the interplay of these three outcome measures, and we do not rule out the possibility of considering other primary outcomes than FM-UE scale in a confirmatory phase III study.

## 2.3 Risk/Benefit Assessment

### 2.3.1 Known Potential Risks

- **General Risk Loss of Confidentiality:** It is expected that this risk will be low because of numerous safeguards that will be in place, including standard data management procedures at the NDMC.
- **tDCS:** Extensive animal and human evidence and theoretical knowledge indicate that tDCS protocols up to 2 mA are safe and tolerable[75-77]. Recently, a Phase I study evaluated the safety and tolerability of tDCS doses up to 4mA in patients with ischemic strokes; the study used a 3+3 study design and escalate dose to 4mA without meeting pre-specified safety rules. Overall, tDCS incurs no significant risk to human subjects per FDA determination. Commonly reported side effects in the literature are as follows: skin redness under the electrode, skin irritation with itching, tingling, burning sensation, and transient headache (which is mostly due to the tight elastic headband that holds the electrode in place). Any skin injury is a very rare side effect (1 out of 1000). Theoretical risks at high dose (e.g., 4mA) might involve second degree skin burns and clinical seizures, but these risks were not observed in the phase 1 safety study. Subjects will be carefully monitored for any potential safety or tolerability issues during each interventional session.

- **MRI:** MRIs will be done using equipment similar to that used for clinical MRI (either 1.5 or 3.0 T). The current revision of the US Food and Drug Administration guidelines (US Department of Health and Human Services, Food and Drug Administration, Center for Devices and Radiological Health, Guidance for the submission of premarket notifications for magnetic resonance diagnostic devices, Washington, DC. November 14, 1998) applies to this study. These guidelines state that magnetic resonance imaging systems with main static magnetic field strengths of 4.0 T (a measure of magnetic strength) and less, such as the ones used for this study, can qualify as non-significant risk devices. Because the MRI machine acts like a large magnet, it could move iron-containing objects in the MRI room during the examination, which could, in the process, possibly harm the subject. Magnetic media such as credit cards, etc. and watches near the coil may also be damaged. Precautions have been taken to prevent such an event from happening; loose metal objects, like key chains or paper clips, are not allowed in the MRI room. If a subject has a piece of metal in your body, such as a fragment in your eye, aneurysm clips, ear implants, spinal nerve stimulators, or a pacemaker, subject will not be allowed into the MRI room and cannot have an MRI. At times during the test, subject may be asked not to swallow for a while which can be uncomfortable.
- **TMS:** Single or paired-pulse TMS (not rTMS) will be used to determine the resting motor threshold as well as the amplitude of motor evoked potential on the affected and unaffected hemisphere. Although extremely rare, seizures have been described with single-pulse TMS, mainly in subjects who have a history of seizure. In some case reports, it was difficult to differentiate seizures from fainting or a convulsive syncope. The TMS coil makes noise, much like a loud pop when it produces its magnetic energy. Subject may or may not feel thumb twitch depending on the strength of the TMS pulse, but subject might also feel your facial muscles twitch slightly just around the eye. It is not painful. TMS can cause heating or movement of metallic objects in or near the head. In addition, the inactivation of pacemakers, medication pumps, cochlear prostheses and other implantable hardware may occur but subject with these metal implants is typically excluded from the study.
- **Modified constraint-induced movement therapy (mCIMT)**: The modified version of CIMT is about 120 minutes of therapy per session; it is less intensive than the original version of CIMT but still more intensive than conventional occupational therapy. It has been widely used in the clinical practice by rehabilitation therapists. It may cause muscle fatigue or mild soreness as a result of exercise which usually resolves within 12-24 hours.
- **Motor Assessments:** Motor assessments may occasionally cause fatigue.

### 2.3.2 Known Potential Benefits

Every subject will receive 120 minutes of mCIMT per session for 10 sessions over the two-week study period regardless of which tDCS dose group they are randomized. mCIMT is proven to be effective in improving upper extremity motor function by a prior NIH-funded multi-center study called The Extremity Constraint-Induced Therapy Evaluation (EXCITE) Trial.[19] The modified version was also demonstrated in the VECTOR trial.[14] It is likely that subject will experience some degree of motor improvement by participating in this study.

Additionally, it is hoped that the study team will gain valuable knowledge from the study. Successful completion of the study will help the researchers better understand the transcranial direct current stimulation, choose a right dose/current group, select the right stroke subjects based on neuroimaging or electrophysiological tool, and potentially develop a new rehabilitation modality for post-stroke motor recovery in the future.

### 2.3.3 Protection of Potential Risks

- **General Risk Loss of Confidentiality:** Subject confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. Therefore, the study protocol, documentation, data, and other information generated will be held in strict confidence. No information about the study or data will be released to any unauthorized third party without prior written approval of the sponsor.
  - The study monitor, other authorized representatives of the sponsor, and representatives of the NCC, NDMC, cIRB may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records for the subjects in this study. The clinical study site will permit access to such records.
  - The study subject's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for the duration specified by the StrokeNet Standard Operating Procedure (SOP) or longer as dictated by cIRB and local institutional regulations.
  - Study subject research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored for the duration of the study and analysis at the NDMC. The study data entry and study management systems used by clinical sites and by the NDMC research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and a Public Use Dataset (PUDS) will be archived with NINDS.
- **tDCS:** In order to protect research subjects, study staff have to strictly follow inclusion and exclusion criteria of this study (see 5.3.3. for exclusion criteria). We will record all adverse events during the intervention phase of this study and serious adverse events during the follow-up period. In addition, subject will fill out a tolerability questionnaire for every tDCS session.
- **MRI:** In order to protect research subjects, study staff have to strictly follow inclusion and exclusion criteria of this study (see 5.3.3. for exclusion criteria). *A trial site specific MR* screening form is used before MRI scan to determine MRI eligibility. The subjects will be excluded from the study if he/she has contraindication for MRI or cannot tolerate the MRI scanner. Headphones or earplugs and padding around the head are provided to the study subject to minimize the noise associated with the MR scanning and increase a subject's comfort.
- **TMS:** In order to protect research subjects, study staff have to strictly follow inclusion and exclusion criteria of this study (see 5.3.3. for exclusion criteria). In addition, sites need to follow site specific TMS policy and procedure to ensure the safety of subjects. TMS is applied by only trained staff. There will be a training workshop at the beginning of the study. The risk of seizures with single or paired pulse TMS is extremely rare. Subject with a history of seizures will be excluded from this study.
- **mCIMT:** Each therapist will be trained with mCIMT protocol prior to treating any subject and will expect to adhere to the training manuals. A short break of up to 5mins can be offered by the therapist during the session if the subject expresses being fatigued.
- **Motor assessment:** Fatigue is unlikely during the motor assessments. However, if it does happen, then study staff will adjust the pace of the assessments.

## 3 OBJECTIVES AND ENDPOINTS

| OBJECTIVES | ENDPOINTS | JUSTIFICATION FOR ENDPOINTS |
|---|---|---|

| | | |
|---|---|---|
| Primary | | |
| To determine whether there is an initial overall treatment effect among 3 dosing groups:<br>● sham + mCIMT<br>● 2 mA + mCIMT<br>● 4 mA + mCIMT<br>Efficacy is measured at day 15 after the initiation of the intervention. | FM-UE scale | FM-UE scale is the primary outcome and a measure of motor impairment |
| | WMFT time score | WMFT is a secondary outcome and a measure of functional motor activity |
| | SIS Hand Subscale | SIS Hand subscale is a secondary outcome and a measure of the quality of life |
| Secondary | | |
| To confirm that the proposed intervention is safe, tolerable, and feasible to administer in a multi-site trial setting | Rate of adverse events | Safety needs to be monitored in a phase II study |
| | Visual Analog Scale | Visual Analog Scale measures tolerability |
| | Treatment completion rate | 80% of treatment completion rate across sites without unexplained/unresolved variability is a measure of feasibility. |
| Tertiary/Exploratory | | |
| To examine whether wCST-LL (structural assessment of integrity of descending motor tract) or MEPs (functional assessment of integrity of descending motor tract) or combination of both are correlated | wCST-LL (structural) | wCST-LL is a structural measure of integrity of descending motor tract using brain MRI that has shown promise in predicting motor outcomes. |

| | | |
|---|---|---|
| with changes in FM-UE scale, and evaluate the utility of these measures as biomarkers for subject selection criteria in the future confirmatory Phase III study | MEPs (functional) | MEPs is a functional measure integrity of descending motor tract using TMS that has shown promise in predicting motor outcomes, but two of them have not been collected and compared in the same study. |
| To examine whether functional or structural changes in motor tracts correlate with changes in impairment and functional motor activity induced by the intervention. | Motor Evoked Potentials (MEPs) and Diffusion tensor imaging MRI. | MEPs can change in response to rehabilitation; Functional and structural aspects of motor tracts and the interaction between motor regions change after intervention. This change can be captured by comparing TMS and MRI measures before and after treatment |

# 4 STUDY DESIGN

## 4.1 OVERALL DESIGN

The **TRANSPORT2** (**TRANS**cranial direct current stimulation current for **P**ost-stroke m**O**tor **R**ecovery – a phase II s**T**udy) trial will test an **overall hypothesis** that a combination of bihemispheric tDCS stimulation at 2 mA or 4 mA, along with mCIMT, will lead to a greater sustained motor improvement on day 15 (± 2 days) after the start of the intervention as compared to sham stimulation. Sustained benefits will be assessed at day 45 (± 5 days) and day 105 (± 10 days) after the start of the intervention. This multicenter, phase II, sham-controlled 3-arm study will randomize 129 subjects in a 1:1:1 allocation ratio per arm (sham, 2 mA, or 4 mA tDCS), and treat subjects with the assigned dose of tDCS for 30 minutes (± 6) and mCIMT for 120 minutes (± 24) of active time, for 10 sessions over a 14 day period. However, the total time of mCIMT will be less than 150 minutes.

## 4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

Motor impairment is the most common deficit after stroke, and effective motor recovery therapies are still lacking[95, 96]. The degree of structural and/or functional injury to the descending motor pathways, quantified by the weighted corticospinal tract -lesion load (wCST-LL) and motor evoked potentials (MEPs), determines the natural outcome and the response to an experimental intervention[16, 17, 20, 97-99]. Other forms of neuroplasticity contributing to recovery include unmasking of pre-existing or new synaptic contacts through axonal sprouting, changes in myelination, and reorganization of peri-lesional or homologous areas of the contralesional hemisphere[23, 30, 100-102]. Plasticity-enhancing tools that have an additive effect are needed. One such tool is transcranial direct current stimulation (tDCS), a non-invasive brain stimulation technique, which can modulate motor cortical excitability, modify a stroke-induced abnormal inter-hemispheric imbalance, and increase synaptic plasticity when combined with a peripheral sensorimotor stimulation[21, 37, 43, 47, 76, 103]. Although several single-center proof-of-concept studies have demonstrated promising results for tDCS' potential to reduce motor impairment, results are mixed with a wide-range of effect sizes and standard deviations[32, 34, 36-38, 42, 88, 104].

The promise of tDCS is unlikely to be realized without a well-designed multi-center phase II study that increases the level of scientific rigor by addressing the following deficiencies from previous studies: (1) Applying higher stimulation dosage: A meta-analysis suggests a dose-response relationship between current density and motor improvement[39]. We recently showed that 4 mA (double the previously used maximal dose) was safe and tolerable to stroke patients in a phase I dose escalation study; (2) Using a bihemispheric stimulation montage as literature[39-41] indicated that bihemispheric stimulation (anodal over affected motor region and cathodal over contralesional region) is better than unihemispheric stimulation; (3) Improving Blinding of tDCS devices to eliminate bias from investigator, therapist and subject; (4) Adding central adjudication of outcomes; and (5) Choosing an effective, standardized and quantifiable peripheral rehabilitation therapy- Constraint-Induced Movement Therapy(CIMT)[19], as the control treatment; (6) Choice of comprehensive outcomes measures.

### 4.3 Justification for Dose

As stated above in §4.2 (1) and (2), up to 4 mA was safe and tolerable for stroke patients in a published phase I dose escalation study. Dose-response relationship and superior efficacy of bihemispheric montage by prior meta-analysis motivated us to design TRANSPORT2 to compare the 4 mA tDCS with the traditional 2 mA tDCS and sham stimulation as a control, where all 3 groups receive mCIMT as adjunctive peripheral rehabilitation therapy. 10 sessions (30 minutes of tDCS stimulation ± 6 minutes and 120 minutes ± 24 minutes of mCIMT per session) will be applied over a 14 day period.

### 4.4 End of Study Definition

Subject is considered to complete the study if he/she has completed the last visit on day 105.

## 5 STUDY POPULATION

### 5.1 Inclusion Criteria

**Each subject must meet <u>all</u> of the following criteria to participate in this study:**
1) 18-80 years old; and
2) First-ever unihemispheric ischemic stroke radiologically verified and occurred within the past 30-180 days; and
3) >10° of active wrist extension, >10° of thumb abduction/extension, and > 10° of extension in at least 2 additional digits; and
4) Unilateral limb weakness with a Fugl-Meyer Upper Extremity score of ≤ 54 (out of 66) to avoid ceiling effects; and
5) An absolute difference of FM-UE scores between the two baseline assessments that is ≤ 2 points indicating stable motor impairment; if subject is not stable, then he/she will be invited for a reassessment after 7-14 days (but no more than 3 reassessments); and
6) Pre-stroke mRS ≤2; and
7) Signed informed consent by the subject or Legally Authorized Representative (LAR).

### 5.2 Exclusion Criteria

**Each Subject who meets <u>any</u> of the following criteria will be excluded from the study:**

1) Primary intracerebral hematoma, subarachnoid hemorrhage or bi-hemispheric or bilateral brainstem ischemic strokes;
2) Medication use at the time of study that may interfere with tDCS, including but not limited to carbamazepine, flunarizine, sulpiride, rivastigmine, dextromethorphan;
3) Other co-existent neuromuscular disorders (pre- or post-stroke) affecting upper extremity motor function;
4) Other neurological disorders (pre- or post-stroke) affecting subject's ability to participate in the study;
5) Moderate to severe cognitive impairment defined as Montreal Cognitive Assessment (MOCA) score < 18/30;
6) History of medically uncontrolled depression or other neuro-psychiatric disorders despite medications either before or after stroke that may affect subject's ability to participate in the study;
7) Uncontrolled hypertension despite medical treatment(s) at the time of randomization, defined as SBP≥185 mmHg or DBP≥110 mmHg (patient can be treated, reassessed and randomized later);
8) Presence of any MRI/tDCS/TMS risk factors including but not limited to:
    8a) an electrically, magnetically or mechanically activated metallic or nonmetallic implant including cardiac pacemaker, intracerebral vascular clips or any other electrically sensitive support system;
    8b) a non-fixed metallic part in any part of the body, including a previous metallic injury to eye;
    8c) pregnancy (effects of MRI, TMS, and tDCS on the fetus are unknown);
    8d) history of seizure disorder or post-stroke seizure;
    8e) preexisting scalp lesion under the intended electrode placement or a bone defect or hemicraniectomy;
9) Planning to move from the local area within the next 6 months;
10) Life expectancy less than 6 months;
11) Has received Botulinum toxin injection to the affected upper extremity in the past 3 months prior to randomization or expectation that Botulinum will be given to the Upper Extremity prior to the completion of the last follow-up visit;
12) Concurrent enrollment in another investigational stroke recovery study;
13) Doesn't speak sufficient English to comply with study procedures;
14) Expectation that subject cannot comply with study procedures and visits.

## 5.3 Screen Failures

Screen failure is defined as a subject that signs a consent form but not randomized. The primary reason for screen failure is mainly due to inclusion/exclusion criteria. A minimal set of screen failure information is required to ensure transparent reporting of screen failure subjects, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demographics, details of screen failure, and which eligibility criteria were not met.

## 5.4 Strategies for Recruitment and Retention

We will recruit 129 stroke subjects at approximately 15 sites over a 3.5-year period. In this section, we will address the possibility of insufficient subject recruitment/retention and quality control issues that we have already installed and others that could come up during this trial. **(1) Detailed pre-submission epidemiology assessment, feasibility survey, and site selection:** Based on the detailed feasibility assessment through StrokeNet, our prior experience with stroke recovery trials including the completed Telerehabilitation Trial, patient volume and the experience with tDCS/TMS/mCIMT at StrokeNet sites, and enthusiasm for participation, approximately 15

sites that responded to the survey who are capable of conducting this trial based on the criteria will be selected. We will initiate the trial with 12 sites with an expected 4-6 subjects enrolled per site per year to ensure 129 patients' enrollment over the 3.5-year recruitment period. **(2) Careful budgetary consideration**: Funds are specifically allocated for the study coordinator to pre-screen potential subjects at the beginning of the trial. Subjects will be adequately reimbursed for the cost of traveling and parking given the number of intervention sessions of this trial. This will alleviate the burden on the participant side and improve enrollment and retention. **(3) Close monitoring of subject enrollment/retention:** We will utilize central risk-based monitoring to rapidly identify sites which deviate from the protocol and work closely with the NINDS Stroke Trials Network NCC and NDMC to address potential issues with subject enrollment, retention. Sites who fail to recruit or have a higher than expected drop-out rate after recruitment will be put on hold until an appropriate corrective action plan is developed and implemented. If the problem subsequently persists, the site will be replaced with a new site from the pool that can meet the requirements of the trial. **(4) Multi-level quality control process**: First; a required in-person workshop for investigators and team members from each site and subsequent annual online certification and recertification in the primary outcome measure; Second; selected site visits if needed by co-investigators to ensure the adherence of mCIMT protocol. Additional quality control of data entry vetting will be implemented through NDMC; Third; videotaping of primary outcome assessment for use in central adjudication purpose. Lastly, a strict blinding process will be implemented to ensure local investigator, therapist, subjects and outcome assessor are all separated and blinded to treatment assignments. **(5) Sharing of Best Practices:** We will plan for "share and learn" sessions on the topic of patient recruitment and quality control within TRANSPORT2 sites as well as other trials within StrokeNet. We will promote effective subject recruitment and quality control strategies throughout the study period.

Specific recruitment plans are as follows:

- Subjects will be identified in both the inpatient hospital setting or outpatient clinics or research subjects database;
- Clinicians and study personnel involved in clinical care of the subjects will identify the potential subjects for the study, evaluate the fit by checking the matching selection criteria, introduce the study to the subjects, offer further details of the study if the subject is interested.
- Once enrolled, study coordinators will send regular reminders of appointment dates, times and locations by approved communication channels like phone and mail address
- Throughout the trial, the recruitment of women and minorities will be closely monitored and compared to expected rates as specified in the following enrollment table; specifically the trial anticipates that males and females will be enrolled at approximately equal rates; approximately 85% of subjects will be non-Hispanic or Latino of which approximately 2% American Indian or Alaskan native, 9% Asian, 2% native Hawaiian or other pacific islander, 25% black or African American, and 63% White; among the approximately 15% of Hispanic or Latino subjects all are anticipated to be White". If at any point recruitment has significantly deviated from these targets, corrective action measures including, but not limited to, consultation with local patient advocacy and minority interest groups, retraining of study coordinators, and the development of racially/gender sensitive recruitment material will be considered. These strategies will be considered before adding sites with a disproportionate catchment of the target racial group, as this strategy would resolve imbalances within the trial but does not address the

equity/accessibility concerns within each site's community.

| Racial Categories | Ethnic Categories | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| | Not Hispanic or Latino | | | Hispanic or Latino | | | Unknown/Not Reported Ethnicity | | | |
| | Female | Male | Unknown/ Not Reported | Female | Male | Unknown/ Not Reported | Female | Male | Unknown/ Not Reported | |
| American Indian/Alaska Native | 1 | 1 | | 0 | 0 | | | | | 2 |
| Asian | 5 | 5 | | 0 | 0 | | | | | 10 |
| Native Hawaiian or Other Pacific Islander | 1 | 1 | | 0 | 0 | | | | | 2 |
| Black or African American | 13 | 14 | | 0 | 0 | | | | | 27 |
| White | 35 | 35 | | 9 | 9 | | | | | 88 |
| More than One Race | 0 | 0 | | 0 | 0 | | | | | 0 |
| Unknown or Not Reported | | | | | | | | | | |
| **Total** | 55 | 56 | | 9 | 9 | | | | | 129 |

A pre-screen interview will be conducted via phone or telehealth/ video software, for those who are interested in the clinical trial. The process may take 10-15 minutes and screen the subject with the following questions:

(1) age between 18-80 years old;

(2) date of stroke onset is less than 180 days;

(3) first-ever stroke;

(3) has minimal movement of wrist and at least 2 fingers;

(5) can speak sufficient English;

(6) can commit to 10 research sessions (5 days per week for 2 weeks; each session lasts approximately 2-3 hours);

(7) has no metallic (including shrapnel injuries) or electronic implants;

(8) can provide a list of currently taken medications;

If the answer to these 8 questions is "yes", then the potential participant will be invited to the local study site for the baseline assessment for consent and further eligibility determination. The IRB-approved prescreening interviewscriptwill be recommended to administer the screening questions. The purpose of prescreening prior to the in-person baseline visit is to reduce the number of screen failures.

## 5.5 PARTICIPANTS IN A RESEARCH TRAINING STUDY

We will recruit up to 12 participants per site in this research training study to train and standardize mCIMT, outcome assessments (FM-UE scale, WMFT and the SIS) and diagnostic tests (TMS, MRI) and tDCS prior to the first enrollment in the TRANSPORT2 clinical trial. In addition, after every 4th participant is enrolled/randomized in the clinical trial, each site will be required to demonstrate fidelity in these study procedures again. The participants for the research training study can be either stroke patient or healthy control. Each participant may consent for one or more study procedure. The same participant can be recruited again for ongoing fidelity

assessments. Any stroke patient who is consented to be part of the Research Training Study CAN NOT be recruited for the TRANSPORT2 clinical trial.

**The inclusion and exclusion for the research training study participant if <u>he/she is a participant with a stroke</u> are as followed:**

**Inclusion criteria:**
Each participant must meet <u>all</u> of the following criteria to participate in this study:
1) 18-80 years old of any gender; and
2) history of stroke with various degree of upper extremity weakness
3) Signed informed consent by the subject or Legally Authorized Representative (LAR).

**Exclusion criteria:**
Each participant who meets <u>any</u> of the following criteria will be excluded from the study:
1) Presence of any MRI/TMS/tDCS risk factors including but not limited to:
    a) an electrically, magnetically or mechanically activated metallic or nonmetallic implant including cardiac pacemaker, intracerebral vascular clips or any other electrically sensitive support system;
    b) a non-fixed metallic part in any part of the body, including a previous metallic injury to eye;
    c) pregnancy (effects of MRI or TMS on the fetus are unknown);
    d) history of seizure disorder or post-stroke seizure;
    e) preexisting scalp lesion under the intended electrode placement or a bone defect or hemicraniectomy;
2) Concurrent enrollment in another investigational stroke study;
3) Doesn't speak sufficient English to comply with study procedures;

**The inclusion and exclusion for the research training study participant if <u>he/she is a healthy participant</u> are as followed:**

**Inclusion criteria:**
Each participant must meet <u>all</u> of the following criteria to participate in this study:
1) 18-80 years old of any gender; and
2) Signed informed consent by the subject or Legally Authorized Representative (LAR).

**Exclusion criteria:**
Each participant who meets <u>any</u> of the following criteria will be excluded from the study:
1) Presence of any MRI/TMS/tDCS risk factors including but not limited to:
    a) an electrically, magnetically or mechanically activated metallic or nonmetallic implant including cardiac pacemaker, intracerebral vascular clips or any other electrically sensitive support system;
    b) a non-fixed metallic part in any part of the body, including a previous metallic injury to eye;
    c) pregnancy (effects of MRI or TMS on the fetus are unknown);
    d) preexisting scalp lesion under the intended electrode placement or a bone defect or hemicraniectomy;
2) Doesn't speak sufficient English to comply with study procedures;

For the mCIMT and outcome assessments, each site will enroll ≥ 1 participant with a stroke to train and standardize the intervention and outcome assessments. The intervention session and evaluation sessions will be videotaped and uploaded to a secure and HIPAA compliant site for

the central evaluators to determine if local therapists and evaluators meet fidelity criteria. Feedback will be provided to the local therapists and evaluators. A second study participate will be enrolled to repeat the training process above until the fidelity criteria are met. We anticipate that up to 3 participants will be needed for training and standardization purposes of mCIMT and the outcome assessments.

For the TMS procedure, each site will enroll ≥ 1 participant with a stroke or healthy participant to train and standardize the TMS procedure. The local investigator and team members will demonstrate their ability to apply TMS to obtain resting motor threshold and other TMS parameters. The data collection process will be videotaped and the TMS data will be uploaded to a secure and HIPAA complaint site and then evaluated by the study team, and feedback will be provided to the sites.

For the MRI procedure, each site will enroll ≥ 1 participant with a stroke or healthy participant to train and standardize MR imaging procedure. The local investigator and team members will demonstrate their ability to use the selected MR sequences and successfully obtain MR images. The MRI data will be uploaded to a secure and HIPAA compliant site and then evaluated by the study PIs and feedback will be provided to the sites. If the study PIs detect any problems with the data or insufficiencies in the data acquisition, then the site PI will be asked to recruit another pilot participant to demonstrate efficiency and fidelity with the TMS and/or MRI acquisition.

For the tDCS, each site will enroll ≥ 1 participant with a stroke to train and get familiar with the device. The local investigator and team members will demonstrate their ability to use the device. A co-investigator is available over the phone or a HIPPA compliant video software for question or feedback or troubleshooting.

The research MRI scans performed for this study are different from those that would be used for a clinical evaluation. Since the MRI scans are not designed for clinical use. We will not provide any medical reading interpretation of the scans to the participants and not including any reading of the scans into the medical records. Nevertheless, should the neurologist, who has extensive experience in neuroimaging including MR images find anything unusual in the images, we will communicate this to the participant and to the primary care physician of the participant if the participant instructs us to do so.

# 6 STUDY INTERVENTION

## 6.1 STUDY INTERVENTION(S) ADMINISTRATION

### 6.1.1 STUDY INTERVENTION DESCRIPTION

**Transcranial Direct Current Stimulation (tDCS):**

tDCS will be delivered to the study subjects through two electrodes (5 × 7=35 cm²) via a direct current delivery device. In the bihemispheric montage, the anodal electrode will be placed over the ipsi-lesional and the cathodal electrode over the contra-lesional motor cortex (C3 and C4 using the international 10 –20 EEG electrode systems). Electrode pads will be disposed after each week or 5 sessions. Pads will not be shared across subjects. Stimulation will last for 30 minutes ($\pm$ 6)minutes for each session. Subject will receive either sham, 2 mA or 4 mA. Records of current will be checked and kept separately without unbinding the sites.

**mCIMT:**

The mCIMT protocol in this study is adapted from a protocol published in the EXCITE and VECTOR trials[14, 19]. The premise of the strategy is that repetitive object grasping and manipulation practice reduces the effects of "learned non-use." mCIMT will be delivered 120 minutes ($\pm$ 24)minutes a day (with the first 30 minutes ($\pm$ 6)minutes simultaneously with tDCS stimulation) for 10 sessions over 2 weeks with the unaffected arm in a constraining mitt for at least 6 hours/day during waking hours. Subjects will not be allowed to use the constrained hand within the task effort. The tasks attempted during the therapy session will be menu driven. Activities will be selected from a task menu, and an activity log kept demonstrating what tasks have been attempted. Each activity will be carried out for 15-30 minutes and activities selected should be challenging yet feasible for the subject, contextually appropriate (i.e., with regards to interests) and challenge movements that need improvement.

Training will primarily consist of task practice (TP) activities designed to promote increased use of the affected upper extremity during functional activities. These activities simulate activities of daily living (ADL) and instrumental activities of daily living (IADL), i.e., common daily activities. If a subject is unable to perform TP activities, the trainer may select activities from a menu of adaptive task practice (ATP) activities. ATP activities are comprised of the basic units or components of larger activities employed in TP. ATP activities focus on improving skills such as grasp, manipulation, dexterity, gross motor control, endurance, timing, and active range of motion. Whenever possible, ATP should be transitioned into TP activities as the subject's skill level increases. The subjects will be provided with feedback (specific knowledge of results about a subject's performance over a session); coaching (suggestions verbally to improve performance); modeling (physically demonstrate a task with the purpose of improving a subject's performance); and encouragement (providing motivation to subjects verbally to promote maximal effort). Patients will wear the mitt for at least 6 hours of waking hours at home. Behavioral techniques to enhance mitt use outside of the research laboratory will include the use of a behavioral contract daily schedule, and encouragement from the supervising therapist to practice 2 to 3 specific tasks daily at home. A behavior contract will be provided to the subject at the end of the first intervention session.

#### 6.1.2 Dosing and Administration

Subjects will be 1:1:1 randomized to sham, 2 mA, or 4 mA group. Investigator, therapist and the subject will not be informed of the group assignment. All subjects will be prepared in the same way. tDCS will be executed by entering WebDCU-generated code in the computer, which will initiate one of the three interventions according to the group to which the subjects was randomized: sham, 2 mA or 4 mA. Thirty minutes of tDCS (including sham) will be accompanied by 120 (±24) minutes of mCIMT with both starting at the same time, overlapping for the first 30 (±6) minutes, and leaving the remaining 90 minutes for mCIMT only. tDCS is delivered by a small, battery-operated device, portable (e.g., can be mounted to a belt) and it does not interfere with mCIMT.

### 6.2 Preparation/Handling/Storage/Accountability

#### 6.2.1 Acquisition and accountability

In order for a new medical product to reach patients, it must first be proven safe and effective and then be approved for use by the governing regulatory body. Clinical trials are the vehicle to bring these innovative technologies to subjects and evaluate them in support of regulatory approval. In clinical research the products are still investigational, these products are treated differently than

those items used in general medical practice. After all, using an unapproved product that is not fully vetted for safety and efficacy outside of the trial protocol requirements could result in risks to subjects.

Failure to account for and manage study materials could affect the acceptability of the data collected from a trial, or even termination of a study completely. Both sponsors and investigators have responsibility for device accountability and can be held accountable if problems are identified.

Accountability:

- The site investigator is responsible for the investigational product/device accountability at the trial site
- The site investigational product/device should be stored in accordance with the applicable regulatory requirements.
- The site investigator should ensure that the investigational product/device are used only in accordance with the approved protocol.

### 6.2.2 Product Storage and Stability

The device should be accessible by the study team only. The device, including the battery, wires, and electrode pads will be checked before and at the end of each session. Each site will be dispensed with two tDCS systems (one for use and another for backup). Any issue should be reported immediately to the site investigator and further to the tDCS core team

### 6.2.3 Preparation

tDCS core personnel will prepare and test the tDCS system before dispensing to the site staff. The site staffs will attend a required onsite training workshop to gain knowledge how to operate the tDCS system.

## 6.3 Measures to Minimize Bias: Randomization and Blinding

On the randomization day, a study coordinator or a team member will log into WebDCU™ to randomize subjects who have been consented and found eligible. Briefly, at the beginning of each intervention session, the coordinator will complete tDCS Treatment Activation Code Request to obtain a new tDCS treatment activation code daily. Each day, the coordinator will need to enter the new code into the tDCS machine which will translate the code to the appropriate stimulation voltage using a pre-loaded script **(Fig 8, right)**. The treating therapist is only to conduct mCIMT on the study subject, another study team member should conduct concurrent tDCS stimulation session to avoid potential bias.

Subjects will be allocated equally among the three treatment arms using a covariate adaptive randomization algorithm which controls for serious imbalances in covariates considered to have an impact

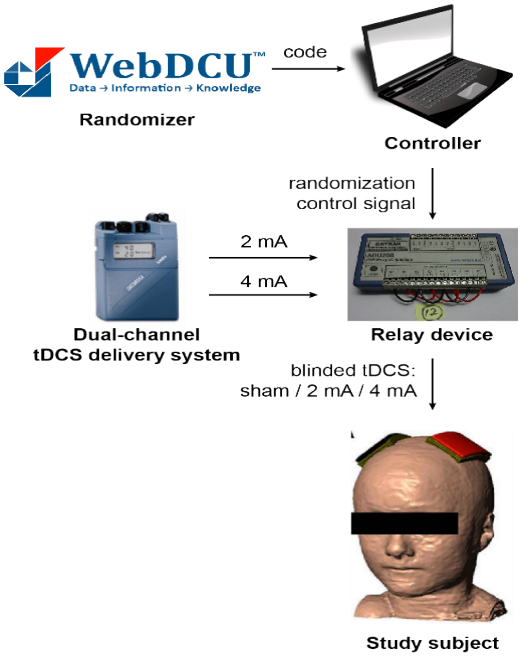



**Fig 8. Schematic of interface between tDCS control, dual-channel tDCS delivery system, and WebDCU™ interface.**

on outcome; specifically, study site, time from stroke, and baseline motor impairment measured using FM-UE scale. At the time of randomization, serious imbalances in these covariates will be accounted for using a minimal sufficient balance approach[105]. That is, at the time of randomization, an overall treatment imbalance and imbalance in each of the covariates is assessed. If no imbalances exceed the tolerated threshold, the subject is randomized according to the specified allocation ratio (1:1:1). If an unacceptable level of imbalance exists, the algorithm will determine whether the current allocation ratio improves or exacerbates the current imbalance and adjust the ratio accordingly. The detailed randomization scheme and source codes will be provided in the Randomization Plan document.

### 6.4 Study Intervention Compliance

Compliance with study treatment will be centrally monitored.

## 7 STUDY INTERVENTION DISCONTINUATION AND SUBJECT DISCONTINUATION/WITHDRAWAL

### 7.1 Discontinuation of Study Intervention

Discontinuation from tDCS and mCIMT intervention does not mean discontinuation from the study, and remaining study outcome assessments should be completed as indicated by the study protocol. If a clinically significant finding is identified (including, but not limited to changes from baseline) after enrollment, the investigator or qualified designee will determine if any change in subject management is needed. Any new clinically relevant finding will be reported as an adverse event (AE). An investigator may discontinue or withdraw a subject from study intervention for the following reasons:

- Pregnancy, but it is unlikely as woman of child-bearing potential will have urine pregnancy test to confirm or exclude the possibility of a pregnancy.
- Significant study intervention non-compliance, defined as missing >2 sessions out of 10 sessions and unable to make it up within the 2 week/14 days intervention period;
- If any adverse event (AE), laboratory abnormality, other medical condition or situation occurs such that continued participation in the study intervention would not be in the best interest of the subject.
- Disease progression (e.g., 4 points or more increases in the NIHSS score or recurrent stroke) which requires discontinuation of the study intervention.
- If a subject meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation.
- Subject suffers another stroke, neurological or neuromuscular disease during the study period that may have an impact on motor recovery.

The reason for intervention discontinuation from the study will be recorded on the Case Report Form (CRF). The subject should continue to be followed and have all study assessments.

### 7.2 Subject Withdrawal from the Study

Subjects are free to withdraw from participation in the study at any time upon request. The reason for subject withdrawal from the study will be recorded on the Case Report Form (CRF). Subjects who sign the informed consent form, and are randomized and receive the study intervention, and subsequently withdraw will not be replaced.

### 7.3 LOST TO FOLLOW-UP

A subject will be considered lost to follow-up if he or she fails to return for three scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a subject fails to return to the clinic for a required study visit:

- The site will attempt to contact the subject and reschedule the missed visit within 2 weeks and counsel the subject on the importance of maintaining the assigned visit schedule and ascertain if the subject wishes to and/or should continue in the study.
- Before a subject is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the subject (where possible, ≥3 telephone calls if necessary). These contact attempts should be documented in subject's study file.

## 8 STUDY ASSESSMENTS AND PROCEDURES

### 8.1 EFFICACY ASSESSMENTS

**Primary Objectives (Efficacy Outcomes):** To determine whether there is an overall intervention effect among 3 dosing groups (sham+mCIMT, 2 mA+mCIMT, and 4 mA+mCIMT) at day 15 after initiation of the intervention in the Fugl-Meyer Upper-Extremity (FM-UE) scale[106], a measure of motor impairment. FM-UE scale consists of a 33-item assessment which provides a global assessment of UE motor impairment. A rater observes 30 voluntary UE motions and 14 voluntary LE motions, 6 tendon tap responses, and provides an ordinal rating (2=near normal ability/response, 1=partial ability, 0=unable to perform/no response). FM-UE scale is a proven scale with excellent intra-rater reliability (0.99), inter-rater reliability (0.99), test-retest reliability (0.94 –0.99), and internal consistency (0.97). [107-109]

Secondary efficacy outcome measures include the Wolf-Motor-Function-Test (WMFT)[110-112], a measure of functional motor activity, and the Stroke-Impact-Scale (SIS)[113], a measure of the quality of life at day 15 after initiation of the intervention. These efficacy outcomes will be collected at the baseline, day 15, day 45 and day 105. WMFT quantifies upper extremity (UE) motor ability through timed and functional tasks. It is a 17-item assessment providing a measure of UE functional ability. In this test, participants are timed as they complete 17 tasks that involve movements and interactions with objects (e.g., placing hand on table, picking up a soda can). These activities begin with those involving only shoulder movements and progress to those requiring distal control of fine motor movements. In addition, subject's movement will be assessed for meeting "**Essential Elements**" (specific elements) that must be accomplished in order for the task to be deemed complete" vs. "**Desired Elements**" which are other qualitative elements that should be included in the task but are not necessary for completion" (please refer to details in the Outcome Assessments MOP, located in the WebDCU Toolbox). [115]

SIS is a stroke-specific, self-report, health status measure assessing multidimensional stroke outcomes.[113, 114]The current 3.0 version includes 59 items and assesses 8 domains: strength (4

items), hand function (5 items), Activities of Daily Living / Instrumental Activities of Daily Living (ADL/IADL, 10 items), mobility (9 items), communication (7 items), emotion (9 items), memory and thinking (7 items), and participation (8 items). The SIS hand function domain is a valid and reliable measure that is well aligned with TRANSPORT2 specific aims. It has face validity for clinical meaningfulness and this self-report measure of hand function corresponds well with other outcomes. While the specific effects of the Intervention are expected to Influence the hand function domain the most, we also expect several non-specific effects on health status generally and the composite physical performance and social participation domains contained in the full SIS. This was true for the EXCITE trial which established the efficacy of mCIMT.

### 8.1.2 Exploratory AIMs Measures

#### 8.1.2.1 Imaging Measures

We will determine **two imaging measures**: weighted corticospinal tract lesion load (**wCST-LL**) that be derived from the pre-intervention MRI and the **fractional anisotropy (FA) measure that** will be derived from the pre- and post-intervention MRI. These imaging measures support the exploratory aims/objectives outlined in section 3 above.

**wCST-LL:** Three MRI sequences (T1-weighted, T2-weighted, and FLAIR imaging) will be used to define and quantify the stroke lesion. A 3D map of this lesion will be overlaid onto a canonical corticospinal tract which was derived from a group of elderly healthy control subjects and exists in our lab as a representative map of the CST; (see Feng et al., 2015 for details). The overlap area between the lesion and the canonical CST will constitute our measure of wCST-LL. The weighting of the lesion load is related to the probabilistic nature of the tract and its geometric changes while it traverses from the cortex through the internal capsule into the brainstem. The **wCST-LL** is a continuous measure expressed in cubic centimeter (cc) with 2 decimals.

**Corticospinal Tract - fractional anisotropy values (CST-FA):** FA values are derived from an MRI sequence called diffusion tensor imaging (DTI). FA values will be determined in maps of the CST. Canonical maps of the CST (stretching from the posterior limb of the internal capsules to the lower brainstem) exist in our laboratory and have been previously described (see Zheng and Schlaug, 2015 for details). FA is a measure of tract integrity or how well fibers are aligned in one direction in a tract. Values are between 0-1. Fractional anisotropy values tell us something about the direction of water flow. If the value is closer to 1, water flows along major fiber tracts in one particular directions, which means that the fibers are healthy and well aligned. Typically, on the side of the stroke, we might see a decrease in FA or water not only flowing in one direction but multiple directions, reflecting a degeneration of a fibers in a tract as an effect of the stroke. FA values derived from a template of the CST on the lesional and contralesional hemisphere will be determined in the pre-intervention MRI and post-intervention MRI and changes in these FA values will be correlated with behavioral changes within the three treatment groups as specified in the SAP.

#### 8.1.2.2 TMS Measures

We will determine **two TMS measures**: presence of **Motor Evoked Potential (MEP)** and amplitude of MEPs. These TMS measures support the exploratory aims/objectives outlined in section 3 above.

**Presence or absence of Motor Evoked Potential (MEP):** The resting motor threshold (rMT) is the lowest stimulus intensity of TMS that gives a recordable MEP from abductor pollicis brevis (50 $\mu$V peak-to-peak). It is a measure of global motor system excitability at both cortex and spinal cord level. This parameter will be categorized into a binary variable (Present/1 or Absent/0) for the purpose of analysis. Present/1 means there is a recordable MEP, Absent/0 means there is no recordable MEP. Presence of MEP in a subject with a stroke typically suggests some degree of preservation of the corticospinal tract. It is possible that MEP is absent on the affected limb despite applying the stimulus on the lesional hemisphere if the CST is severely injured. rMT from non-paretic side is collected as well and serves as a reference to the paretic side. It is estimated that ~20% of patients may not have a recordable MEP from this study. If it turns out that only < 10% of patients do not have MEP and the number of subjects in the absent group is too small. We will use an alternative to analyze the data. We will use asymmetric index {i.e. (absolute value rMT on the affected side – absolute value of rMT on the unaffected side) / absolute value rMT on the affected side). If MEP is absent, a value of 100 % maximum stimulator output (MSO) will be assigned as rMT. If MEP is present, the rMT (% MSO required to produce 50 $\mu$V peak-to-peak using PEST algorithm) will be compared between baseline and post-therapy sessions.

**Size of the Motor Evoked Potential (MEP)** The size of MEP (i.e. the peak to peak amplitude of a stable MEP that is ≥1 mV) provides an estimate of the extent of corticospinal tract and motor neuron activation by the magnetic stimulation. This is only done if the patient has a recordable MEP first. If patient does not have a recordable MEP, the amplitude of MEP will be set as 0. If the patient has a recordable MEP, the % MSO required to produce 1 mV peak-to-peak using PEST algorithm will be determined on baseline and post-therapy sessions. This value will be used as a testing stimulus (TS) in the paired-pulse stimulation (below) and therefore will be called as testing motor threshold (tMT). Patients with non-recordable 1 mV peak-to-peak MEP will be applied 100% MSO as TS in paired-pulse stimulation routine. 20 MEP at tMT with 4-7 seconds inter-pulse interval will be collected to determine the size of MEP. Comparison of MEP size will be performed between baseline and post-therapy sessions using baseline visit tMT to ensure that same stimulus intensity when comparing MEP size. The size of MEP from the non-paretic side will be collected as well and serves as a reference to the paretic side.

## 8.2 Adverse Events and Serious Adverse Events

A tDCS questionnaire will be used in each intervention session to assess the common adverse events or tolerability issues associated with its use. Adverse events, including serious adverse events will be collected during the intervention period. Only serious adverse events or clinically related (possibly or definitely) adverse events will be collected after intervention period. Please refer to AE reporting section for details.

### 8.2.1 Definition of Adverse Events (AE)

An adverse event (AE) is defined as any new untoward medical occurrence or worsening of a preexisting medical condition in a clinical investigation subject administered study intervention and that does not necessarily have a causal relationship with this intervention. An AE can therefore be any unfavorable and unintended sign (such as an abnormal laboratory finding), symptom, or disease temporally associated with the use of the investigational intervention, whether or not considered related to the investigational intervention. Adverse events can be spontaneously reported or elicited during open-ended questioning, examination, or evaluation of a subject.

### 8.2.2 Definition of Serious Adverse Events (SAE)

A SAE is any untoward medical occurrence that:

- results in death;
- is life-threatening (defined as an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe);
- requires inpatient hospitalization or causes prolongation of existing hospitalization;
- results in persistent or significant disability/incapacity;
- is a congenital anomaly/birth defect;
- is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention (e.g., medical, surgical) to prevent one of the other serious outcomes listed in the definition above. Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.)

The definition of SAE excludes the following hospitalizations:

- A visit to the emergency room or other hospital department < 24 hours, that does not result in admission (unless considered an important medical or life-threatening event);
- Elective surgery, planned prior to signing consent;
- Admissions as per protocol for a planned medical/surgical procedure;
- Routine health assessment requiring admission for baseline/trending of health status (e.g., routine colonoscopy);
- Medical/surgical admission other than to remedy ill health and planned prior to entry into the study (appropriate documentation is required in these cases);
- Admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (e.g., lack of housing, economic inadequacy, caregiver respite, family circumstances, administrative reason).

### 8.2.3 Classification of an Adverse Event

#### 8.2.3.1 Severity of Event

The severity of AEs will be reported using the grading system outlined in the NCI Common Terminology Criteria for Adverse Events Version 4.03 (CTCAE). The CTCAE provides a grading (severity) scale for each AE term and AEs are listed alphabetically within categories based on anatomy or pathophysiology. The CTCAE displays Grades 1-5 with unique clinical descriptions of severity for each AE based on this general guidance:

The complete definitions of these grades are:

- Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated AE.
- Grade 2: Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.).
- Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living (bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden).

- Grade 4: Life-threatening consequences; urgent intervention indicated.
- Grade 5: Death related to AE.

#### 8.2.3.2 Relationship to Study INTERVENTION

All adverse events (AEs) must have their relationship to study intervention assessed by the clinician who examines and evaluates the subject based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

1. Unrelated: The temporal relationship between treatment exposure and the adverse event is unreasonable or incompatible and/or adverse event is clearly due to extraneous causes (e.g., underlying disease, environment);

2. Unlikely: Must have both of the following 2 conditions, but may have reasonable or only tenuous temporal relationship to intervention:
    - Could readily have been produced by the subject's clinical state, or environmental or other interventions.
    - Does not follow known pattern of response to intervention.

3. Reasonable Possibility: Must have at least 2 of the following 3 conditions:
    - Has a reasonable temporal relationship to intervention.
    - Could not readily have been produced by the subject's clinical state or environmental or other interventions.
    - Follows a known pattern of response to intervention.
4. Definitely: Must have all 3 of the following conditions:
    - Has a reasonable temporal relationship to intervention.
    - Could not possibly have been produced by the subject's clinical state or have been due to environmental or other interventions
    - Follows a known pattern of response to intervention.

#### 8.2.3.3 Expectedness

In general, expected adverse reactions are AEs that are known to occur for the study intervention being studied and should be collected in a standard, systematic format using a grading scale based on functional assessment or magnitude of reaction. Identify the source of the reference safety information used to determine the expectedness of the AE (e.g., IB, approved labeling). Expectedness is assessed based on the awareness of AEs previously observed, not on the basis of what might be anticipated from the properties of the study intervention. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

### 8.2.4 Time Period and Frequency for Event Assessment and Follow-Up

The IMSM who is independent of any involvement with the study will monitor the study with regard to the safety concerns when adverse events or serious adverse event occurs. The IMSM will review all SAEs and determine whether they are related to study drug administration (as described above); a query to the site will be generated to amend the AE narrative if necessary for

any questions or clarifications regarding an event. Periodically throughout the study, the Executive Committee and the IMSM will review reports on the incidence rates of all reported AEs, whether serious or not and blinded to treatment assignment. Should such monitoring uncover issues that may threaten subject safety (e.g., unexpectedly high rate of AEs), a notification will be sent to the DSMB liaison for review proposing further actions to be taken, if any. Two statistical reports will be generated semiannually (unless requested at an alternative interval by the IMSM or DSMB) – an open report to be distributed to the Executive Committee and IMSM, and a closed report to be distributed only to the DSMB. Each semi-annual report will provide cumulative summary statistics on enrollment, subject status in the study, baseline characteristics, protocol violations, safety data (including a summary of the most frequent and most serious AEs, and a listing of all subjects who were terminated from the study and the reason for termination), and data management/quality information. The open report statistics will be provided for the overall study with no separation of intervention groups. The closed report will provide cumulative summary statistics by partially blinded intervention group to DSMB members, the NIH liaison, and the project's unblinded statistician. All people with access to the closed reports are fully independent of trial operation and have no impact on subject recruitment, intervention, and assessment. If the DSMB wishes to be completely unblinded for these reports, a sealed identification envelope will be provided to the DSMB liaison; this envelope can be opened at the discretion of the DSMB.

#### 8.2.5 Adverse Event Reporting

Required reporting must be submitted through the StrokeNet WebDCU™ as described and within the timeframes described in NIH StrokeNet Network Standard Operating Procedure ADM 12 Central Institutional Review Board (cIRB) Reporting https://www.nihstrokenet.org/docs/default-source/strokenet-sops/adm12_cirb_reporting_060314.pdf?sfvrsn=2.

#### 8.2.6 Serious Adverse Event Reporting

All SAEs are required to be reported in WebDCU™ within 24 hours of the study site being made aware of the occurrence of the SAE. The investigators are required to provide relevant information such as description of the SAE, date/time of onset and resolution, severity and seriousness, action taken, and suspected relationship to the study intervention. Reporting of SAEs will trigger notification of the event to the Project Manager (PM). After reviewing the SAE for completeness and accuracy, the PM will forward the SAE to the MSM who will conduct an independent review of each SAE to determine its relationship to the study intervention along with other elements. Within 72 hours of receipt of the SAE for review, the IMSM will enter his opinion into WebDCU™ as to whether the SAE is, in fact, serious, unexpected, and related to the study drug. After the submission of the initial SAE Report, the site investigator at the corresponding clinical site will be responsible for obtaining follow-up information about the event and reporting it in WebDCU™.

### 8.3 Unanticipated Problems

#### 8.3.1 Definition of Unanticipated Problems (UP)

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to subjects or others to include, in general, any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;

- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

This definition could include an unanticipated adverse device effect, any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects (21 CFR 812.3(s)).

### 8.3.2 Unanticipated Problem Reporting

The investigator will report unanticipated problems (UPs) to the cIRB. The UP report will include the following information:

- Protocol identifying information: protocol title and site name;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents a UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are serious adverse events (SAEs) will be reported to the cIRB within 10 days of the investigator becoming aware of the event.
- Any other UP will be reported to the DCC/study sponsor within 5 days of the investigator becoming aware of the problem.
- All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), and the Office for Human Research Protections (OHRP) within 1 month of the IRB's receipt of the report of the problem from the investigator.

### 8.3.3 Reporting Unanticipated Problems to Participants

Unanticipated problems judged after review by the TRANSPORT2 protocol PIs, cIRB, DSMB, or NINDS to require changes to the informed consent, study protocol, or background knowledge and training provided to sites will be communicated to all participating site PIs by the National Coordinating Center (NCC) or NDMC. This will be accomplished by email and may be supplemented by request for confirmation of receipt and follow-up by phone, all with assistance of the relevant Regional Coordinating Center when necessary. Any change in protocol or risk of the research will be communicated by site staff, by phone (supplemented by other means as necessary), to active intervention arm participants or their caretakers/other contacts.

## 9 STATISTICAL CONSIDERATIONS

## 9.1 Statistical Hypotheses

- Primary Efficacy Endpoint(s):

The primary null hypothesis is that there is no difference in the least-squares mean estimate of the change in FM-UE scale on Day 15 after the initiation of the 14 day intervention among the three dose groups.

$$H_0 : \hat{Y}_{sham,15d} = \hat{Y}_{2\,\mathrm{mA},15d} = \hat{Y}_{4\,\mathrm{mA},15d}$$

$$H_A : \text{The means are not all equal}$$

- Secondary Efficacy Endpoint(s):

The secondary null hypothesis is that there is no difference in the least-squares mean estimate of the change in Wolf-Motor Functional Test (WMFT) time score and Stroke Impact Scale (version 3.0, SIS) hand subscale.

## 9.2 Sample Size Determination

A change of 4.25-7.25 points on the FM-UE scale is considered to be a meaningful clinically important difference (MCID)[116]. This study is powered under the assumption that mCIMT alone, will at least achieve this intervention effect (4.5) and furthermore intervention with either 2 mA or 4 mA tDCS will further increase the change in FM-UE scale from the baseline by 4.5 points (i.e., a minimum intervention effect of 9.0). Secondly, based on the meta-analysis of previous trials assessing tDCS in stroke patients[39], a conservative estimate of the intervention variability is defined as SD = 7. Based on these assumptions, the current study requires 31 subjects per arm to estimate the 90% confidence interval for the treatment effect in each arm with a standard error of ± 1.3, assuming an ANOVA-type testing approach which is more conservative than the proposed analysis model which includes multiple time points and adjusts for covariates.

This level of precision was determined to be sufficiently informative to plan a Phase III trial. Specifically, the confidence interval for a clinically meaningful treatment effect will exclude the hypothesized null effect size of 4.5; that is, the estimate and 90% confidence interval for a clinically meaningful treatment might be 9.0 (6.9, 11.1). Moreover, the choice of standard error represents a balance between the level of precision in the estimate and the number of subjects/resources expended during this phase of investigation **(Fig. 9, below).** Further, if the estimate of the standard deviation were overly conservative, the precision of the treatment effect estimate would increase. (N.B. Planning estimates of the null treatment effect size, 4.5, do not impact power as long as the MCID from stimulation remains at 4.5 points.)

In addition, with a sample size of 31 subjects per group, a two-sided type I error rate of 10%, and standard deviation of 7, if the true pattern of mean changes is 4.5, 9.0, and 9.0 for the sham, 2 mA, and 4 mA groups respectively, we would have 83% power to reject the null hypothesis. A 10% type I error rate was selected in consideration of the track record for safety in the intervention, the relative cost of committing a type I and type II error with respect to trial duration, subject utilization, and the remaining need for a subsequently planned Phase III trial should the current trial demonstrate promising results. Based on a conservative estimate from the EXCITE and VECTOR trial[14, 19], 8% of subjects are expected to complete less than 10 full sessions of tDCS + mCIMT in the specified 14 day time-window. Thus, the sample size is inflated for the cross-over in treatment effect using an inflation factor of $1/(1-0.08)^2$.[117] In addition, up to 15% of subjects may be lost to follow-up prior to collection of the primary outcomes at the 3-month follow-up visit.

Therefore, an inflation of 115% is used to account for the missing samples in the ITT primary analysis. As a result, the final estimated sample size is 43 per group (or 129 subjects in total). Finally, considering the longitudinal nature of the design and adjustment for key covariates, the power will be maintained when using an auto-regressive (1) correlation structure regardless of the degree of correlation between FM-UE scale measured on proximal visits or the strength of the covariates as predictors. We elected not to further reduce the sample size both because a robust estimate of these quantities is not currently available, and to retain sufficient power to evaluate secondary hypotheses.

## 9.3 Populations for Analyses

- Intent-to-treat (ITT) population

All analyses will be conducted under the ITT principle. That is, the evaluable sample includes all randomized subjects, regardless of whether or not the subjects completed the study protocol; where subjects are classified according to the intervention assignment, they are randomized into.

- Per-protocol (PP) population

Sensitivity of the primary findings will be assessed using a per-protocol sample defined as all subjects who (a) complete at least 8 out of 10 sessions of the intervention; (b) complete the FM-UE scale with all three post-intervention visits; (c) do not experience a recurrent clinical stroke or other pre-specified illness known to impact upper extremity motor functioning during the study period (i.e., Bone fracture in the affected arm, newly diagnosed neuromuscular disease, cervical spine injury.); and (d) do not receive any other forms of rehabilitation therapy after 2 weeks of intervention. The intent of this analysis sample is to assess the maximum possible treatment effect achievable with tDCS at a dose of either 2 mA or 4 mA. It is anticipated that <50% of subjects will receive additional forms of rehabilitation therapy after the 14 day intervention (subjects enrolled in the subacute phase are the ones who have likely received additional rehabilitation therapy), however, if this percentage exceeds 50% the PP sample will exclude criteria (d).

## 9.4 Statistical Analyses

Details of the statistical analyses are provided in the Statistical Analysis Plan.

### 9.4.1 General Approach

The goal of this proposed phase-2 clinical trial is assessing whether bihemispheric transcranial Direct Current Stimulation (tDCS) at 2 mA or 4 mA, when combined with modified constraint-induced movement therapy (mCIMT) will evoke a motor impairment reduction as measured by the Fugl-Meyer Upper Extremity (FM-UE) scale immediately after the 14 day intervention and at the time of 1 and 3 months follow-up when compared to sham stimulation combined with mCIMT. The central hypothesis is that tDCS, a non-invasive brain stimulation technique, can enhance current post-stroke motor recovery by inducing plasticity and potentiating the brain to be receptive to proven rehabilitation techniques (i.e., mCIMT). However, it is not known whether the current maximum dose (2 mA) is sufficient or whether a higher dose (4 mA) may evoke a better response while maintaining a similar safety and tolerability profile.

### 9.4.2 Analysis of the Primary Efficacy Endpoint(s)

The change in FM-UE Scale in each treatment arm will be modeled using generalized linear mixed effects repeated measures model, where the dependent variable is the change from baseline in

FM-UE to post-intervention visit; the model is adjusted for intervention arm, baseline FM-UE, time from stroke, visit and site; and the primary outcome is the fitted estimate at day 15. Model assumptions, including normality, will be assessed and appropriate transformations will be used in keeping with best practices and conventional approaches in the literature. If the primary null hypothesis is rejected, all pairwise secondary hypotheses will be tested using a Dunnet type multiple comparisons correction[118, 119].

### 9.4.3 Analysis of the Secondary Endpoint(s)

Aim 1 will also test the secondary null hypotheses that there is no difference in the least-squares mean estimate of the change in Wolf-Motor Functional Test (WMFT) time score and Stroke Impact Scale (SIS, version 3) hand subscale at day 15. The procedure described above will be repeated for each of the secondary endpoints with the type-I error rate adjusted using a Bonferroni type gate-keeping procedure to sequentially test the hierarchical null hypotheses[120, 121]. That is, if the primary null hypothesis (i.e., no difference in the change in FM-UE Scale between the three groups) is rejected, the secondary null hypothesis of no difference with respect to WMFT time score will be assessed; if that null hypothesis is also rejected, then the null hypothesis of no difference in the SIS hand subscale will be tested.

### 9.4.4 Safety Analyses

***Safety: Analysis Method***

IMSM and Data and Safety Monitoring Board (DSMB) will receive periodic safety reports of all reportable adverse events including serious adverse events (SAEs) if there are any. All clinical safety endpoints and SAEs will be summarized by AE code (as provided on the AE CRF) regarding the frequency of the event, the number of subjects having the event, severity, and relatedness to the study treatment. Clinically important adverse events include:

- Severe headache
- Second-degree skin burn
- Clinical seizure
- Neurological deterioration (≥ 4-point increase in NIHSS)

The proportion of subjects experiencing each of these events will be provided in the closed report by intervention arm with two-sided 95% CIs and unadjusted relative risks. Based on the Phase I dose-escalation study, no subjects experienced any of the clinically important adverse events. Fisher's exact tests will be used to assess intervention group differences in the rates of clinically important adverse events.

***Tolerability: Analysis Method***

Tolerability will primarily be measured through the use of the Visual-Analog scale (VAS), a 10-point scale ranging from 0 (No Discomfort) to 10 (Extreme Discomfort). For the primary analysis, any subject who requests to be withdrawn from the study due to procedure related issues will be imputed as a 10 on the Visual-Analog Scale. Differences in tolerability will be assessed using a Kruskal-Wallis test at a 10% type-I error rate.

### 9.4.5 Baseline Descriptive Statistics

The characteristic of subjects at baseline will be tabulated ordered by 3 dose groups. p value is based on ANOVA if the variable is continuous and is based on Chi-squared test if the variable is categorical.

#### 9.4.6 Sub-Group Analyses

In pre-specified subgroup analyses, the primary analysis (§9.4.2) will be repeated but include an interaction effect between sex/gender or race/ethnicity and intervention arm. Differential effects of tDCS by sex/gender or race/ethnicity are not anticipated, and therefore the trial is not powered specifically for these subgroup analyses, but these subgroup analyses will allow any unexpected large variations to be identified and regardless of outcome will assist with planning a Phase III trial which will account for differences if identified here.

Additional analyses are specified in the statistical analysis plan (SAP).

#### 9.4.7 Tabulation of Individual participant Data

Individual subject data will not be listed or tabulate by measure and time point. Only summarized data by dose group and time points and measures will be presented.

## 10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

### 10.1 Regulatory, Ethical, and Study Oversight Considerations

#### 10.1.1 Informed Consent Process

##### 10.1.1.1 Consent/assent and Other Informational Documents Provided to participants

Consent forms describing in detail the study intervention, study procedures, and risks are given to the subject, and written documentation of informed consent is required prior to starting intervention/administering study intervention.

##### 10.1.1.2 Consent Procedures and Documentation

Informed consent is a process that is initiated prior to the individual's agreement to participate in the study and continues throughout the individual's study participation. Consent forms will be Institutional Review Board (IRB)-approved, and the subject will be asked to read and review the document. The investigator will explain the research study to the subject/LAR and answer any questions that may arise. A verbal explanation will be provided in terms suited to the subject's comprehension of the purposes, procedures, and potential risks of the study and their rights as research subjects. Subject/LAR will have the opportunity to carefully review the written consent form and ask questions before signing. Subject/LAR should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. Subject/LAR will sign the informed consent document prior to any procedures being done specifically for the study. Subject who is cognitively capable will sign informed consent; If subject is unable to provide written informed consent due to cognitive impairment (e.g., NIH Stroke Scale Level of Cognition Questions 1-3 combined score >= 3), a Legally Authorized Representative (LAR) will sign informed consent instead. During the follow-up visit, subject who was initially cognitively impaired

will be reassessed his/her cognition by study team member, if subject regains cognitive capability, he/she will be given an opportunity to decide if he/she still wants to remain in the study. Subject/LAR must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the subject/LAR for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed before the subject undergoes any study-specific procedures. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

In an effort to review informed consent forms in a timely manner, enrolling sites will upload a pdf of the signed informed consent form, into the password protected clinical trial management system, WebDCU™. The PDF file will be linked to the subject ID but will be stored on a secure server separate from the study's CRF data. The secure server on which these files are stored is not backed up to prevent copies of files containing individually identifiable health information from being copied and stored on non-NDMC back up servers. The files on these servers can only be accessed by designated NDMC study personnel. NDMC staff will remotely monitor the informed consent forms and issues identified will be relayed to the clinical site for corrective and preventative action. After remote monitoring is complete, the PDF file containing the informed consent form will be permanently deleted from the secure server. If a subject must be re-consented, the process will repeat itself.

### 10.1.2 Study Discontinuation and Closure

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study subjects, investigator, funding agency, and regulatory authorities if applicable. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study subjects, the cIRB, and sponsor and will provide the reason(s) for the termination or suspension. Study subjects will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to subjects
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance with protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

The study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, and/or the cIRB.

### 10.1.3 Confidentiality and Privacy

Subject confidentiality and privacy are strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their interventions. The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study, or the data will be released to any unauthorized third party without a prior written approval of the sponsor. All research activities will be conducted in as private a setting as possible.

The IMSM, other authorized representatives of the sponsor, representatives of the NDMC, NCC, and cIRB, regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this study. The clinical study site will permit access to such records.

The study subject's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for the duration specified by the StrokeNet Standard Operating Procedure (SOP) or longer as dictated by cIRB and local institutional regulations.

Study subject research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the NDMC. Subjects and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by NDMC research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and a Public Use Dataset (PUDS) will be archived at the NINDS data repository.

### 10.1.4 Future Use of Stored Specimens and Data

Data collected for this study will be analyzed and stored at the NDMC. After the study is completed, the de-identified, archived data will be transmitted to and stored in the NINDS Data Repository, for use by other researchers including those outside of the study, per StrokeNet SOP ADM 04 and in accordance with de-identification procedures of GCP 05. Permission to transmit data to these NINDS repositories will be included in the informed consent.

### 10.1.5 Study Governance and organizational structure

The research team will be governed by an Executive Committee, which will oversee several other Committees and Cores. The committees will provide oversight of specific aspects of data collection, management, analysis, interpretation, and manuscript preparation, and will convene regularly by web-assisted teleconferences, and in person at annual meetings. Apart from the Data and Safety Monitoring Board (DSMB) appointed by NINDS, the committees will include the PIs from MUSC and BIDMC, the NCC and NDMC, and representatives from the participating sites. **Four committees** will be set up to oversee the trial - Executive Committee; Advisory Committee; Publications Committee and Data and Safety Monitoring Committee (NINDS-appointed)

### 10.1.6 Safety Oversight

The NINDS will create the DSMB for the TRANSPORT2 trial. Their main responsibilities will include review of the research protocol and ongoing study activities, including review of data quality and completeness; review of fidelity to the study protocol; review of adequacy of subject recruitment and retention; review of AEs; making recommendations to the NINDS and the study Co-PIs concerning trial continuation, modification, or conclusion. The DSMB will meet regularly in person or by teleconference, typically on a semi-annual basis, to monitor the cumulative safety data during subject follow-up. The in-person format is recommended for the initial meeting and then annually, when possible. In no instance, should more than 12 months elapse between DSMB reviews of cumulative safety data after the first subject has enrolled.

The DSMB will monitor the study according to the guidelines specified in the study protocol and the operating procedures established at the initial meeting unless the DSMB determines during

the course of the trial that modification of the guidelines is in the best interest of the study and its subjects. Except as explicitly authorized by the DSMB, it is critical that study investigators remain masked to the interim data because knowledge of emerging trends between intervention arms may influence subject enrollment, management, and evaluation, thus compromising the study by introducing bias. The DSMB decides in their first meeting if DSMB members will be unmasked. If the DSMB decides to remain masked, they should consider assigning one DSMB member, when possible a clinician, to be unmasked to intervention assignment. The unmasked DSMB member may decide to unmask other DSMB members as indicated, for example, based on concerns over imbalances between study groups in rates of SAEs. Transcranial direct current stimulation is classified as "non-significant risk" device per FDA regulation**,** there have been no SAEs reported in the literature. However, we still implement the process to systematically monitor safety events for the protection of study subjects.

### 10.1.7 Clinical Monitoring

Clinical site monitoring is conducted to ensure that the rights and well-being of human subjects are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with GCP, with applicable FDA regulations (21 CFR 312), and with the FDA's "Guidance for Industry Oversight of Clinical Investigations — A Risk-Based Approach to Monitoring."

- Monitoring for this study will be performed by the NDMC centrally, on site, and remotely.
- Per the study's monitoring plan, monitoring will include a combination of **on-site monitoring** (to verify data entered into the WebDCU™ database against source documents and query inaccuracies between the source documents and WebDCU™ database), **remote monitoring** (source document verification, including verification of written consent, may be performed remotely by reviewing source documents that have been uploaded into WebDCU™ or via remote access to electronic medical records), and **central monitoring** (using web-based data validation rules, data manager review of entered data, statistical analysis, and ongoing review of site metrics).
- The NDMC, study co-PIs, and the appropriate site PIs will be provided copies of monitoring reports within 30 days of site visits.

Further details of clinical site monitoring are documented in the study's Monitoring Plan. The Monitoring Plan describes in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports.

### 10.1.8 Quality Assurance and Quality Control

Site staffs are required to attend an onsite in-person workshop (training on the study protocol, tDCS system operation, TMS procedure, MRI protocol and mCIMT protocol) before initiating the study. Subsequently, staffs are required to take annual online certification and recertification in primary outcome measure. Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution. Following written procedures as detailed in the monitoring plan, the monitors will verify that the clinical trial is conducted, and data are generated, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonization Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP),

Good Manufacturing Practices (GMP)). The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

### 10.1.9 Data Handling and Record Keeping

#### 10.1.9.1 Data Collection and Management Responsibilities

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site principal investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Black ink is required to ensure clarity of reproduced copies. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. DO NOT ERASE, OVERWRITE, OR USE CORRECTION FLUID OR TAPE ON THE ORIGINAL. Copies of the electronic CRF (eCRF) will be provided for use as source documents and maintained for recording data for each subject enrolled in the study. Data reported in the eCRF derived from source documents should be consistent with the source documents, or the discrepancies should be explained and captured in a progress note and maintained in the subject's official electronic study record. Clinical data will be entered into WebDCU™. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

#### 10.1.9.2 Study Records Retention

Study documents should be retained for the duration specified by the StrokeNet SOP. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

### 10.1.1.10 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, International Conference on Harmonization Good Clinical Practice (ICH GCP), or Manual of Procedures (MOP) requirements. The noncompliance may be either on the part of the subject, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly after review and approval by the NCC.

These practices are consistent with ICH E6

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site investigator to use continuous vigilance to identify and report deviations within 10 working days of identification of the protocol deviation, or within 10 working days of the scheduled protocol-required activity. All deviations must be addressed in study source documents and reported t via WebDCU for review by the TRANSPORT2 NCC Project Manager. Protocol deviations must be sent to the cIRB within 10 days if deviation requires prompt reporting. All other deviations will be reported at the time of continuing review. The site investigator is responsible for knowing and adhering to the reviewing cIRB requirements. Further details about the handling of protocol deviations are included in the MOP.

#### 10.1.1.11 Publication and Data Sharing Policy

TRANSPORT2 study will be conducted in accordance with the following publication and data sharing policies and regulations. National Institutes of Health (NIH) Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication. This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals. Data from this study may be requested from other researchers two years after the completion of the primary endpoint by contacting the NINDS (CRliason@ninds.nih.gov ).

Data generated through this project will be shared according to StrokeNet SOP ADM 04 regarding the Network Data Sharing Policy Data Sharing Policy. In summary, the goals of this Data and Resource Sharing Policy are to make available final data from StrokeNet clinical trials to the research community, while safeguarding the privacy of trial subjects and protecting confidential and proprietary data.

Upon database lock, the NDMC statisticians will generate data files from each data table corresponding to each electronic CRF in the database. In compliance with the HIPAA regulations, each data table will be stripped of any and all personal identifiers and will undergo a de-identification process. Furthermore, the NDMC statisticians will create a minimum number of derived variables that would be necessary to ensure reproducibility of the primary analysis.

Within 1 year from the acceptance of the primary manuscript for publication OR no later than 2 years from the database lock the PUDS will be submitted to the NINDS data repository, along with the final version of the study protocol, the data dictionary, and a user guide (or a “Readme” file) regarding the data files, including an explanation of any derived variables. Once PUDS are available, any researcher (study investigator or otherwise) wishing to receive them can contact the NINDS (CRLiaison@ninds.nih.gov). All manuscripts, abstracts, and press releases using the study data must acknowledge the StrokeNet investigators and the NINDS as the study sponsor with the relevant grant numbers.

To expedite and track external data sharing, the TRANSPORT2 team plans to create a website that serves as a resource and informational site for potential external investigators and collaborators. The website will include the TRANSPORT2 publication policy, the data sharing procedures and policies, timelines, and contact information. All published TRANSPORT2 manuscripts, abstracts, and brief descriptions of ongoing projects will be posted on the website maintained by the StrokeNet NCC.

#### 10.1.12 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the NINDS has established policies and

procedures for all study group members to disclose all conflicts of interest and will create a mechanism for the management of all reported dualities of interest.

## 10.2 Additional Considerations

Source Documents and Access to Source Data:
Each participating site will maintain appropriate medical and research records for this trial, in compliance with ICH E6 and regulatory and institutional requirements for the protection of confidentiality of subjects. As part of participating in an NINDS-sponsored trial, each site will permit authorized representatives of NINDS and regulatory agencies to examine (and when permitted by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety, progress, and data validity.

Data will be collected using CRFs whenever possible, but source data to be collected will also include copies of provider notes, laboratory results, and imaging reports. Subjects' participation in the study will be documented in the electronic medical record unless prohibited by local regulations.

In an effort to review informed consent forms in a timely manner, enrolling sites will upload a pdf of the signed informed consent form, into the password protected clinical trial management system, WebDCU™. The PDF file will be linked to the subject ID but will be stored on a secure server separate from the study's CRF data. The secure server on which these files are stored is not backed up to prevent copies of files containing Individually identifiable health information from being copied and stored on non-NDMC back up servers. The files on these servers can only be accessed by designated study personnel upon entry of a second password. NDMC staff will remotely monitor the informed consent forms and issues identified will be relayed to the clinical site for corrective and preventative action. After remote monitoring is complete, the PDF file containing the informed consent form will be permanently deleted from the secure server. If a subject must be re-consented, the process will repeat itself.

## 10.3 Abbreviations

| | |
|---|---|
| ADL | Activities of Daily Living |
| AE | Adverse Event |
| CFR | Code of Federal Regulations |
| CST-FA | Corticospinal Tract - fractional anisotropy values |
| CONSORT | Consolidated Standards of Reporting Trials |
| CRF | Case Report Form |
| DCC | Data Coordinating Center |
| DSMB | Data Safety Monitoring Board |
| | |

| | |
|---|---|
| eCRF | Electronic Case Report Forms |
| FDA | Food and Drug Administration |
| FFR | Federal Financial Report |
| FM-UE | Fugl-Meyer Upper Extremity |
| GCP | Good Clinical Practice |
| GLP | Good Laboratory Practices |
| GMP | Good Manufacturing Practices |
| HIPAA | Health Insurance Portability and Accountability Act |
| IADL | Instrumental Activities of Daily Living |
| IDE | Investigational Device Exemption |
| IND | Investigational New Drug Application |
| IRB | Institutional Review Board |
| ITT | Intention-To-Treat |
| mCIMT | modified Constraint-Induced Movement Therapy |
| MEP | Motor evoked potential |
| MOP | Manual of Procedures |
| MSDS | Material Safety Data Sheet |
| MUSC | Medical University of South Carolina |
| NDMC | National Data Management Center |
| NCT | National Clinical Trial |
| NIH | National Institutes of Health |
| NIH IC | NIH Institute or Center |
| NINDS | National Institute of Neurological Disorders and Stroke |
| OHRP | Office for Human Research Protections |
| PI | Principal Investigator |
| QA | Quality Assurance |
| QC | Quality Control |

| | |
|---|---|
| SAE | Serious Adverse Event |
| SAP | Statistical Analysis Plan |
| SMC | Safety Monitoring Committee |
| SOA | Schedule of Activities |
| SOP | Standard Operating Procedure |
| tDCS | Transcranial direct current stimulation |
| TMS | Transcranial magnetic stimulation |
| TRANSPORT2 | TRANScranial direct current stimulation for POst-stroke motor Recovery – a phase II sTudy |
| UP | Unanticipated Problem |
| US | United States |
| wCST-LL | weighted CorticoSpinal Tract – Lesion Load |
| WMFT | Wolf Motor Function Test |

## 10.4 PROTOCOL AMENDMENT HISTORY

| Version | Date | Description of Change | Brief Rationale |
|---|---|---|---|
| 5 | 11/9/20 | Version and Date Update on Title Page | |
| 5 | 11/9/20 | Update number of sites to 15 throughout protocol | 3 additional sites were added to original 12 |
| 5 | 11/9/20 | Clarified schema and schedule of activities 1st qualifying baseline days -20 through -7 and 2nd qualifying baseline days -6 through 1 | Clarification |
| 5 | 11/9/20 | Added that baseline visit 2 can be broken up over several days. All baseline activities need to take place prior to treatment visit activities start. | Clarification |
| 5 | 11/9/20 | Superscript 4 in schedule of activities denotes an activity that only takes place at final treatment session. | Clarification |
| 5 | 11/9/20 | 30 minute dose of tDCS updated to include (± 6) minute window throughout | Although variation unlikely it is possible due to interruption and technical variation, a deviation of less than 20% (24-36 minutes) is considered within the tolerated window of receiving a full dose |
| 5 | 11/9/20 | 120 minute dose of mCIMT updated to include (± 24) minute window throughout protocol | Although variation unlikely it is possible due to interruption and technical variation, a deviation of less than 20% (96- 144 minutes) is considered within the tolerated window of receiving a full dose |
| 5 | 11/9/20 | Phone screen was updated to prescreen interview throughout protocol | Allows for the use of video for prescreening |
| 5 | 11/9/20 | Updated the number of possible training subjects from 5 to 12 | Staff changes have required more training subjects than originally thought |
| 5 | 11/9/20 | Removed randomization and generate a code corresponding to in the following sentence.<br>tDCS will be executed by entering WebDCU-generated code in the computer, which will initiate one of the three interventions according to the group to which the subjects was randomized | Clarification |
| 5 | 11/9/20 | Added: On the randomization day, a study coordinator or a team member will log into WebDCU™ to randomize subjects who have been consented and found eligible. Briefly, at the beginning of <u>each</u> intervention session, the coordinator will complete tDCS Treatment Activation Code Request to obtain a new tDCS treatment activation code daily. Each day, the coordinator will need to enter the new code into the tDCS machine which will translate the code to the appropriate stimulation voltage using a pre-loaded script | Clarification |
| 5 | 11/9/20 | Removed: In addition, real-time confirmation of the correct randomization code will occur as | Clarification |

| | | | |
|---|---|---|---|
| | | follows. First, at the time of randomization, the study coordinator or any designed person will receive a randomization code that will be entered into the controller. The controller will return a confirmation code to be entered into WebDCU. This will ensure that the randomization code was entered correctly, and the subject is receiving the correct dose before the initiation of intervention. Each site will only enroll one subject at a time. | |
| 5 | 11/9/20 | Added: overall treatment imbalance and<br>That is, at the time of randomization, an overall treatment imbalance and imbalance in each of the covariates is assessed. | Clarification |
| 5 | 11/9/20 | Added: In an effort to review informed consent forms in a timely manner, enrolling sites will upload a pdf of the signed informed consent form, into the password protected clinical trial management system, WebDCU™. The PDF file will be linked to the subject ID but will be stored on a secure server separate from the study's CRF data. The secure server on which these files are stored is not backed up to prevent copies of files containing individually identifiable health information from being copied and stored on non-NDMC back up servers. The files on these servers can only be accessed by designated NDMC study personnel. NDMC staff will remotely monitor the informed consent forms and issues identified will be relayed to the clinical site for corrective and preventative action. After remote monitoring is complete, the PDF file containing the informed consent form will be permanently deleted from the secure server. If a subject must be re-consented, the process will repeat itself. | |
| 5 | 11/9/20 | Removed: The informed consent will be obtained by either the site PI or other members of the study team who are designed to perform this task on the Delegation of Authority Log. Subject/LAR will be asked to explain back the study to confirm his/her understanding of the study and its procedures. In an effort to review informed consent forms in a timely manner, enrolling sites will upload a pdf of the signed informed consent form, into the password protected clinical trial management system - WebDCU™. | |
| 5 | 11/9/20 | Removed: DCU Added: NDMC | |
| 5 | 11/9/20 | Removed Data Coordination Unit from abbreviations | |
| | | | |
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