Study Protocol

Transcranial electrical Stimulation in Stroke EaRly After onset Clinical Trial_ Bridging and Adjunctive neuroprotection (TESSERACT-BA)

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Investigational Plan:
Protocol: Transcranial electrical Stimulation in Stroke EaRly After onset Clinical Trial – Bridging and Adjunctive neuroprotection (TESSERACT-BA)

Overview of Protocol of TESSERACT-BA

Title	Transcranial electrical Stimulation in Stroke EaRly After onset Clinical Trial - Bridging and Adjunctive neuroprotection (TESSERACT-BA)
Objective	To evaluate the safety, tolerability, and feasibility, and preliminarily explore the potential efficacy, of cathodal transcranial direct current stimulation (C-tDCS) as an innovative non- invasive neuroprotection technique in acute ischemic stroke patients before and after endovascular thrombectomy
Study Design	A single site, sham- controlled, dose escalation study
Population Studied	24-48 acute ischemic stroke patients from Ronal Regan Medical Center (RRMC) Emergency Department or RRMC Inpatient hospital with: 1) New focal neurologic deficit consistent with acute ischemic stroke; 2) NIHSS ≥4; 3) Age ≥18; 4) Last known well time within 24 hours of randomization stimulation initiation; 5) ICA or M1 or M2 MCA occlusion on MRA/CTA; 6) Pre-stroke modified Rankin Scale≤ 3; 7) Ineligible for intravenous thrombolysis; 8) Patient have undergone thrombectomy with less than a complete reperfusion (TICI < 2c,3); 9) Undergoing endovascular thrombectomy for patients enrolled in 5 and 6 dose tiers; 10) Signed informed consent obtained from the patient or patient's legally authorized representative.
Intervention	3:1 randomization to active treatment with C-tDCS vs sham control
Device	Transcranial Direct Current Stimulation (tDCS)

A. Specific Aims

Stroke is a devastating condition and continues to be a leading cause of adult death and disability.[1] Endovascular thrombectomy (ET) has become standard of care for acute ischemic stroke due to large vessel occlusion. [2]However, ET has limitations that would be aided by neuroprotective interventions. ET fails to achieve substantial reperfusion in 20-30% of patient. Even among reperfusing patients, rates of excellent outcome are low - only 20-25% achieve freedom from disability (modified Rankin Scale 0-1), due to infarct growth prior to reperfusion and delayed and reperfusion injury after reperfusion. [3-5] Therefore, bridging neuroprotective therapies prior to ET, can preserve more viable penumbra to rescue intervention by reperfusion and, therefore, enhancing its clinical benefit. While adjunctive neuroprotective strategies after ET can protect the brain against reperfusion injury and infarct growth due to incomplete reperfusion.[6]

Multiple candidate neuroprotective agents have failed in translation from animal to human studies.[7,8] Amongst the missing intervention properties in the failed neuroprotection trials, two offer the most significant prospect of success: 1) having a pleiotropic effect, interdicting multiple pathways in the ischemic cascade, and 2) delivery by a direct transcranial, rather than intravascular, route, with fast and direct delivery to neural tissues regardless of cerebral perfusion. Transcranial direct current stimulation (tDCS) offers promise as just such an approach. In pre-clinical acute ischemic stroke models, cathodal tDCS (C-tDCS) applied to the ischemic hemisphere, has been shown to have a substantial neuroprotective effect with widely pleiotropic neuroprotective molecular mechanisms of action and can be delivered to the salvageable ischemic tissue beyond the occlusive thrombus despite reduced blood flow.[9,10]

This study proposes to conduct the first-in-human, sham-controlled, dose-escalation, safety, feasibility, and potential efficacy trial of C-tDCS as a bridging and adjunctive neuroprotection before and after thrombectomy in acute ischemic stroke. To fully assess the potential safety aspects of adjunct C-tDCS to ET, we will exclude patients who receive intravenous thrombolysis.

Specific Aim I: To establish the safety and tolerability of C-tDCS in AIS as an adjunct and bridge to endovascular thrombectomy (EVT).

Hypothesis I-a: C-tDCS will be safe in AIS patients receiving EVT with no increased risk of symptomatic intracranial hemorrhage (SICH) within the 24-hour period after EVT. Hypothesis I-b: C-tDCS will not increase the rates of asymptomatic intracranial hemorrhage, early neurological deterioration (NIHSS during the 24-hour period after stimulation), 90-day mortality, and all serious adverse events. Hypothesis I-c: Majority of AIS patients (≥ 90%) will tolerate the C-tDCS by completing at least 75% of the stimulation period with no major stimulation related pain, discomfort and skin changes.

Specific Aim II: To determine the feasibility of delivering C-tDCS as an adjunct and bridge to EVT.

Hypothesis II-a: Adjunctive C-tDCS after endovascular thrombectomy will be started more rapidly after the end of ET procedure over the course of the study and in the last 10 patients, median time from end of ET procedure to C-tDCS initiation will be ≤ 10 minutes. Hypothesis II-b: Bridging C-tDCS prior to endovascular thrombectomy will be started more rapidly over the course of the study and in the last 4 patients, median time from randomization to C-tDCS initiation will be ≤ 10 minutes.

Specific Aim III: To explore the potential imaging and clinical efficacy of tDCS in AIS an adjunct and bridge to EVT.

1) Early and late penumbral salvage, defined as proportion of penumbral tissue on baseline multimodal MRI that does not proceed to infarct on 2-hour and 24-hour MRI (PWI); **2)** Early and late collateral enhancement, evidenced by reduced perfusion lesion volume on 2-hour and 24-hour perfusion-weighted MRI (PWI); **3)** Early and late reduction in infarct growth from baseline to 2-hour and 24-hour MRI; 4) Early improvement in neurologic deficit (NIHSS at 24h); **5)** Improvement in final 3-month disability and quality of life (modified Rankin scale, AMC Linear Disability Scale, EQ-5D, and Barthel Index)

Impact

This in-hospital safety trial, will identify C-tDCS dose regimens that are feasible, well-tolerated, and preliminarily safe in patients with acute ischemic stroke who undergo ET. Study finding will identify the most promising dose regimens to advance to phase 2b and pivotal phase 3 efficacy trials for three patient populations with pressing need for improved therapies: 1) thrombectomy-eligible patients arriving directly to thrombectomy-capable hospitals who would benefit from post-arrival brain protection; 2) thrombectomy-eligible patients arriving initially

to non-thrombectomy-capable hospitals who would benefit from brain protection during interfacility transfer; and 3) thrombectomy-eligible patients initially care for in Mobile Stroke Units who would benefit from brain protection during transport to, and early post-arrival course at, thrombectomy-capable hospitals.

B. Background and Significance

Transcranial direct current stimulation (tDCS) is a non-invasive neuromodulation method that delivers a weak electrical current to the brain via scalp electrodes.[11] Rather than directly eliciting a neuronal response, tDCS modulates neuronal excitability in regions of the brain depending on the polarity of stimulation. By altering the resting membrane potential cathodal tDCS (C-tDCS) reduces neuronal excitability [in contract to anodal tDCS (A-tDCS) which increases excitability]. [12,13] tDCS has been extensively investigated in humans for decades, as a neuromodulatory intervention to treat depression and diverse other neuropsychiatric disorders, as a neuroplasticity-enhancing intervention for chronic stroke patients, and as a tool to change cognition and behavior in healthy individuals. [14-17]Thus far, tDCS has been found safe and tolerable with no reported serious adverse events across multiple clinical and preclinical studies. [18,19] In addition to these established applications of tDCS, tDCS studies in patients with acute ischemic stroke. C-tDCS is of substantial promise for acute cerebral ischemia due to large vessel occlusion, based on preclinical studies.

Stroke is a leading cause of mortality and morbidity across the world. Large vessel occlusion (LVO) is accounted for 30-40% of acute ischemic strokes and foretells a poorer prognosis compared to non-LVO strokes.[20] While endovascular therapy (EVT) has fundamentally improved outcomes of AIS patients with LVO, patient outcomes even with EVT remain suboptimal; with only 20-25% achieving a disability-free outcome.[3,5] Therefore, even in the modern thrombectomy era, the need to develop complementary neuroprotective therapies exists. These therapies interrupt the cellular, biochemical and metabolic process that mediate hypoxic and reperfusion cellular injury.

There are two stages of care in endovascular thrombectomy patients in which effective neuroprotection therapies could synergistically further improve tissue salvage and functional outcome: 1) bridging neuroprotection prior to EVT start, and 2) adjunctive neuroprotection immediately after EVT completion. Bridging neuroprotective treatments applied prior to start of the endovascular thrombectomy procedure could preserve neural parenchyma and the neurovascular unit until reperfusion and resolution of ischemia is achieved by EVT. Bridging neuroprotective therapies would be beneficial in at least 3 settings: i) started soon after patient arrival at the thrombectomy-capable hospital during the 60-120 minute window from door to recanalization; ii) started in Mobile Stroke Units prior to arrival at thrombectomy-capable hospitals; and iii) started at outside, non-thrombectomy hospitals prior to patient interfacility transfer to a thrombectomy center. If a neuroprotective treatment could completely “freeze” stroke progression until reperfusion was achieved, with every 15 minutes in which therapy was delivered, among 1000 treated patients, an estimated 39 patients would have less disabled outcome at 3 months, including 25 more who will be functionally independent (mRS 0-2).[3] Similarly, adjunctive neuroprotective therapies, begun soon after EVT procedure completion, could be helpful in three additional ways :i) among patients with incomplete reperfusion (less than TICI 2c-3), adjunctive neuroprotective therapies could permit tissue tolerance of ongoing ischemia while spontaneous further recanalization proceeds; ii) among patients with substantial (TICI 2b) or excellent/complete (TICI 2c-3) reperfusion, adjunctive neuroprotective therapies could prevent delayed post-ischemia elaboration of injury through apoptotic and secondary injury cascades; and iii) adjunctive neuroprotective therapies could prevent reperfusion injury arising from reperfusion oxidative injury, inflammation, and other mechanisms.[6]

While many past attempts at developing neuroprotective therapies in human have been disappointing, they had the following shortcomings:

1) The great preponderance tested pharmacologic agents that affected only one or two molecular ischemic pathways. **2)** The majority of the neuroprotection therapies were administered intravenously, reducing delivery to brain areas with low blood flow, and, even there, requiring passage through the blood-brain barrier.[7,21] **3)** Few trials of neuroprotection in human have used penumbral imaging for patient selection. [22] Therefore, the use of penumbral imaging to limit the patient selection to those for whom the intervention is most likely to be of benefit is crucial in studies of neuroprotection, and lastly **4)** The neuroprotection therapies have been tested as stand-alone treatments without definitive reperfusion. While neuroprotection is intrinsically a temporizing therapy, enabling brain cells to tolerate an ischemic episode longer, reperfusion is the ultimate therapy for AIS that finally resolves the ischemic episode. Hence, combining neuroprotection with reperfusion therapies in the post-thrombectomy era is not only feasible but also increases the chance of proving clinical benefits.

Our study addresses all these challenges by: **a)** Testing C-tDCS as a multi-mechanistic neuroprotective method, **b)** Delivering the neuroprotection transcranially to ischemic tissue beyond occlusive thrombus**, c)** Using penumbral imaging prior to enrollment, and **d)** Using C-tDCS as a bridge and adjunct to thrombectomy.
In pre-clinical studies, multiple investigators in several independent labs worldwide have found evidence of a neuroprotective effect of C-tDCS in different animal models of acute cerebral ischemia:

B.1. Proposed neuroprotective mechanisms of cathodal direct current electrical stimulation based on experimental pre-clinical models of acute cerebral ischemia

B.1.1. Inhibition of peri-infarct excitatory depolarizations

During the acute stages of cerebral ischemia, an excitotoxic cascade will be triggered by the excess glutamate and other excitotoxic amino acids that are released as the result of cellular necrosis.[23] Experimental models of acute middle cerebral artery occlusion (MCAO) have shown that the excitotoxicity generates recurrent spontaneous waves of depolarization also known as peri-infarct depolarizations (PIDs).[24,25] The PIDs occur soon after the MCA occlusion and spread across the penumbra to the normally perfused tissue. The infarct growth correlates with the number and duration of PIDs and the basis for this relationship has been related to: 1) An abnormal vasoconstriction in response to depolarization 2) An imbalance between increased metabolic overload, induced by the depolarization, and blood supply in acute ischemic stroke.[26] Notturno et al. studied the effect of cathodal stimulation on PIDs in 3-vessel permanent occlusion rat model, with cumulative stimulation durations of 120 and 180 mins (15' on-15' off cycles).[9] They found that cathodal stimulation applied to the ischemic MCA territory significantly reduced PIDs, and reduced infarct volume by 20-30%. They found no effect on brain edema between the stimulated and control groups and no stimulation induced macroscopic or microscopic lesion or hemorrhagic transformation.

B.1.2. Anti-inflammatory, Anti-apoptotic, and Angiogenic effect

Beyond the activation of the excitotoxic cascade, an inflammatory response and programmed cellular apoptosis will result in a secondary damage and expansion of the irreversibly damaged core (Figure1). [23] Therefore, suppression of the innate pro-inflammatory cells and the cellular apoptotic cascade results in the reduction of the infarct size and cerebral edema. Peruzzotti et al. studied cathodal direct current stimulation in acute stroke mice models with temporary MCAO (reperfusion models) applied for 40 minutes (20'on-20off-20'on). [10] Cathodal stimulation of the ischemic hemisphere reduced final infarct size, with lowering of cortical glutamate synthesis, downregulation of N-methyl-D- aspartate (NMDA) receptor (NR2B) expression, and reduction in peri-ischemic inflammatory response and apoptotic markers.

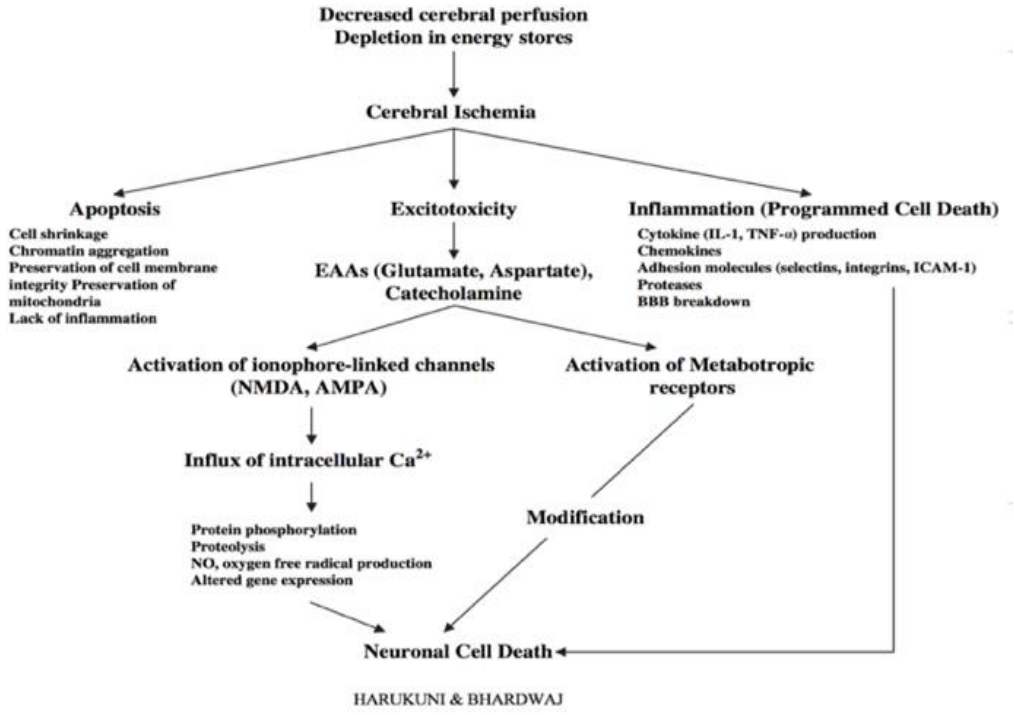

Figure 1. This schematic shows the events leading to ischemic brain injury.

Furthermore, a significant functional amelioration and improvement of cerebral edema were observed even when stimulation was applied hours after the MCAO. No macroscopic or microscopic lesion or hemorrhagic transformation induced by cathodal stimulation was found.

Baba et al. showed in their study that a low-frequency (2 and 10 Hz) electrical cortical stimulation may exert neuroprotective effects reflecting by attenuation in both necrotic and apoptotic cell deaths, blockade of microglial/astrocytic activation and expression of trophic factors. They also demonstrated an increase in cerebral blood flow of the electrically stimulated animals. [27] No neuroprotective effect was observed at higher frequency (50Hz).

B.1.4. Preservation of neuronal axons

Kim et al. studied the neuroprotective effect of cathodal and anodal direct current stimulation on axons and myelin integrity. They delivered stimulation for 30 minutes two days after MCAO. They found significant amelioration of axonal damage and preservation of white matter axonal integrity in rat models of cerebral ischemia mainly after anodal stimulation. [28]

B.1.3. Direct vasodilatory effect

In addition to the direct neuroprotective effects, direct current may confer benefit in AIS via vasodilation and collateral blood flow enhancement. In fact, Fox et al. found a direct vasodilatory effect of electrical stimulation when applied over the basilar artery. [29]This effect was more pronounced with cathodal compared to anodal stimulation.

C. Preliminary studies

C.1. Experience with transcranial neuromodulation in human subjects

Our group at UCLA has extensive experience with transcranial neuromodulation using direct electrical and magnetically induced currents in human subjects. Over the past 10 years, we have performed transcranial neuromodulation in over 500 subjects.[30-35] This substantial experience provides a firm foundation for undertaking

C.2. A meta-analysis of preclinical studies using electrical stimulation as neuroprotection in acute cerebral ischemia

To assess the neuroprotective effect of electrical stimulation in AIS, we performed a systematic review of all preclinical acute cerebral ischemia studies using electrical stimulation as a neuroprotective method. Our systematic search identified 28 experiments in 21 studies, including a total of 350 animals, of electrical stimulation in preclinical acute cerebral ischemia. Studies were categorized among 6 strategies. Three strategies applied different stimulation subtypes to tissues within the ischemic zone [cathodal hemispheric stimulation (CHS), anodal hemispheric stimulation (AHS), and pulsed hemispheric stimulation (PHS)] and three strategies applied deep brain stimulation to different neuronal targets remote from the ischemic zone [fastigial nucleus stimulation (FNS), subthalamic vasodilator area stimulation (SVAS), and dorsal periaqueductal gray stimulation (DPAGS)]. Random effects meta-analysis assessed electrical

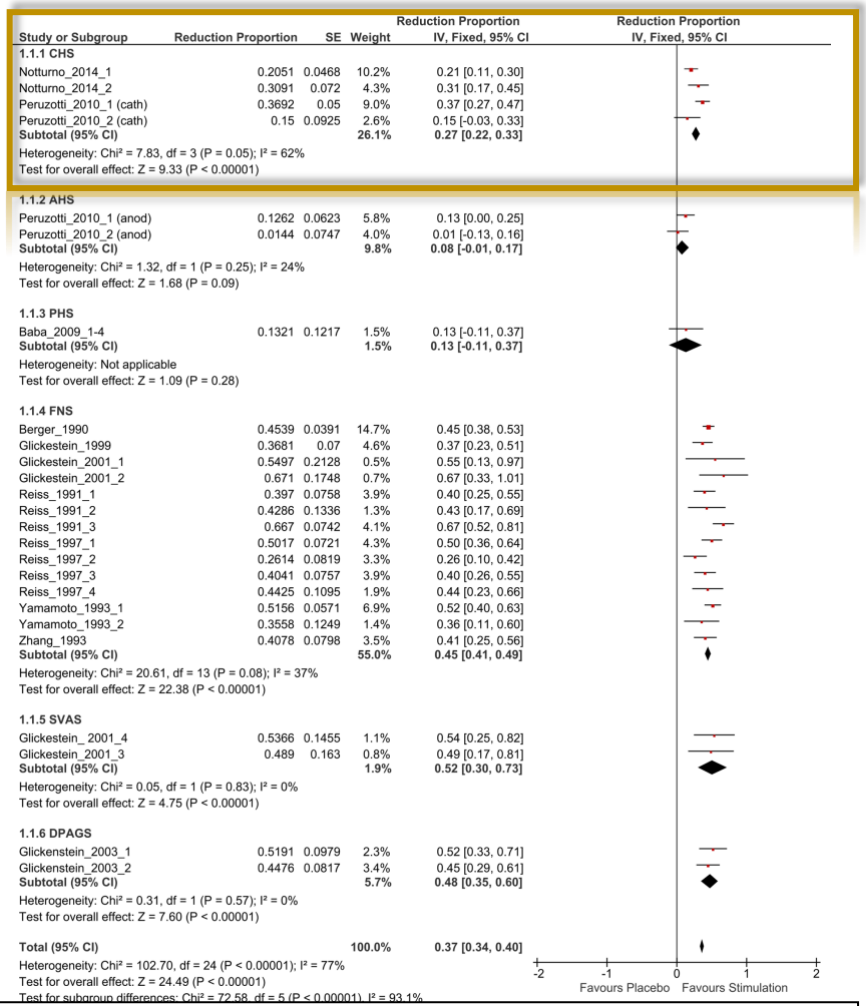

Figure 2. Forest plot shows the neuroprotective effect of electrical stimulation across 28 pre-clinical experiments. Within the highlighted square are the studies using direct current stimulation as neuroprotection.

stimulation modification of final infarct volume. Study-level risk-of-bias and intervention-level readiness-for-translation were assessed using formal rating scales. Overall, in animals undergoing electrical stimulation, compared with control, final infarct volumes were reduced by 37% (CI 95%; 34-40% P< 0.00001). There was evidence of heterogeneity of efficacy among stimulation mode subgroups (I^2 = 93.1%, $p_{heterogeneity}$ < 0.0001). Amongst the within-ischemic zone stimulation techniques, only CHS significantly reduced the infarct volume (27 %, CI 95%; 22-33%, P< 0.00001); amongst the remote-from ischemic zone techniques, all (FNS, SVAS, and DPAGS) reduced infarct volumes by approximately half. On formal rating scales, CHS studies had the lowest risk-of-bias and CHS had the highest overall quality of intervention-level evidence supporting readiness to proceed to clinical testing. We concluded that electrical stimulation reduces final infarct volume across preclinical studies. Cathodal hemispheric direct current stimulation shows the most robust evidence and is potentially appropriate for progression to early stage human clinical trial testing.

C.3. Vascular response associated with Direct Current

A preliminary study in human volunteers by Marom Bikson (Co-mentor of the current proposal) demonstrated that direct current produces vasodilation of cerebral vessels (Figure 3). Furthermore, the vasodilatory response to tDCS is evident by its known mild dose-dependent effect in causing skin erythema. The cerebral vasodilatation is likely partially due to non-specific polarization of vascular system. Bikson and colleagues also demonstrated that 10 minutes of tDCS resulted in up-regulation of endothelial nitric oxide synthase (eNOS) gene expression and increase production of nitric oxide (NO), a known vasodilator. [36] Samdani et al. have demonstrated in their study that the endothelial NO upregulation favorably affects outcomes by the accentuation of the cerebral blood flow and attenuation of platelet aggregation, platelet adhesion, and NMDA current. [37] These findings support that tDCS could augment blood flow through the stimulated vasculature. Leptomeningeal collateral networks, peripherally located, are particularly accessible to the electric field. The potential vasoactive effects of tDCS also raise the possibility that stimulation will affect the blood-brain barrier. While no hemorrhagic transformation with C-tDCS was noted in preclinical models, this potential effect supports the approach of undertaking dose escalation safety trial as the first study in AIS patients, even though all studied dose tiers are within ranges safe in chronic stroke and other brain disease patients.[11 19]

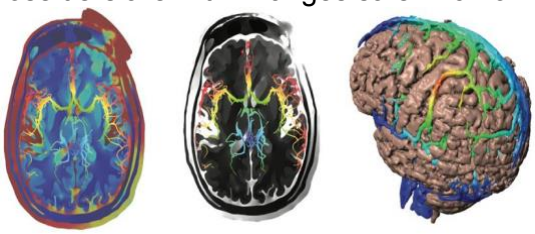

Figure 3. Shows models of intra-cranial current flow during tDCS is concentrated by the vasculature

C.4. Feasibility of neuroprotection therapies in acute ischemic stroke

Our group has extensive experience with the conduct of clinical trials of drug and device neuroprotective therapies for acute ischemic stroke, both as standalone interventions and as a complement to reperfusion treatment. We recently completed the NIH Field Administration of Stroke Therapy-Magnesium phase 3 trial, enrolling 1700 patients. [8] Of particular relevance to the current proposal, we have conducted trials of transcranial delivery of acute neuroprotective intervention using laser [38], and trials of collateral enhancement interventions [39] and trials and studies using MRI penumbral imaging and permeability imaging as technical efficacy and safety end points.

D. Trial Design and Methods

D.1. Study overview

This proposal is a prospective, single-center, dose-escalation safety, tolerability and feasibility study of C-tDCS as adjunctive and bridging neuroprotection in acute stroke patients undergoing endovascular thrombectomy. Patients with less than a complete EVT reperfusion (the thrombolysis in cerebral infarction (TICI) grading of <2c and 3) will be receiving at least one cycle of adjunct C-tDCS after EVT and at higher dose tiers, one cycle of bridging C-tDCS will be administered before EVT and if EVT results in TICI < 2c/3, then two cycles of adjunct EVT will be applied after EVT. The primary safety endpoints will be symptomatic intracranial hemorrhage during the 24-hour period after thrombectomy. Secondary measures of safety will include asymptomatic intracranial hemorrhage, early neurological deterioration, 3-month mortality and all serious adverse events. Tolerability will be judged based on the percentage of the patients completing the protocol-assigned stimulation treatment and secondarily, the rate and severity of cutaneous, neurologic, nociceptive or

other adverse effects will be assessed. Feasibility endpoints will analyze the speed with which tDCS will be implemented. Finally, we will explore signals of potential efficacy by examining the imaging evidence of infarct growth, and clinical outcomes of early neurologic deficit evolution, and 3-month global disability and health-related quality of life.

D2. Technology

D.2.1. tDCS Device and Stimulation Parameters

The study will employ a Soterix™ high-definition DC-Stimulator, owned by the principal investigator. This tDCS unit consists of a stimulator, 4x1 HD interface and an adjustable cap with pre-maid openings that quickly and easily fits different head sizes. (Figure 5). The cap will be loaded with plastic electrode holders. These will be filled with conductance gel (Signa® gel), 1 cm^2 electrodes will be placed in the holders, and the holder then locked.

D.2.2. Montage

The electrode positioning montage will be a 4 to 1-ring configuration, with the center or active electrode connected to cathode and the 4 reference or return electrodes connected to anode. The 4 to 1-ring configuration is chosen to focus the stimulation to the penumbral region. (Fig 6)

For electrodes positioning location, we use one of the 3 predefined positionings according to the part of the middle cerebral artery (MCA) territory at risk. These electrode positionings are based on computational modeling of 3 electrical fields concentrated over different parts of the MCA territory: electrical field covering territory of MCA-M1 branch (Fig 5A, a); electrical field covering territory of MCA-M2 superior branch (Fig 5B,b); and electrical field covering territory of MCA-M2 inferior branch (Fig 5C,c).

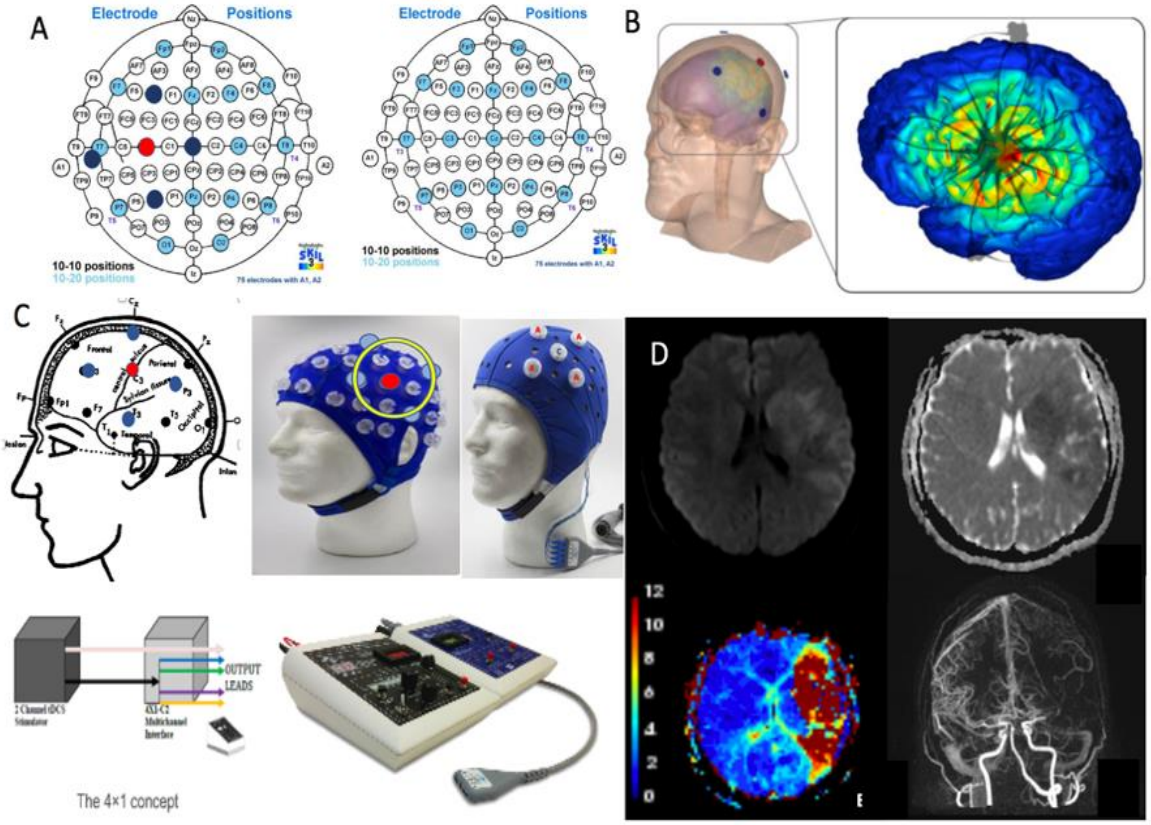

Figure 5. Shows an example of electrode positioning in a 4 to 1-ring configuration in a patient with left M1 occlusion. **A)** The reference electrodes (anode-blue) are positioned on F3, T7, Cz and P3 and the center electrode (cathode-red) is positioned over the C3 (central sulcus). **B)** Shows the computational modeling of the electrical field, concentrated over the MCA territory. **C)** Shows the electrode positioning on the tDCS HD cap, the schematic of the tDCS stimulator connected to the HD interface, and the Soterix™ HD tDCS unit (stimulator + interface) that will be used in this study. This stimulator connects to the adjustable cap. **D)** Shows the penumbral region on the perfusion MRI of the patient with L MCA-M1 occlusion.

D.2.3. Sterility and Quality Assurance

Prior to stimulation, all the electrodes will be monitored for any sign of damage such as chipping. The electrodes will be discarded after being exposed to 5 cycles of stimulation. We will avoid any metal to contact the electrodes. The stimulation cap and the electrodes will be sanitized prior to each subject use.

D.2.4. Device training

Device use will be performed by physician investigators, technologists, and research staff who have completed training and certification in safe tDCS use. Training will include instructions on different components of the device, installing and assembling the components, charging the device prior to the first use, recharging the device after each procedure, verifying the device is charged prior to each procedure, recognizing and addressing the different Warning/Error indications (e.g., battery light flashing blue, indicating that the battery is low but can still perform at least one procedure, battery light steady red, indicating battery low error – not enough battery power to run a complete procedure, etc.) and specific instructions for returning the device to the sponsor in the event of an error notification that cannot be addressed by the site personnel, or a failure of the device to charge after three hours. All device training will be documented in a training log that will be maintained in the site regulatory binder.

D3. Subjects

Twenty four to 48 acute ischemic stroke patients who are undergoing endovascular therapy and meet study inclusion/exclusion criteria will be enrolled from Ronald Reagan Medical Center (RRMC) Emergency Department or RRMC inpatients. At last two dose tiers, patients arriving in Mobile Stroke Unit (MSU) with large vessel occlusion who are candidates for EVT will also be included for bridging stimulation en route to RRMC.

Based upon acute stroke patient referral rates to UCLA over the past 3 years, we anticipate *24 enrollments per year.* Therefore, we estimate that the study enrollment will take 2 years to complete. Enrolled subjects will be randomized to active versus sham stimulation in 3:1 ratio.

D.3.1 Entry Criteria

Inclusion criteria

1) New focal neurologic deficit consistent with AIS
2) Age≥18
3) NIHSS ≥ 4
4) ICA or M1 or M2 MCA occlusion on pre-thrombectomy MRA or CTA
5) Onset (last-seen-well) time to randomization time within 24 hours
6) Pre-stroke modified Rankin Scale≤ 3
8) Patient ineligible for IV tPA, per national AHA/ASA Guidelines
9) Having undergone endovascular thrombectomy with less than a complete reperfusion (<TICI 2c, 3) for receiving post-thrombectomy adjunct C-tDCS
10) Undergoing endovascular thrombectomy, per national AHA/ASA Guidelines for patients who are assigned to pre-thrombectomy bridging session at Tiers 5, 6
11) A signed informed consent is obtained from the patient or patient's legally authorized representative

Exclusion criteria

1) Acute intracranial hemorrhage
2) Evidence of a large Ischemic core volume (ADC < 620 $\mu m^2/s$ or rCBF< 30%) ≥ 100 ml
3) Presence of tDCS contraindications - electrically or magnetically activated intracranial metal and non-metal implants
4) Pregnancy
5) Severe contrast allergy or absolute contraindication to iodinated contrast preventing endovascular intervention
6) History of seizure disorder or new seizures with presentation of current stroke
7) Evidence of any other major life-threatening or serious medical condition that would prevent completion of the study protocol including attendance at the 3-month follow-up visit
8) Concomitant experimental therapy
9) Preexisting scalp lesion at the site of the stimulation or presence of skull defects (may alter current flow pattern)
10) Preexisting coagulopathy, consist of platelet count of ≤ 100, INR ≥ 3, PTT ≥ 90
11) Patients suspected of having infective endocarditis and ischemic stroke related to septic emboli
12) Patients suspected or known to be infected with coronavirus 2019 (CoVID-19)

13) Patient with radiographic evidence or suspicion of chronic conditions that may predispose them to intracranial hemorrhage including brain arteriovenous malformations, cerebral cavernous malformations, cerebral telangiectasia, multiple previous intracerebral hemorrhages (amyloid angiopathy)
14) Suspected cerebral vasculitis based on medical history and CTA/Magnetic Resonance Angiogram (MRA)
15) Suspected cysticercosis
16) Suspected cranial dural arteriovenous fistula
17) Cerebral venous thrombosis
18) Head trauma causing loss of consciousness, concussion, confusion, or a headache within the past 30 days
19) Patient has suffered a hemorrhagic or ischemic stroke within the last three (3) months
20) History of a cancer known to cause hemorrhagic metastases, e.g., melanoma, renal cell carcinoma, choriocarcinoma, thyroid carcinoma, lung carcinoma, breast carcinoma, and hepatocellular carcinoma
21) History of left atrial myxoma
22) Evidence of dissection in the intracranial cerebral arteries
23) Suspicion of aortic dissection
24) Significant mass effect with midline shift
25) The patient is in a coma

Additional Inclusion Criterion for Penumbral Salvage Substudy

1) Presence of salvageable penumbra with Tmax> 6 sec/ ischemic core volume (ADC < 620 $\mu m^2/s$ or rCBF< 30%) ≥ 1.2
2) Dose tier to which patient will be assigned includes a pre-thrombectomy bridging active or sham stimulation session

D.3.2.Enrollment and Consent

All acute ischemic stroke patients within 24 hours of their symptoms onset who present to Roland Reagan Medical Center (Emergency Department or inpatient hospital if they are already admitted for a different indication) and meet the study inclusion/exclusion criteria will be identified by a study physician-investigator and subsequently offered enrollment in the trial. Prospective subjects will be provided with written and verbal information regarding the nature of the study, the procedures and evaluations involved, and the potential risks and benefits. All participating patients or their legally authorized representatives will provide explicit written informed consent. Not all adult subjects will have the capacity to give informed consent. The likely range of impairment includes stupor and aphasia. By interviewing the patient, the investigators will assess whether the affected individual understands the central elements of the study procedures and has the capacity for informed consent, using the recommended approach of the institution's Institutional Review Board, such as the UCLA Office of Human Research Protection Decision-Making Capacity Assessment Tool.

Patients with capacity to consent will be invited to participate by the PI. In patients without the capacity to consent, the patient's legally authorized representative will be asked to provide consent for participation. The investigator will inform the patient or legally authorized representative of the availability of the study as follows: "You (your relative) is having a stroke. We are doing a research study of a new treatment for stroke. Here is an informed consent form that describes the study. Please read it. After you are finished, I will answer any questions you may have." Once subjects or their legally authorized representatives have read and understood the IRB-approved consent form, and had all their questions answered, written informed consent will be elicited.

D.4. Dose Tiers and Randomization

We will implement a traditional 3+3 (rule-based, modified Fibonacci) dose escalation design, with 3:1 randomization to active treatment vs sham control. There will be 6 dose tiers, reflecting increasing intensity and duration of stimulation:

Tier 1 – 1 mA, single 20 - min cycle, will start at the conclusion of the EVT procedure in patients with less than a complete EVT recanalization (the thrombolysis in cerebral infarction (TICI) grading of <2c and 3) and negative immediate post-EVT CT scan for a definitive evidence of intracranial hemorrhage (ICH).

Tier 2– 2 mA, single 20 min cycle, will start at the conclusion of the EVT procedure in patients with TICI<2c and 3 and negative immediate post-EVT CT scan for a definitive evidence of ICH.

Tier 3 – 1 mA, in 2 treatment cycles. First cycle will be a 20 min cycle, administered after the conclusion of the EVT procedure in patients with TICI<2c and 3 and negative immediate post-EVT CT scan for a definitive evidence of ICH. The second cycle will start 20 minutes after the end of the first cycle, and will last 20 minutes.

Tier 4– 2 mA, in 2 treatment cycles. First cycle will be a 20 min cycle, administered after the conclusion of the EVT procedure in patients with TICI<2c and 3 and negative immediate post-EVT CT scan for a definitive evidence of ICH. The second cycle will start 20 minutes after the end of the first cycle, and will last 20 minutes.

Tier 5 - 1 mA, in 3 treatment cycles. First cycle will be a 20 min cycle, administered after initial imaging either in the Roland Reagan Medical Center or mobile stroke unit and prior to arterial puncture (cycle will be shorter than 20 minutes if EVT puncture is ready to proceed, in order not to delay EVT). The second cycle will start at the conclusion of the EVT procedure in patients with TICI<2c and 3 and negative immediate post-EVT CT scan for a definitive evidence of ICH (and not sooner than 20 minutes after the end of the first cycle), and will last 20 minutes. The third cycle will start 20 minutes after the end of the second cycle, and will last 20 minutes.

Tier 6 - 2 mA, in 3 treatment cycles. First cycle will be a 20 min cycle, administered after initial imaging either in the Roland Reagan Medical Center or mobile stroke unit and prior to arterial puncture (cycle will be shorter than 20 minutes if EVT puncture is ready to proceed, in order not to delay EVT). The second cycle will start at the conclusion of the EVT procedure in patients with TICI<2c and 3 and negative immediate post-EVT CT scan for a definitive evidence of ICH (and not sooner than 20 minutes after the end of the first cycle), and will last 20 minutes. The third cycle will start 20 minutes after the end of the second cycle, and will last 20 minutes.

Patients in the sham stimulation arm at all the tiers will have the cap and electrodes in place, but without any delivered electrical stimulation. While the highest dose tier in this study is expected to be fully safe, based on preclinical and clinical studies [11,18], since this is a first-in-human study in acute stroke, a formal escalation to higher dose tiers is prudent.

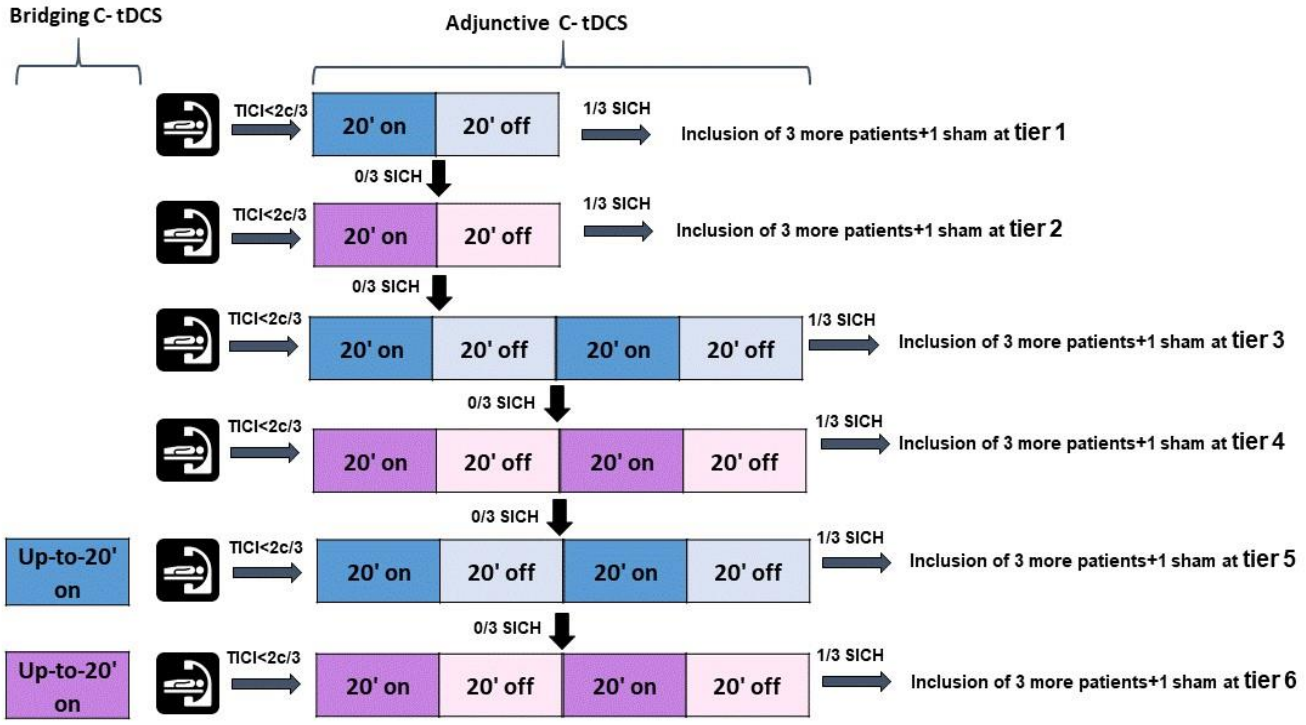

Figure 7. This figure shows the 6 escalating tiers. Blue represents 1 mA, purple shows 2 mA of C-tDCS. The black/white neuroangio suite icon represents the endovascular thrombectomy (EVT) procedure, occurring before the adjunctive C-tDCS at all tiers if EVT results in a less than a complete recanalization (TICI <2c, 3). At tiers 5 and 6, bridging C-tDCS occurs for up to 20' before EVT. Symptomatic ICH (SICH) in none of the three C-tDCS patients in a tier will result in escalation. However, if SICH occurs in one C-tDCS patient, 3 more C-tDCS patients and 1 more sham will be enrolled at the same tier.

D.4.Management pre, during, and post adjunctive stimulation period

As soon as the endovascular thrombectomy (EVT) ends, a CT head will be obtained in patients with less than a complete EVT reperfusion (TICI< 2c,3) and if negative for definitive evidence of intracranial hemorrhage, then the patient will be randomized, and the adjunct stimulation will be initiated in neurosurgical intensive care unit according to the tier that patient is assigned to.

After randomization and during the stimulation, all patients will be monitored closely by a physician-investigator. NIHSS will be obtained at the end of each 20-minute stimulation cycle and a visual inspection of the skin and rate degree of any potential erythema under the electrode will be performed. Then, a tolerability form will be completed based on validated cutaneous, neurological, and pain items of the PRO-CTAE (Patient-Reported Outcomes version of the Common Terminology Criteria for Adverse Events) (Figure 8).[40,41]

After the stimulation, nursing neurologic assessments will be performed by Neurointensive Care Unit with extensive experience in monitoring acute stroke patients. Specifically, patients will be monitored for any signs of neurological worsening and will be queried on development of new or worsening neurological symptoms, including headache, dizziness, numbness, or weakness, etc. If any sign or symptom of neurological worsening is suspected, the patient will be immediately assessed by the on-call neurologist and an immediate brain imaging (CT or MRI) will be obtained. Neuro-checks will be performed at 1h and 2h after stimulation and then every 2 hours. Subsequent care will be continued in these settings, including medical management per national guidelines for acute ischemic stroke management issued by the American Stroke Association.[42] Patients will not receive NSAIDs as concomitant therapy for the first 7 days after enrollment. All patients will undergo a multimodal MRI or CT including standard parenchymal images, non-invasive angiography, and perfusion studies and formal NIHSS assessment at 2-hour and 24-hour following the end of the stimulation tier as part of the study protocol.

D.5. Management pre, during, and post bridging stimulation

At tiers 5 and 6, hospital arriving patients who are candidates for EVT with an LVO detected on CT angiography and are being transferred to agio suite for EVT procedure will receive up-to-20 minutes of stimulation. See section D.4. for care during and post-stimulation.

Figure7: Technician-filled tolerability form	*Presence*		*Severity*			*Effect on the study treatment (Shortened the stim duration)*		*Duration/Frequency*			*Treatment*		
Adverse Events	**A**	**P**	**M**	**Mod**	**S**	**Y**	**N**	**< 50 % of stim period**	**≥50% of stim period**	**lasted after stim period (min)**	**None**	**Con**	**Med**
Rash													
Skin dryness													
Hair loss													
Itching													
Hives													
Skin Sensitivity to light													
Skin soreness													
Skin redness													
Skin darkening													
Numbness													
Tingling													
Dizziness													
General Pain													
Headache													

***A**=Absent, **Con**=Conservative therapy such as cold pack and lotion**, M**= Mild, **Mod**=Moderate, **Med**= Medication such as topical steroid, **N**= No, **P**= Present**, R**: Resolved, **S**: Severe, **Stim**= Stimulation, **Y**=Yes.

Figure 8. Shows the technician-filled tolerability form. The selected items are based on the most common reported adverse effects associated with tDCS.

D.6. Study Visits and Data Acquisition

D.6.1 Schedule of Events

	Baseline	**End of each 20-min stimulation cycle***			**Before/ After ET**	**2-hr**	**24-hr**	**Day 4**	**Day 30**	**Day 90**
		1(Bridging)	**2 (adjunctive)**	**3 (adjunctive)**						
Consent	✔									
History / Exam	✔									
Routine Labs	✔									
Vital Signs	✔	✔	✔	✔	✔					
Tolerability form/Skin Inspection*	✔	✔	✔	✔						
Multimodal MRI/CT	✔					✔	✔			
NIHSS	✔	✔	✔	✔	✔	✔	✔			✔
Modified Rankin Scale (mRS)	✔							✔	✔	✔
Barthel Index (BI)										✔
EuroQol (EQ-5D)										✔
AMC Linear Disability Scale										✔
Interval serious adverse events		✔	✔	✔	✔	✔	✔	✔	✔	✔
Concomitant/Interval medications/procedures	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔

* Skin Inspection will be performed before each stimulation period

D.6.2. Baseline Evaluation

- Demographics (age, sex, race)
- Last known well time
- Past medical/surgical history including vascular diagnoses and risk factors (stroke, TIA, carotid stenosis, myocardial infarction, atrial fibrillation, peripheral arterial disease, hypertension, diabetes, dyslipidemia)
- Medications, including antithrombotics, antihypertensives, statins, anti-arrhythmics Family history of vascular disease;
- Tobacco (including timing, duration, amount), alcohol, and illicit drug use;
- Vital signs (systolic blood pressure, diastolic blood pressure, pulse, respiratory rate, oxygen saturation);
- Premorbid global disability (modified Rankin Scale);
- Neurological deficits severity (NIHSS);
- Laboratory results (CBC, platelet count, glucose, lytes, INR, PTT, LFTs.

D.6.3. C-tDCS Treatment

- Skin inspection before each cycle of stimulation
- Tolerability/AE Form – after each cycle of stimulation
- NIH Stroke Scale (NIHSS) after each cycle of stimulation

D.6.4. Endovascular thrombectomy (EVT)

- Records of all the time metrics related to EVT: time of decision to take to catheter angiography suite for EVT, time of arrival at the catheter angiography suite, time of Groin Puncture, the time that C-tDCS was aborted prior to EVT, door to puncture time, on clot time, time first device deployed, time of first device passed (the times for each pass and deployment will be recorded), Total duration of EVT.
- Type of device uses (stent-retriever vs aspiration catheter)
- Total number of device passes
- NIHSS before and after EVT
- Interval serious adverse events during EVT
- Interval medications/procedures during EVT

D.6.4. Early (2 Hours) After EVT

- NIHSS,
- Multimodal MRI/CT
- Interval serious adverse events
- Interval medications/procedures

D.6.5. Late (24 Hours) After EVT

- NIHSS
- Multimodal MRI/CT
- Interval serious adverse events
- Interval medications/procedures

D.6.6. Day 4

- Modified Rankin Scale (mRS)
- Interval serious adverse events
- Interval medications/procedures

D.6.7. Day 30

- mRS (phone)**,**
- Interval serious adverse events
- Interval medications/procedures

D.6.8. Day 90

- NIHSS,
- Modified Rankin Scale (mRS)
- AMC Linear Disability Scale (granular disability)
- Barthel Index (BI) (activities of daily living)
- EuroQol (EQ-5D) (health-related quality of life)
- Interval serious adverse events
- Interval medications/procedures

D.6.9. Imaging Assessments

- Baseline

Emergent multimodal MR and CT is currently acquired as the routine initial imaging study in all acute stroke patients at UCLA. In patients receiving MRI, a 1.5 T or 3 T scanner equipped with echo-planar imaging capability is used for rapid acquisition of diffusion and perfusion scans. The standard clinical MRI protocol includes Gradient Recall- Echo (GRE), DWI, FLAIR (Fluid-Attenuated Recovery Image), PWI and MR angiography. The ADC (apparent -diffusion coefficient) values derived from DWI acquisition (b=0, 1000 s/mm^2 applied in each of three principal gradient directions) will be used to delineate the volume of ischemic core. The tissue with ADC values of ≤ 620 μm^2/s will be considered ischemic core, indicative of tissue with advanced, irreversible bioenergetic compromise. FLAIR delineates early parenchymal signal abnormality associated with ischemia and slow retrograde flow in leptomeningeal collaterals appearing as FLAIR vascular hyperintensity (FVH). The GRE sequence is used to evaluate the presence of intracranial hemorrhage and deoxygenated leptomeningeal collaterals appearing as GRE vascular hypointensity (GVH). PWI is acquired with sequential T2*-weighted (gradient echo) EPI time sequence scanning. Early in the time series, a bolus (0.1 mmol/kg) of MRI contrast material is rapidly infused (5 ml/sec through an 18 or larger gauge angiocatheter) using a power injector. The perfusion lesion volume (tissue at risk volume) will be the region with Tmax ≥ 6sec (Tmax = the time delay from

the arrival of contrast to its peak concentration at the tissue vasculature). [43,44] Intracranial CEMRA (Contrast-Enhanced MRA) will identify large vessel occlusion.
In patients receiving CT, the standard clinical CT protocol includes non-contrast CT (NCCT), multiphase CT angiography, CT perfusion (CTP) processed through RAPID software. NCCT is used to rule out acute intracranial hemorrhage appearing as hyperdensity. The tissue with rCBF < 30% on CTP will be considered ischemic core and the perfusion lesion volume (tissue at risk volume) will be the region with Tmax ≥ 6sec. CT angiography (CTA) will identify large vessel occlusion.

- Endovascular Thrombectomy

Patients will be undergoing cerebral angiography as part of the endovascular thrombectomy for treatment of acute ischemic stroke due to large vessel occlusion. The following data from pre-reperfusion cerebral angiography images will be collected: Target vessel occlusion for mechanical thrombectomy procedure, collateral grade prior to EVT (measured with the ASITN collateral grading scale). The following data will be collected from the post-reperfusion cerebral angiography images: arterial occlusion lesion: 1) Reperfusion, as measured with the expanded thrombolysis In cerebral infarction scale (eTICI) after final device pass (TICI 0, TICI 1, TICI 2a, TICI 2b,TICI 2c, TICI3); and 2) Recanalization, as measured with the arterial occlusive lesion (AOL) scale (0-No reduction of target thrombus, 1 – Reduced thrombus size but no recanalization, 2 – Partial recanalization, 3 – Complete recanalization).
TICI scale will be defined as the following: 0 – No perfusion, 1 – Antegrade reperfusion past the initial occlusion, but limited distal branch filling with little or slow distal reperfusion, 2a – Antegrade reperfusion of less than half of the occluded target artery previously ischemic territory (eg, in 1 major division of the MCA and its territory), 2b – Antegrade reperfusion of 51-90% of the previously occluded target artery ischemic territory, 2c – Anterograde perfusion of >90% but less than TICI 3 (i.e. near complete reperfusion), 3 – Complete antegrade reperfusion of the previously occluded target artery ischemic territory, with absence of visualized occlusion in all distal branches.[45]

- 2h and 24h Post- Endovascular Thrombectomy

Follow-up MR/CT multimodal imaging will be obtained routinely at UCLA at 2h and 24h post-thrombectomy (supported by Radiology Dept. research funds). MRI Imaging sequences will include DWI/FLAIR/GRE/PWI/CEMRA and CT imaging sequences will consist of NCCT/CTA/CTP. RAPID image processing software will be applied to the above images to quantify, at the baseline, 2h, and 24h time-points: 1) Early penumbra preservation: Volume of baseline penumbra tissue not progressing to ischemic core at 2h; 2) Penumbral salvage: Volume of baseline penumbra tissue not progressing to ischemic core at 24h; 3) Early collateral flow enhancement: Perfusion lesion volume at baseline – Perfusion lesion volume at 2h; 4) Sustained collateral flow enhancement: Perfusion lesion volume at baseline – Perfusion lesion volume at 24h; 5) Early infarct growth: Ischemic core lesion volume at 2h – Ischemic core lesion volume at baseline; and 6) Final infarct growth: Ischemic core lesion volume at 24h – Ischemic core lesion volume at baseline. Additional location and extent of ischemic injury at baseline, 2h, and 24h will be rated using the ASPECTS scoring system.

D.6.10. Adverse Events

D.6.10.1 Serious Adverse Events (SAEs)

All serious adverse events occurring during the 90 days of study participation will be recorded. A serious adverse event is any adverse event that is fatal, is life-threatening, is permanently or substantially disabling, requires or prolongs hospitalization, or requires medical or surgical intervention to prevent one of the above outcomes.[18]

D.6.10.2 Symptomatic intracranial hemorrhage

The lead safety endpoint adverse event is symptomatic intracranial hemorrhage (SICH), defined using the SWIFT PRIME trial criteria [46]: an increase of 4 or more points on the NIHSS within 24 hours of stimulation associated with parenchymal hematoma type 1 (PH1), parenchymal hematoma type 2 (PH2), remote intraparenchymal hemorrhage (RIH), subarachnoid hemorrhage (SAH), or intraventricular hemorrhage (IVH). In addition, all hemorrhages, both symptomatic and asymptomatic, will also be separately classified and analyzed by radiologic subtype, as hemorrhagic infarction type 1 (HI1), hemorrhagic infarction type 2 (HI2), PH1, PH2, RIH, SAH, or IVH.[46] A central neuroimaging core lab, blinded to treatment assignment, will review all brain scans obtained at 24h and rate presence and type of radiologic hemorrhagic transformation. In addition, in patients who experienced worsening by 4 or more NIHSS points in the first 24 hours, they will review any and all additional brain MRI or CT scans obtained during the 24h time period,

SICH is the primary safety endpoint of the current trial, but trial results will be assessed on a variety of additional safety, feasibility, and tolerability results as well. It is anticipated that, after completion of the current trial, the judgement of whether to proceed directly to a pivotal trial, to proceed to a larger safety and preliminary efficacy trial, or to not proceed with further development, will rest on a considered and informed assessment of all outcome measures. It is important, therefore, to collect data on a wide range of safety endpoints and a wide range of measures of functional outcome, as is planned in this proposal. In making the selection of a primary safety endpoint, an emphasis was placed on ensuring patient safety throughout the course of the trial by choosing an endpoint with uncontestable clinical relevance (Symptomatic Intracranial Hemorrhage).

D.6.10.3 Additional Adverse Events with Specific Interrogation

In addition to general screening for all serious adverse events and focused elicitation of symptomatic intracranial hemorrhage events, the following events will be specifically interrogated for and recorded in the case report forms: skin redness, scalp rash, hair loss, seizure, headache, sensitivity to light, new ischemic stroke, deep venous thrombosis, pulmonary embolism, pneumonia, acute MI.

E. Safety Monitoring

E.1. Data and Safety Monitoring Plan

The trial will be monitored by an Independent Data and Safety Monitoring Board (DSMB). Dr. Bruce Dobkins from department of neurorehabilitation will lead the DSMB. DSMB will assess for the causal relationship of the serious adverse event to the study treatment as definite, probable, possible, unlikely, or unrelated.

DSMB will meet at the completion of each dose tier, review all safety data, and determine whether the study will proceed to the next dose tier. DSMB deliberations will be guided by: 1) a formal stopping/escalation rule, based on the occurrence of the lead safety endpoint, symptomatic intracranial hemorrhage (SICH), and 2) The DSMB members' clinical judgement upon review of all other safety outcomes. In addition, the Food and Drug Administration (FDA) will be notified if there is one occurrence of SICH prior to repeating the tier or escalating to a higher tier. The DSMB, along with the investigators, will also continuously monitor door-to-puncture and imaging-to-puncture times in enrolled patients, comparing them with national guidelines from American Heart/American Stroke Association (AHA/ASA), the Society of Neurointerventional Surgery (SNIS), the Society of Vascular and Interventional Neurology (SVIN) and academic medical center comparator hospitals to ensure that start of thrombectomy is not being delayed by study procedures.

E. 2. Statistical Design and Analyses Plan

E.2.1. Sample size

The study sample size derives from the use of the 3+3, rule-based, modified Fibonacci, dose escalation design, with 3:1 randomization to active treatment vs sham control. There will be 6 dose tiers, reflecting increasing intensity and duration of stimulation (Figure 7). The 3+3 study design (3 patients and 1 sham) with 6 dose tiers yields a sample size of at least 24 and potentially up to 48. The 3+3 design is the classical approach to dose escalation in first-in-human studies. [47,48] While newer, adaptive designs for dose escalation trials have been developed, they are more complex and have limited advantages when major toxicities are not expected. [49] Therefore, the rule-based, 3+3 design remains the dominantly employed approach in current dose-escalation studies. We will take every step to avoid missing data by scheduling of the follow-up visits early in visit time windows and readiness to travel to the patient's location to perform needed assessments. Should any missing data occur, primary analyses will be performed using multiple imputation, with sensitivity analyses including complete case and worst possible outcome analyses. [50]

E.2.2. Baseline characteristics

The demographic and baseline clinical characteristics of the study population will be delineated with standard descriptive statistics. Categorical variables describing the clinical history, examination findings, and initial treatment will be summarized by frequencies. Continuous variables such as vital signs, laboratory results, and time variables will be characterized by means, standard deviations, and 95% confidence intervals (CI). Ordinal and non-normally distributed variables (such as the NIHSS) will be characterized by medians and interquartile ranges. Baseline characteristics will be compared between the tDCS stimulation group with sham group to assess covariate balance. Wilcoxon Rank-Sum tests will be used for continuous or ordinal variables; Fisher's exact tests and chi-square tests will be used for grouped or nominal categorical variables.

E.2.3. Safety and Tolerability (Specific Aim 1)

E.2.3.1 Dose Escalation

The study Data and Safety Monitoring Board (DSMB) will meet at the completion of each dose tier, review all safety data, and determine whether the study will proceed to the next dose tier. DSMB deliberations will be

guided by: 1) a formal stopping/escalation rule, based on the occurrence of the lead safety endpoint, symptomatic intracranial hemorrhage (SICH), and 2) The DSMB members' clinical judgement upon review of all other safety outcomes. The formal dose escalation rule uses SICH frequency to gate the occurrence and pace of escalation through the 6 dose tiers. If no SICH occurs in the 3 active patients at a dose tier, enrollment may escalate to the next dose tier. If one SICH occurs, 3 more active (and 1 more control) patient will be enrolled at that dose tier before escalation. If 2 SICHs occur at a dose tier, further study enrollment is held until detailed review by the DSMB (Fig 6). In addition, whenever the formal SICH criteria for dose escalation has been met, the DSMB will formally meet, review the SICH data and all other safety data, and advice regarding proceeding to the next tier, continuing at the current tier, or placing the study on hold.

E.2.3.2. Primary Safety Endpoint Analysis

For the final statistical analysis of the primary SICH safety endpoint, a chi-square test will be used to detect differences in the rate of SICH between the active treatment and sham patients and higher and lower dose tiers. The treatment will be considered to have exhibited adequate safety in the current trial to proceed to future, larger, pivotal efficacy trials if tDCS results in lower or equivalent rates of SICH compared to sham.

E.2.3.3. Secondary Safety Endpoints Analysis

In secondary safety endpoint analyses, the following will be compared between the active treatment and sham patients, and between higher and lower dose tiers, using chi-squared tests: **1)** Asymptomatic ICH by 24h (intracranial hemorrhage not associated with NIHSS worsening ≥ 4); **2)** Early neurologic deterioration (worsening ≥ 4 on NIHSS during the 24-hour period after stimulation, with or without intracranial hemorrhage); **3)** All-cause mortality at day 90 (mRS); and **4)** All serious adverse events.

E.3.3. Tolerability Endpoint Analysis

The lead tolerability endpoint is completion of the protocol-assigned stimulation treatment without early cessation due to cutaneous, neurologic, nociceptive or other adverse effects. Experience with tDCS in post-stroke patients indicates only infrequent cutaneous (itching, tingling) adverse effects are likely to occur. Accordingly, for the current study, a patient will be considered to have tolerated the procedure if at least 75% of the stimulus period was completed. The treatment will be considered generally tolerable if, among all enrolled patients, tolerated procedures are achieved in ≥90% of patients, assessed with a one-sided p value of 0.025. Secondary tolerability endpoints will be the rate and severity of cutaneous, neurological, and pain items of the technician-filled (Figure 7), descriptively compared between active treatment and sham patients, and between higher and lower dose tiers.

E.3.4. Feasibility Endpoint Analysis (Specific Aim 2)

A time-motion analysis and mock run-throughs will be conducted prior to first enrollment. Nonetheless, it is anticipated that processes to optimize rapid placement of the cap and electrodes will continue to improve with experience gained from initially enrolled patients. The predefined success threshold for feasibility will be median times from randomization to bridging C-tDCS initiation and the time form end of EVT procedure to adjunctive C-tDCS initiation ≤ 10 minutes in the last 4 and 10 enrolled patients, respectively.

E.3.5. Exploratory Efficacy Endpoints Analysis (Specific Aim 3)

E.3.5.1. Exploratory Clinical Efficacy Endpoints

This study is underpowered to definitively determine efficacy, so all clinical efficacy analyses will be purely exploratory and descriptive. Four clinical outcome measures were selected based on their reliability, familiarity to the neurologic community, and adaptability for use in patients who have had a stroke. These endpoints are: the modified Rankin Scale (mRS), a rating of global disability; the Barthel Index (BI), a measure of instrumental activities of daily living; the National Institutes of Health Stroke Scale (NIHSS), a measure of neurologic deficit severity; and the EuroQol (EQ-5D), an assessment of health-related quality of life; and AMC Linear Disability Scale, a granular degree of disability.

Clinical efficacy endpoints will be characterized in the active and sham patients, and in higher and lower dose tiers. Early course clinical efficacy endpoints of greatest interest that will be explored are: **1)** Normalized change in neurologic deficit from baseline to 24h (normalized delta NIHSS – linear variable, analyzed with means and 95% CIs; and **2)** Degree of neurologic deficit at 24h (NIHSS – quasi-linear variable, analyzed with means and 95% CIs). Final outcome clinical efficacy endpoints of greatest interest that will be explored are: **1)** Degree of disability at 90 days, assessed across all 7 levels of the modified Rankin Scale – ordinal variable, analyzed with medians (IQRs) and means (95% CIs); **2)** Functional independence (mRS 0-2) at 90 days – binary variable, analyzed with rates and 95% CIs; **3)** Granular degree of disability at 90 days (AMC Linear Disability Scale) –

linear variable, analyzed with means and 95% CIs; and **4)** Health-related quality of life (EQ-5D) – linear variable, analyzed with means and 95% Cis.

E.3.5.2. Exploratory Imaging Biomarker Efficacy Endpoints

This study is underpowered to definitively determine efficacy, so all imaging efficacy analyses will be purely exploratory and descriptive. The imaging efficacy analysis will be done on the subgroup of patients (including tier 5 and 6 patients who have presence of salvageable penumbra in their entry criteria) with baseline pre-EVT penumbral imaging demonstrating presence of salvageable penumbra with Tmax> 6 sec/ ischemic core volume of ≥ 1.2.

Imaging biomarker efficacy endpoints will be characterized in the active and sham patients, and in higher and lower dose tiers, using means and 95% confidence intervals. The six imaging efficacy endpoints of greatest interest that will be explored are: early and late penumbral salvage, early and late collateral flow enhancement, and early and late infarct growth.

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