**Experimental Protocol**

|  |  |
| --- | --- |
| **#1** | **INFORMATION** |

|  |  |  |
| --- | --- | --- |
| **Experiment Title** | | **Site Protocol Stage 2** |
| **Originators** | | **SPAN Coordinating Center** |
| **Creation/Revision Date** | | **2/7/21; 4/21/21** |
| **#2** | **SCOPE** | |
| This Stage 2 protocol applies to all study sites. | | |
| **#3** | **PURPOSE** | |
| This SOP details the experimental protocol to be followed by sites during Stage 2. | | |
| **#3** | **ROLES AND RESPONSIBILITIES** | |
| SPAN. Coordinating Center: draft all SOPs  Study Site Principal Investigator: read and distribute all SOPs to relevant study team members and assure compliance. | | |
| **#4** | **REFERENCES TO APPLICABLE SOPS** | |
| SPAN SOP2 Animal Acquisition  SPAN SOP3 RapID Ear Tagging  SPAN SOP4 Mouse Enrollment  SPAN SOP5 Intention to Treat  SPAN SOP 6 Mouse Middle Cerebral Occlusion  SPAN SOP7 Intravenous Treatment Preparation  SPAN SOP8 Behavior Testing Room Setup  SPAN SOP9 Neurological Deficit Score  SPAN SOP10 Mouse Corner Test Assessment and Recording  SPAN SOP11 Corner Test Scoring  SPAN SOP12 Mouse Grid Walking Assessment and Recording  SPAN SOP13 Grid Walk Scoring  SPAN SOP14 Mouse Hanging Wire Assessment and Scoring  SPAN SOP16 Intravenous Treatment  SPAN SOP17 Reperfusion  SPAN SOP18 Intraperitoneal Treatment  SPAN SOP19 Behavior Video Naming Convention  SPAN SOP20 Abnormal Event Report  SPAN SOP21 Early Dropout Report  SPAN SOP22 Experimental Endpoint  SPAN SOP23 Intraperitoneal Treatment Preparation  SPAN SOP 25 Remote Ischemic Conditioning (RIC) Treatment  SPAN SOP26 Surgical Certification  SPAN SOP27 RapID Bluetooth Pairing  SPAN SOP28 Behavior Video Upload (to be re-written for Video Upload 2.0)  SPAN SOP30 Redcap Account Setup  SPAN SOP33 MRI upload  SPAN SOP35 MRI acquisition (new Stage 2 MRI acquisition SOP to be drafter after run-in)  SPAN SOP36 Edit Closed Animal Record  SPAN SOP38 RapID Barcode Scanning  SPAN SOP 40 Rat Middle Cerebral Occlusion  SPAN SOP41 IDA Account Setup  SPAN SOP 45 Rat Corner Test Assessment and Recording (pending feedback during run-in)  SPAN SOP 46 Rat Grid Walking Assessment and Recording (pending feedback during run-in)  SPAN SOP 52 Obesity Induced Hyperglycemic Mouse  SPAN SOP 53 Aging Model  SPAN SOP 54 Spontaneously Hypertensive Rat (SHR) | | |
| **#5** | **TERMS AND ABBREVIATIONS [update by Jessica at end]** | |
| SOP: standard operating procedure  NINDS: National Institutes for Neurological Disorders and Stroke  SPAN: Stroke Preclinical Assessment Network  ARRIVE: Animal Research: Reporting of in Vivo Experiments | | |
| **#6** | **Training and Requirements** | |
| **Training**  Site animal handling training  RapID training video  SPAN REDCap database training  Behavior assessment training and certification  **Requirements**  Surgeon will complete Surgical Certification (SPAN SOP 26 Surgical Certification)  REDCap Users will have a REDCap account (SPAN SOP 30 REDCap Account Setup)  IDA Users will have an IDA account (SPAN SOP 41 IDA Account Setup)   |  |  | | --- | --- | | **Location Where Records Maintained:** | Site | | | |

|  |  |
| --- | --- |
| **#10** | **SPECIFIC PROTOCOL** |
| |  |  | | --- | --- | | **Description** | **Notes** | | 1. Order an equal number of male and female animals from the closest laboratory vendor site.   Obese/Hyperglycemic Animal (OB) C57BL/6J Stock No: 000664 Order OB mice that are 4-5 wks. old  Aging Model: In-house aging C57BL/6J Stock No: 000664 Target Age at stroke 15-17 mths.  Spontaneously Hypertensive Rat (SHR) Charles River Stock No: SHR/NCrl . Sites are permitted to order SHR from other vendors to meet sample size. Target Age at stroke is 15-17 wks. | SPAN SOP2 Animal Acquisition  SPAN SOP 52 Obese/Hyperglycemic Animal  SPAN SOP 53 Aging Model  SPAN SOP54 Spontaneously Hypertensive Rat (SHR)  Please note that these animals must be ordered far enough in advance to induce the comorbidity. | | 1. Place RapID ear tag in ears of animals **upon arrival** | SPAN SOP27 RapID Bluetooth Pairing  SPAN SOP3 RapID Ear Tagging | | 1. Enroll mouse or rat in SPAN. Weigh the animal and enter the weight in the enrollment form. | SPAN SOP38 RapID Barcode Scanning  SPAN SOP 34 Animal Enrollment  **Note:** Rat may need to be placed under brief anesthesia for RapID Eartag placement. | | 1. At minimum 12 weeks before planned surgery and no more than 13 weeks prior to surgery date, start feeding OB mouse model high fat diet. Begin diet prior to mouse turning 6 weeks of age. Record pertinent information regarding the high fat diet on the enrollment form. | SPAN SOP 52 Obese/Hyperglycemic Animal | | 1. Perform corner test the week prior to planned MCAo | SPAN SOP8 Behavior Testing Room Setup  SPAN SOP10 Mouse Corner Test Assessment and Recording  SOP 45 Rat Corner Test Assessment and Recording  SPAN SOP19 Behavior Video Naming Convention  SPAN SOP28 Behavior Video Upload | | 1. One week before surgery, submit Redcap ITT form indicating plan to perform surgery. Complete one form per animal.   If site is in possession of the RIC instrument, indicate that RIC surgery is being performed. The animal will be randomized to either RIC or RIC-sham.  Sites performing surgery on SHR will request that vendor send blood pressure measurements and date of measurement on first 10 animals (5M/5F) SHRs shipped to site. Site will record mean arterial pressure (MAP) on the enrollment REDCap form for the 10 animals.  Sites performing surgery on OB mice will measure blood glucose using recommended glucometer on the first 10 animals (5M/5F) on HFD and 5 controls prior to surgery day to reduce stress on animal. Sites to collect blood glucose on week of baseline assessments for first 5 animals. during surgery. If there is a low or abnormal value, site will need to continue glucose testing. Site to record blood glucose measurements on ITT. | SPAN SOP5 Intention to Treat  **Note:** Stage 2 ITT has additional requested fields for weight and blood pressure.    **Note:** There may be a threshold for SHR blood pressure.  See SPAN SOP52 Obesity Induced Hyperglycemic Animal for glucometer recommendations.  **Note:** TheCC will randomize animals daily, sites must submit ITT/enrollment forms for subjects before 3pm PST 1 week prior to surgery date. | | 1. Site will receive randomization email detailing animal’s treatment assignment. Details will include:   Route of injection  Box and Vial ID  Volume of injection  Rate of injection if IV  If RIC, then RIC or RIC Sham |  | | 1. Prior to beginning surgery, prepare IV treatment or IP treatment.   For mouse IP treatments: **Withdraw 4.4 ml of air** and then inject **4.4 ml of sterile wate**r. Verify correct vial is used for correct animal before every injection. IP injection volume will be specified in the randomization email sent by the CC.  For rat IP treatments: **Withdraw 26.4 ml of air** and then inject **26.4 ml of sterile wate**r. Verify correct vial is used for correct animal before every injection. IP injection volume will be specified in the randomization email sent by the CC.  For RIC treatments: Assure machine is in working order. | SPAN SOP7 Intravenous Treatment Preparation  Or  SPAN SOP23 Intraperitoneal Treatment Preparation  Or  SPAN SOP 25 Remote Ischemic Conditioning (RIC) Treatment  **Note:** Volumes of water to add may change. Volume will be included in the randomization email. | | 1. (Surgery Day) Perform a 30-60 min.? Right side occlusion.   Aged Mice: 60 min; site to keep mice awake during occlusion  Obesity Induced Hyperglycemic: 45 min or 60 min? awake during occlusion  Spontaneously Hypertensive Rat: 60 min or 90 min? Sites will choose asleep or awake during occlusion.  There will be a 30-day reperfusion (survival) period. | SPAN SOP6 Mouse Middle Cerebral Artery Occlusion  SPAN SOP 40 Rat Middle Cerebral Artery Occlusion | | 8a. If animal is **awake** during occlusion, perform the Neurological Deficit Score right before re-anesthetizing animal for reperfusion and treatments.  If animal was **kept asleep**, skip the Neurological Deficit Score. | SPAN SOP9 Neurological Deficit Score | | 8b. If randomized to IV treatment:  NOTE: This is a single dose treatment  Load syringe into a syringe pump programmed to deliver rate indicated in randomization email.   |  |  |  | | --- | --- | --- | | Brand | Size | Internal Diameter (ID) | | BD | 1ml TB | 4.71mm | | BD | 3ml | 8.66mm | | BD | 5ml | 12.06 |   Start infusion 5 minutes before reperfusion | SPAN SOP16 Intravenous Treatment | | 8c. If randomized to IP treatment  NOTE: Each animal should receive 6 doses in total.  Animals should receive the first dose 5min. before reperfusion.  (Then the following 5 injections every 12 hours) | SPAN SOP18 Intraperitoneal Treatment | | 8d. If randomized to RIC Treatment:  NOTE: Each animal should receive a total of 6 RIC treatment sessions.  Each session of RIC consists of 5 mins. inflation and 5 mins. of deflation repeated 4 times. This amounts to 40 minutes per session.  If randomized to RIC Sham Treatment:  Cuffs will be placed on hind limbs, but cuffs should not be inflated.  (The first session of RIC occurs immediately after reperfusion. The second session will occur as close as possible to 12 ± 2 hours. Following treatments will occur once per day for a total of 6 treatments) | SPAN SOP 25 Remote Ischemic Conditioning (RIC) Treatment | | 8e. At the end of the specified occlusion period, remove the filament to allow reperfusion. | SPAN SOP17 Reperfusion  Note: duration of occlusion may vary depending on ongoing results at each site | | 9. Post-Operative Day 1: Score neurological deficit at 24 hours after MCAo and administer SQ fluids twice daily. | SPAN SOP9 Neurological Deficit Score | | 9a. IP follow up injections AM and PM or RIC treatment as randomized. | SPAN SOP18 Intraperitoneal Treatment  SPAN SOP 25 Remote Ischemic Conditioning (RIC) Treatment | | 10. Post-Operative Day 2: Score neurological deficit at 48 hours after MCAo and administer SQ fluids twice daily. | SPAN SOP9 Neurological Deficit Score | | 10a. IP follow up injections AM and PM or RIC treatment as randomized | SPAN SOP18 Intraperitoneal Treatment  SPAN SOP 25 Remote Ischemic Conditioning (RIC) Treatment | | 10b. Perform MRI at 48±4 hours after MCAo, Upload MRI scans into the IDA account | SPAN SOP35 MRI acquisition (New Stage 2 SOP to be written. Sites to use Stage 2 Pilot MRI memo )  SPAN SOP33 MRI upload | | 11. (Day 3-5) Perform subsequent treatments for IP injections and RIC as per protocol. | SPAN SOP18 Intraperitoneal Treatment  SPAN SOP 25 Remote Ischemic Conditioning (RIC) Instrument | | 12. (Day 3-7) Administer subcutaneous fluids to the aging mice twice daily and to other models as needed. | See each model SOP for suggestions on Post-Operative supportive care. | | 13. (Day 7) Perform behavioral testing 7±1 days after MCAo, perform Corner Test, grid walk test and hanging wire. | SPAN SOP8 Behavior Testing Room Setup  SPAN SOP 10 Mouse Corner Test Assessment and Recording  SPAN SOP 45Rat Corner Test Assessment and Recording  SPAN SOP12 Mouse Grid Walking Assessment and Recording  SPAN SOP 46 Rat Grid Walking Assessment and Recording  SPAN SOP15 Hanging Wire Scoring  SPAN SOP19 Behavior Video Naming Convention  SPAN SOP28 Behavior Video Upload | | 14. Day 30 ± 2 day days after MCAo, repeat Corner Test, Grid walk test and Hanging wire. | SPAN SOP8 Behavior Testing Room Setup  SPAN SOP 10 Mouse Corner Test Assessment and Recording  SPAN SOP 45Rat Corner Test Assessment and Recording  SPAN SOP12 Mouse Grid Walking Assessment and Recording  SPAN SOP 46 Rat Grid Walking Assessment and Recording  SPAN SOP15 Hanging Wire Scoring  SPAN SOP19 Behavior Video Naming Convention  SPAN SOP28 Behavior Video Upload | | 15. Perform Day 30 ± 2 day MRI | SPAN SOP 35 MRI acquisition (Need to adjust to Rat)  SPAN SOP33 MRI Upload | | 16. Day 30 ± 2 day for Obesity model, blood glucose reading.  Weigh and record the obesity model weight at sacrifice  Sacrifice (tissue banking then perfuse 1u/ml heparinized saline, then 4% PFA, then post fix overnight in PFA, move to 30% sucrose next day) | SPAN SOP 22 Experimental Endpoint  Note: Please note any observations or unusual findings in the comment field on the End of Study Form. | | 17. As necessary, refer to SOPs for:  Animal deaths prior to sacrifice  Something unusual happens during the study  A mistake is noted in the record after it is closed | SPAN SOP21 Early Dropout Report  SPAN SOP20 Abnormal Event Report  SPAN SOP36 Edit Closed Animal Record | | Score behavior videos as assigned and submit scores to the SPAN CC group email [spancc@usc.edu](mailto:spancc@usc.edu) within 7 days of receiving the video assignment. | SPAN SOP11 Corner Test Scoring  SPAN SOP13 Grid Walk Scoring | | |

|  |  |
| --- | --- |
| **#11** | **REVIEWED AND APPROVED BY** |
| *Patrick Lyden, Principal Investigator*  *\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_*  *(Printed Name/Title) (Signature)*  *Jessica Lamb, SPAN Manager*  *\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_*  *(Printed Name/Title) (Signature)*  *\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_*  *(Printed Name/Title) (Signature)*  *\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_*  *(Printed Name/Title) (Signature)*  *\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_*  *(Printed Name/Title) (Signature)*  *\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_*  *(Printed Name/Title) (Signature)*  *\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_*  *(Printed Name/Title) (Signature)*  *\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_*  *(Printed Name/Title) (Signature)* | |