1. Protocol Title: Efficacy and mechanisms of virtual reality treatment of phantom leg pain

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4. Setting of the Research:

The research will be conducted at the Virtual Reality Laboratories of MossRehab/Moss Rehabilitation Research Institute (MRRI), University of Pennsylvania and University of Washington/Harborview Medical Center in Seattle, Washington.

5. Resources Available to Conduct this Research:

Forty individuals with above (AKA) or below (BKA) knee amputation suffering from chronic phantom limb pain (PLP) will be recruited from clinics that specialize in the treatment of patients with amputation at Penn Medicine, MossRehab, and Harborview Medical Center (HMC). Penn Institute for Rehabilitation Medicine site had 75 new outpatient, 75 new inpatient and 300 follow-up visits by patients with lower limb amputation in 2019. The MossRehab Amputation Rehabilitation Center is the largest rehabilitation center in the Delaware Valley Region. There were approximately 120 new inpatient and over 100 new outpatient visits by patients with leg amputation at MossRehab in 2019. Based on our past experience under NIH grant R21NS099645, approximately 50% of subjects will meet inclusion/exclusion criteria for this study. Finally, HMC houses the Limb Preservation and Amputation Services and has 150 major limb amputations performed each year. Multicenter recruitment efforts are expected to provide the required number of suitable subjects to take part in this study during the planned five years' time window. Dr. Buxbaum will be responsible for recruiting at MossRehab/MRRI, Dr. Coslett at the University of Pennsylvania, and Drs. Rombokas and Friedly at Harborview Medical Center/University of Washington. Adequate researchers will conduct the investigations. All research staff will be trained in behavioral testing, administration of virtual reality, human subjects protection and HIPAA regulations. In addition, we will also recruit 30 non-amputee control participants without limb amputation.

6. Inclusion and Exclusion Criteria

Specific inclusion criteria for research subjects include:
1. Age 18 -100
2. Capacity to provide Informed Consent
3. Unilateral above or below knee amputation more than 3 months prior to enrollment
4. Absence of cognitive impairment, operationally defined as a Montreal Cognitive Assessment score of 18 or greater[1].

[1] Should a potential subject fail the telephone version of the MOCA, an in-person, complete MOCA will be administered; the presence or absence of cognitive impairment will be determined according to the norms of Milani et al. (2018) that take age, ethnicity and education into consideration.

5. Pain averaged over the preceding 1 month in the phantom limb rated as greater than 4 on a scale of 0-10.

Specific exclusion criteria for research subjects include:
1. History of significant medical or neurological disorder such as stroke or moderate to severe traumatic brain injury (operationally defined as loss of consciousness for more than 30 minutes)
2. History of significant or poorly controlled psychiatric disorders
3. Current significant depression or anxiety as judged by the Hospital Anxiety and Depression Scale (HADS) (Zigmond & Snaith, 1983).
4. Current abuse of alcohol or drugs, prescription or otherwise
5. Nursing a child, pregnant, or intent to become pregnant during the study

Specific inclusion criteria for control participants include: 1. Age 18 or older
2. Capacity to provide Informed Consent.
3. Absence of lower limb amputation

4. Absence of clinically evident cognitive impairment.

Specific exclusion criteria for control participants include:
1. History of significant medical or neurological disorder such as stroke or moderate to severe

traumatic brain injury (operationally defined as loss of consciousness for more than 30

minutes)
2. History of significant or poorly controlled psychiatric disorders.
3. Current abuse of alcohol or drugs, prescription or otherwise
4. Nursing a child, pregnant, or intent to become pregnant during the study

For the 7T or 3T scanner, specific contraindications to MRI are: 1) Cardiac pacemaker; 2) Other programmable implanted devices, e.g., For the carotid sinus; Insulin pumps and nerve stimulators; Lead wires or similar wires; 3) Optic implant; 4) Implanted cardiac defibrillator; 5) Aneurysm clip; 6) Any electronically, magnetically, and mechanically activated implant; 7) Ferromagnetic implants: coils, filters, and stents; metal sutures or staples; 8) Pregnancy; 9) Claustrophobia; 10) Metal in eye or orbit; 11) Tattooed eyeliner; other non-removable face paintings or tattoos.

7. Vulnerable Populations

Prisoners will not be recruited. Employees of the participating institutions will be permitted to participate but will not be selectively recruited. All participants will have the cognitive capacity to give informed consent.

8. Background:

Almost 2 million people in the US have had an amputation. Major lower limb amputations are performed approximately four times as often as upper-limb amputations, with approximately 75,000 annually in the U.S. (Weeks et al., 2010; Ziegler-Graham et al., 2008). Up to 90% of people with limb amputation experience the persistent sensation of the missing extremity, a phenomenon known as a “phantom limb” (Weeks et al., 2010). Additionally, a significant proportion of individuals with a phantom limb – up to 85% in some studies – experience

persistent and debilitating pain in the missing limb, a condition known as phantom limb pain (hereafter PLP). Although existing therapies provide pain relief in some cases, there is widespread agreement that current approaches fall short of bringing relief to most individuals with PLP (Weeks et al., 2010).

Many accounts of PLP propose that motor commands sent to the amputated limb fail to generate the visual, auditory, proprioceptive, and tactile afferent signals that the brain expects when controlling an intact limb (Bjorkman et al., 2012; Flor et al., 2006; Gragne et al., 2009; Makin et al., 2013; Ramachandran et al., 1995; Reilly & Sirigu, 2008). This lack of correspondence between action plans and sensory feedback from action is hypothesized to introduce imprecision or "noise" in the representation of the extremity, and the imprecision manifests as pain. On such accounts, one might predict that interventions that provide feedback relevant to the planned action of the missing extremity would reduce pain. There is evidence that this strategy is beneficial. Several investigators have demonstrated that the use of a mirror may reduce PLP (Brodie et al., 2007; Seidel et al., 2011). In this treatment, a mirror is placed perpendicular to the subject's midline, and the subject perceives the illusion that movements of the normal limb in the mirror are those of the missing limb. However, many subjects with amputation, particularly those who perceive the limb to be markedly foreshortened (or "telescoped"), fail to benefit from mirror therapy (Foell et al., 2013). Furthermore, an important limitation of mirror therapy is that only symmetric actions can be modeled; for example, one cannot simulate asymmetric actions such as walking. In addition, several prior studies with mirror therapy have reported challenges with compliance, limited adherence to treatment protocols, and high rates of drop-out (Darnall & Li, 2012; Kuys et al., 2012; Rothgangel et al., 2013).

A substantial literature has addressed the neural consequences of loss of sensory inputs in both animals (Kaas et al., 2008) and humans (Makin et al., 2015). Many inconsistencies in the literature remain, however, and several crucial issues have not been adequately explored (e.g., the relationship between sensori-motor cortices and brain structures that process pain).

We recently completed a proof-of-concept study funded by R21NS099645 (Coslett, PI). We hypothesized that a major limitation of prior therapies is the poor verisimilitude of the sensory feedback provided from the missing limb. As argued by Perry et al. (2014), approaches involving virtual reality (VR) that provide more life-like feedback may be substantially more efficacious than mirror therapy because they enable more diverse limb movements and provide richer sensory cues. Indeed, there have been a number of reports of the benefit of VR treatment in patients with PLP (Cole et al., 2009; Murray et al., 2007; Ortiz-Catalan et al., 2014). In our proof-of-concept study, eight subjects with below knee amputations (BKA) played a variety of active games requiring leg movements while receiving high-quality visual feedback of the missing lower leg. Subjects viewed the virtual environment through a head-mounted display that provides a three-dimensional graphical view of the virtual environment. Feedback about leg position was provided via an electromagnetic system using leg sensors (trackSTAR, Ascension Technologies Inc), and the program generated an image of the missing lower leg, visible as a first-person avatar. We developed a suite of 8 engaging games or activities from which subjects could choose during each session (Active VR treatment). This treatment was contrasted with a "Distractor" treatment, in which participants were engaged in a visually immersive virtual reality experience that did not require leg movements (Cool!™). Both treatments were associated with significant reductions in pain intensity, but the Active VR treatment was also associated with reductions in pain interference, depression, and anxiety. Moreover, single subject analyses

(Beeson & Robey, 2006) showed that the Active VR treatment showed superior efficacy in reducing pain intensity as compared to the Distractor treatment in 3 subjects.

9. Objectives:

The specific aims of this study are (i) to replicate our prior observations of efficacy of VR treatment in a larger sample of individuals with BKA; (ii) to test VR therapy in patients with AKA; (iii) to compare the efficacy of Active VR treatment to Distractor VR treatment for PLP on measures of pain as well as psychological health and quality of life, (iv) to determine neuroanatomic correlates of PLP and Virtual Reality treatment response to PLP

10. Study Endpoints

The primary endpoint will be (1) the change in phantom limb pain score pre and post each intervention, at baseline and after the intervention and in the follow-up sessions as assessed by visual analogue scale of the McGill Pain Short Form Questionnaire (Melzack, 1987); (2) change in the pain quality between baseline, post intervention and follow-up sessions using McGill Short Form Questionnaire. The secondary endpoint, assessed before each intervention will be changes in the average pain intensity item from 11-point numerical rating pain scale. (0 – minimum score/no pain; 10 maximum score/pain as bad as you can imagine), In addition, other outcomes measures will be changes in factors assessed by our battery of questionnaires (e.g., mood; see next section for details) between pre- and post-treatment sessions as well as between pre-treatment and the 1 and 8 week follow-up sessions.

To determine neuroanatomic correlates of PLP and Virtual Reality treatment response to PLP, we will first contrast neuroimaging findings of subjects with PLP (n=20) to that of non-amputee (n-20) subjects at two timepoints, we will explore the association between PLP and various measures of brain plasticity. Second, by comparing the performance of subjects with PLP

before and after VR treatment, we will determine the relationship between treatment-related changes in pain severity and changes in specific measures of brain organization.

11. Procedures Involved in the Research

Participants

Subjects at each of the 3 testing sites (MossRehab/MRRI, Penn, Harborview) will be quasi-randomly assigned to Active VR versus Distractor VR treatments in 50:50 ratio. Allocation will be conducted using covariate adaptive randomization (Suresh, 2011) implemented using the carat package in R; covariates will include average pain severity over the last week, limb telescoping (present/absent), level of amputation (AKA or BKA), and prosthesis use (regular, intermittent/occasional, none). Participants' enrollment and randomization will continue for two years. In addition, we will recruit up to 30 total healthy participants without limb amputation, and of similar age and gender, for the imaging portion of the study.

Methods:
VR System. At all sites, the VR system will be composed of an Oculus Quest 2 head mounted display with four modified integrated infrared trackers for BKA and three trackers for AKA. With AKA subjects, the flexion and extension of the knee arises as a synergy from the hip. For BKA subjects, the trackers will provide real-time information on upper and lower leg angles that are

readily used to generate a virtual avatar. Coupling of the sensors to the body (“grounding”) in bony areas is straightforward; for BKA we will create an adaptation of commercially available handheld controllers and a strap that is worn on the residual limb. However, some subjects with AKA, especially those with a short residual limb, offer a strap location with soft tissue that moves relative to the intended motion of the skeleton. We will use a grounding solution based on techniques used in prosthetic socket design. A limb strap resembling a gel sock will be used, incorporating the sensor and a patient-specific stiffness profile generated by 3D printed "boning," an approach similar to that widely-used in preparing orthotic splints. We will use our experience in tissue characterization and grounding to generate an encompassing fit around the residual limb that translates movements into worldframe movements of the sensor that can be detected by headset tracking.

Treatment Protocol.

Both Active and Distractor VR treatment will consist of 8 twice-weekly sessions of 1 hour. Subjects assigned to the Active VR treatment will participate in a variety of games and activities developed and used by our teams including Kick, Quest for Fire, Chess, Checkers, Sudoku, and surfing the internet. Subjects will have substantial flexibility to select games according to their interests but will be required to spend at least 30 minutes in each session in games that require forceful, large amplitude, movements of the amputated lower limb (e.g., Quest for Fire, Kick). Time spent and level of engagement as measured by movements of the avatar or, in the case of chess, checkers, solitaire, Sudoku, and internet surfing, the number of clicks produced with each leg will be recorded for each activity using custom software.

Subjects assigned to the Distractor VR treatment will participate in the *REAL i-Series*® *immersive VR experience*, which has been demonstrated to reduce pain in several studies but lacks the hypothesized “active ingredients” of our Active VR treatment (visual and auditory feedback of movement of an extrapolated amputated limb). Subjects will navigate through a pleasant VR environment; they do not see any rendering of their body and make no movements with their legs.

A study team member will offer encouragement and reminders as needed. Subjects will be monitored for the development of symptoms of cyber-sickness throughout each session. In our pilot study, mild and transient cyber-sickness occurred in 3 subjects, and no subject discontinued the study as a result of cyber-sickness. Should cyber-sickness be observed, the headset will be removed, the subject will be given a break and, when ready, they will resume Active VR treatment involving games/activities that involve less head movement (e.g., checkers, surfing the web). Adverse events will be recorded after each session.

Pre- and post-session testing.
At the start of each session, subjects will be asked to rate their **present pain** severity (Visual analog scale of the McGill Pain Questionnaire; Melzack, 1987) and their **average pain** since the previous treatment on an 11-point numerical rating pain scale; (0 - minimum score/no pain; 10 maximum score/pain as bad as participants can imagine). At the end of each session, subjects will again be asked to rate their present pain using the Visual analog scale of the McGill Pain Questionnaire.

After the first and last treatment session, subjects will complete the Simulator Sickness Questionnaire (Kennedy et al., 1993) which assesses cybersickness and the brief Slater-Usoh-Steed Presence Questionnaire (Usoh et al., 2000; Slater et al., 1998; 1994), which assess the

subject's experience of "presence" during the treatment session. The usability of each game will be assessed using the System Usability Scale (Brooke, 1996) in the last treatment session.

Pre- and post-treatments and follow-up.

An initial testing battery will be administered before starting the treatment and immediately after the 8-weeks treatment is completed; the battery will also be administered 1 and 8 weeks after the treatment is completed.
This battery of questionnaires will include:

1. **The McGill Pain Questionnaire**, Short Form (Melzack, 1987): a series of 4-point scales assessing 15 characteristics of pain (e.g., throbbing, shooting, cramping, etc.). It generates separate scores for Sensory, Affective, and Total pain (maximum 78 points), and correlates strongly with the well-known long form of the questionnaire

2. **The Frenchay Activities Index (FAI) (**Holbrook et al., 1983): a scale measuring the physical function and daily activity that has excellent reliability in subjects with lower-limb amputation in predicting quality of life (Asono et al., 2008).
3. **The 12-Item Short Form Health Survey (SF-12) (**Ware et al., 1996): a brief measure of quality of life and functional capacity that has been validated in patients with amputation and PLP (Padovani et al., 2015; Chen et al., 2012; Pape et al., 2010).

4. **The Pain Interference Scale from the Brief Pain Inventory** (Cleeland & Ryan, 1994): this assesses the degree to which pain interferes with daily activities using a 0-10 numeric rating scale. This measure has been validated for use in limb loss (Cleeland & Ryain, 1994).
5. **The Modified Limb Deficiency and Phantom Limb Questionnaire** (Goller et al., 2013): this questionnaire assesses prosthesis type and usage and non-painful phantom limb experiences, including perceived position (e.g., telescoping) and ability to move the phantom. In addition to providing useful descriptive information, prosthesis use and telescoping will be used in the adaptive randomization algorithm to assign subjects to treatments.

6. **The Hospital Anxiety and Depression Scale (HADS)** (Zigmond & Snaith, 1983)**:** the HADS is a 14- item measure assessing both depressive and anxiety symptoms. In addition to providing separate scores for anxiety and depression, the HADS also provides a measure of global negative affect. This measure has been recommended by the IMMPACT consensus panel for

inclusion in clinical trials given the importance of psychosocial factors in chronic pain and need for understanding inter-patient variability in treatment responding (Edwards et al., 2016). If a subject screens positive for significant depression or anxiety, we will refer him or her for appropriate support.

7. The **13-item Pain Catastrophizing Scale** (Sullivan et al., 1995): this investigates pain catastrophizing, which has been highly associated with pain severity and disability after amputation (Hanley et al., 2004; Whyte & Carroll, 2004; Jensen et al., 2002)
8. The **Insomnia Severity Index (**Bastien et al., 2001): this is a 7-point scale that measure insomnia, a symptom associated with PLP (Durmus et al., 2015).

In addition to this battery, we will administer the **Technology Acceptance Scale** (Morris et al., 1997) to assess perceptions of the VR system before the intervention and obtain a rating of treatment satisfaction (Robinson et al., 2004; Smith et al., 2005) in the one week follow up.

Timeline

After obtaining IRB approval, recruitment will start immediately and will continue for two to three years. Individual participation in the study will take 16-20 weeks.

12. Withdrawal of Subjects

Before the study begins, subjects will be informed that they are free to withdraw from the assessment at any time and that their confidential information will be protected. All questionnaires and other documents containing data will be identified by a subject code that is available only to research staff. Should a subject withdraw from the study, his/her research results will remain part of the study. Subjects may be withdrawn from the study for failing to participate in study activities.

13. Statistical Plan

a. Sample Size Determination
G*power (Faul et al.,2007) was used to determine the number of participants needed to observe a significant effect of the VR treatment and an interaction between groups (Active VR vs. Distractor VR) based on the moderate effect size of .64 observed in our pilot data comparing first versus last session data from the McGill Pain Inventory. The analysis determined that 18 participants per group are required to achieve statistical power of 80% ($p < .05$) for detecting the interaction. Allowing for 10% attrition, we will plan to include 20 participants each in the Active VR and Distractor VR arms.

7T imaging provides an average of 33% improvement in power in hypothesis-driven region-of-interest analyses, with a 300% improvement in sensitivity in some regions (Torrisi et al., 2018). Moreover, single-subject mapping of the sensory representations of adjacent fingertips in area S1 has been detected in samples as small as 6 subjects at 7T (Besle et al., 2014). Thus, our event-related, hypothesis-driven, within-subject analyses are likely to be sufficiently powered. Finally, functional connectivity analyses can yield improved reliability at 7T, and subcortical-cortical connectivity among small gray matter nuclei in the brainstem, thalamus, and cortex is feasible (Tak et al., 2018).

b. Statistical Methods
The dependent measures (see Study Endpoints) will be analyzed using permutation analysis, a rigorous approach favored over older parametric statistics when parametric assumptions are violated (Collingridge,2013; LaFleur & Greevy, 2009; Ludbrook & Dudley, 1998). The primary analyses of all outcome measures will focus on the significance of difference of scores in the baseline versus post-treatment assessments. To assess persistence of effects, we will also conduct analyses of these variables at baseline vs. post treatment, baseline vs. 1 week, and baseline vs. 8 weeks. Finally, we will assess whether pain responds rapidly to single sessions of treatment by comparing pre- and post-session data points for the Pain Intensity Scale of the McGill Questionnaire. All analyses will also include the interaction term (Treatment Group: Active vs Distractor treatment) to determine whether one treatment is significantly more efficacious than the other, and correction for multiple comparisons will be applied. In addition to group-level analyses, we will also conduct single subject analyses of treatment effects with Beeson & Robey's (Beeson & Robey, 2006) modification of the Cohen's D statistic, which quantifies treatment outcomes for single subjects using effect sizes.

c. **Sharing of Results with Subjects**
Results of the study will not be communicated to the participants until the study is completed.

14. Recruitment Methods

Forty individuals with chronic PLP will be recruited from clinics that specialize in the treatment of patients with amputation at Penn Medicine (Penn Institute for Rehabilitation Medicine), MossRehab, and Harborview Medical Center. Participant may also be referred by colleagues throughout the Philadelphia and Seattle region or self-refer after learning of the project. We may also seek referrals from area prosthetic clinics, local amputee support groups, and the Regional Amputee Center. In addition, UPenn and MRRI investigators will recruit up to 30 total healthy participants without limb amputation for the imaging portion of the study.

Candidate participants will be contacted by telephone, email or standard mail by study team members at each site. Mailings will inform patients that they can call for more information or to opt-out of the study. A print Opt-out Letter will be used for mailings to potential participants whose contact information is known; it advises recipients that we will call to ascertain their interest & eligibility for the study unless they request otherwise. A Recruitment Flyer and Opt-in Letter, asks interested individuals to contact the study coordinator; these may be provided to amputee support group leaders, clinicians, companies or other individuals who have agreed to distribute it to their clients/patients. Research study staff may also distribute recruitment information directly at support groups or professional meetings (virtually or in-person). As both MRRI and UPenn serve the greater Philadelphia area, a single recruitment flyer noting both study sites will be used. MRRI/UPenn online recruitment may also take place. Recruitment information will be displayed on a dedicated page of the MRRI website; viewers will be driven directly to the page via social media posts on Facebook or Twitter. MRRI may also recruit participants with amputations using Recruitment Enhancement Services at Jefferson Clinical Research Institute. The Recruitment Enhancement Service provides assistance to the principal investigator in developing the study's recruitment plan and may assist in identifying and contacting potential participants using the Informatics group, internal communication resources, community contacts, and other available resources as outlined in the study team's protocol. MyChart may also be used to identify and generate lists of potential participants. Individuals identified by the RES as meeting eligibility criteria will be contacted by either postal mail or email. As this is a service center, its employees are not considered research personnel and so should not be named as such on the IRB paperwork. Specific to this project: the study will be listed in the Jefferson Clinical Trails finder and emails will be distributed by RES. At the University of Pennsylvania, cohorts of individuals with lower limb amputation will be identified using SlicerDicer. Research staff will extract identified data of potential participants and send them to the Data Analytics Center - Perelman School of Medicine at the University of Pennsylvania. The Data Analytics Center will revise this information and ensure that the participants in the cohort are still part of Pennchart and provide a realistic list of potential participants. Study personnel will then identify these potential participants in MyPennMedicine and send an invitation email through this portal. At the University of Washington, a recruitment flyer may be posted/distributed at locations where potential candidates gather. Additionally, it will be provided to local physicians and clinicians familiar with the study methods for distribution to their patients; physicians may also distribute study information to their patients via their electronic medical records. If interested, candidates will contact the UWash study coordinator.

Healthy volunteers will be recruited to the Imaging portion of the study through use of recruitment flyers and the MRRI Research Registry, a consent-based listing of people who have agreed to be contacted about MRRI-affiliated research projects.

Regardless of how participants learn of the study, those who are interested will be invited to continue over the phone or video conference to hear more details about the study and to undergo screening. The brief screening interview includes questions to confirm eligibility and a description of the study. Upon agreement, the patient will come to the study site and the study team member will initiate the informed consent process. The nature and goals of the proposed research will be explained to subjects, and they will be asked to sign an IRB-approved Informed Consent form, a copy of which will be given to them.

Subjects will be paid for their participation by the site where they enrolled, At MRRI and UPenn subjects will be paid $20 for each assessment and treatment session and will be reimbursed for transportation expenses (up to $100); participants will be paid $40 for each MRI session. At UWash, $45 gift card payments will be issued for each assessment and treatment session. In addition, research participants will PLP may receive two completion bonuses of $100 each (for a total of $200): the first bonus will be given after the completion of the intervention (after the 8th session of the intervention) while the other bonus will be provided upon the completion of the entire study (after the 8-weeks follow-up session).

15. Potential Benefits to Subjects

We hope that subjects with PLP will directly benefit from participation by virtue of pain reduction, as well as improvement of co-morbid symptoms like depression and anxiety. The MRI sessions are not likely to benefit subjects directly but may provide important information regarding human brain plasticity and provide information regarding potential biomarkers that will be of potential use for other subjects with PLP.

16. Risks to Subjects

Risks from the behavioral tests are minimal, but include transient boredom, frustration, or anxiety. Participants will be encouraged to take breaks as needed. Risks from the virtual reality system are also minimal; subjects will be monitored during the entire time that they wear the virtual reality apparatus. Subjects will be seated in a comfortable and stable chair while wearing the device, minimizing the risk of falls. Should subjects report any adverse effects (e.g., cybersickness), the headset will be immediately removed, and subjects will rest quietly until their symptoms resolve. For the Active VR treatment, subjects will be encouraged to play games/activities that involve less head movement (e.g., checkers, surfing the web). Adverse events will be recorded after each session. At the end of the first and last session of the treatment, subjects will be administered a post session cyber-sickness questionnaire.

17. Medical Care and Compensation for Injury

Subjects will be asked to report if they become ill or injured during or as result of their participation. In the event of an injury or illness resulting from their participation in this research project, they will be provided with clinically appropriate medical care for that injury or illness within the capabilities of our clinics. However, we cannot assure that the medical care and treatment will be provided without charge, and the costs incurred may, ultimately be the participants' responsibility.

18. Alternatives

The alternative to participation is to not participate in the study but to seek treatment elsewhere.

19. Provisions to Monitor the Data for the Safety of Subjects

A Data Safety Monitoring Board (DSMB) composed of experts in statistics and experience in clinical trials will review the trial design, data collection system, and advise the principal and co-investigators regarding any perceived deficiencies in safety and overall study plan. The DSMB will also review adverse events and make recommendations regarding stopping or extending the trial. The DSMB will meet bi-annually.

The participants will be instructed to report any adverse effects (AEs) related to the study protocol during or between sessions. The AEs will be classified as serious or non-serious, study-related, possibly study-related, or not study-related by the lead investigator at the site where the subject was enrolled. All Serious study-related AEs will be reported to the Institutional Review Board (IRB) within 48 hours of knowledge of the event; AEs will also be submitted online through the central REDCap database and categorized by MedDRA for internal study reporting purposes. The determination of a probable or possible relationship to the study intervention will be discussed with PIs and Co-Is and the Data Safety Monitoring Board (DSMB) to clarify whether a study hold should be initiated. Reports will be routed to each institution's IRB via institutional PIs if required.

20. Cost to Subjects

Subjects may incur transportation costs in excess of the reimbursed amount. However, there will be no costs to the participant for any tests, procedures, or visits that are solely for research purposes. Participants' standard medical care will remain their responsibility or the responsibility of their insurance provider.

21. Provisions to Protect the Privacy Interests of Subjects

All researchers will have certification of training for protecting the rights of human subjects in research and for maintaining the integrity of the research project. They will take all the possible measures to protect subjects' privacy interests. As noted above, codes will be used in place of names on all research materials; the master spreadsheet linking name and research code will be stored in a password-protected file accessible only to research personnel.

22. Provisions to Maintain the Confidentiality of Data

Data will be collected by the study team (Principal Investigators, co-Investigator, and study staff) familiar with the protocol. All case report forms will be identified only by a unique study number. All information will be both transferred and stored in a coded format. Direct data access to Jefferson REDCap, Penn+Box, and UPenn FlyWheel will be controlled by passwords, stratified by the study role, and will be stored on dedicated and secure server space.

All researchers will have certification of training for protecting the rights of human subjects in research and for maintaining the integrity of the research project. The data quality will be periodically checked by study personnel and discussed by all members of the research team during the monthly videoconference meetings to ensure quality control of data collection and

data entry. Cross-checking of data entry and data fidelity will occur monthly at each site with the site PI. The central REDCap database will conform to policies of the respective institutions to maximize patient confidentiality and privacy. Research personnel will have access in REDCap to identified data of subjects recruited on their site, while only deidentified data will be available for collaborators of the other centers. Data sharing will comply with all HIPAA and institutional rules.

23. Data Management

General data will be shared amongst sites through REDCap (Research Electronic Data Capture) hosted by Thomas Jefferson University; this is HIPAA-compatible, secure web application for building and managing online surveys and data collection instruments, and secure sharing across multiple centers. Research files and records are labeled with a subject code and contain no identifying information.

24. Consent Process

Subjects who are interested in this study be invited for a phone meeting to hear more details about the study and to undergo a screening. The brief screening interview includes questions to confirm eligibility, a description of the study, and the responsibilities and potential risks and benefits of participating. Upon agreement, the patient will come to the study site and the study team member will initiate the informed consent process. The nature and goals of the proposed research will be explained to subjects, and they will be asked to sign an IRB-approved Informed Consent form, a copy of which will be given to them.

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