**Meeting Minutes**

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| Meeting: | SPAN Steering Committee Meeting |
| Date/ Time: | 4/28/21 11am-12pm PT |
| Present: | Patrick Lyden, MB Khan, Ligia Boisserand, Klaus Van Leyen, Andrew Goh, Lydia Obertas, Andreia Morias, Kris Dhandapani, Takahiko Hima, Senthil Gounder, Taylan Erdogan, Nirav Dhanesha, Anjali Chauhan, Jaroslaw Aronowski, Fahmeed Hyder, Anil Chauhan, David Hess, Cenk Ayata, Andre Rogatko, Basav Sanganahalli, Dan Thedens, Francesca Bosetti, Jelena Mihailovic, Jim Koenig, Lauren Sansing, Ligia boisserand, Marcio Diniz, Mariia Kumskova, Raymond Koehler, Ryan Cabeen, Tom Qin, Jessica Lamb, Karisma Nagarkatti |

**Agenda Items**

1. **Interim SPAN Analysis- Stage 1** 
   1. Preliminary snapshot: 902 randomized, 900 surgeries, 870 modified ITT, 713 per protocol
   2. 764 D2 MRI analyzed, 574 Day 30 MRI analyzed
   3. 9885 behavioral ratings
   4. Still ongoing: finalize mITT and PP populations, finalize descript stats, finalize outcomes, run Dunnett’s test on corner, select candidates for Stage 2
   5. Control limit run charts were presented based on Day 2 Lesion Volume. One site appeared to produce lesions outside of the lower control limit. One of the PIs asked whether if site goes outside of the prespecified range, should sites stop enrolling? Dr. Lyden agreed to take this recommendation under advisement.
   6. The controls are all treatments, all animals? Raises the potential for integrating effective treatments have you separated placebo?
   7. Marcio defined upper and lower control limits based on mean/SD lesion volume seen in the pilot study. We did include all the mice in this plot for all the treatments. Going forward we will include only the control mice. Will site be one of the factors in terms of the outcomes of the corner tests when comparing the different drugs vs. placebos? To look at treatment effect.
      1. Dunnett’s test assumes randomization takes care of all the differences. We are also doing a regression model that considers other factors (site, sex, etc.)
   8. One or two sites show time dependent drift? Is there a way in statistics to correct for that?
      1. There is no way to correct for the time drift, but stats can correct for the volume of the lesion. Stats can add volume lesion in the regression model and consider that.
   9. UTH: It is theoretically possible that some drugs may act differently on larger vs smaller lesions.
      1. Dr. Lyden answered that the regression equation may be the most powerful approach to studying an interaction between lesion size and treatment effect. If size interacts with treatment that would indicate that.
   10. In addition, there are two sites with small average lesion size and, 3 sites with large lesion, Parcellation by size based on grouping on the 48 hr. MRI scan to see if we can detect drug differences in one cohort vs. another. Regression model is the most powerful way to do that.
   11. U Iowa: Some sites changed the way we did surgery in Stage 1 vs. pilot. In pilot site did asleep and in Stage 1 site performed awake surgeries and changed the filament used.
   12. Control Limits are there only to generate alerts.
   13. Time dependence will affect RIC differently from other interventions because RIC was grouped during Stage 1.
2. **Stage 1- Tail Issue** 
   1. CC addressed Tail issue with site. Issue was brought to attention based on comments from videos. Dr. Lyden emphasized the need for scrupulous protocol adherence.
3. **Stage 2 Planning**
   1. Start date 7/5/2021
   2. IACUC Protocol once approved send to CC
   3. Run-in phase
      1. ‘Stage 2 Pilot’ Visits are available in IDA for Rat MRI scans and video uploads
      2. Sites will need to alert CC (spancc@usc.edu) when video or MRI scan is uploaded into this visit code
4. **MRI Validation** 
   1. Asking for Histology sections/Image analysis of n=20 (n=3-4 per site) CC selected tissue banked brains. We need morphometry, Cresyl violet is typical but any stain that helps us analyze the lesion volume. We would not suggest embedding this in paraffin. Entire brain needs to be cut serially from frontal poles to posterior lobes. When you send the scanned images do not include the lesion/hemispheric outlines permanently affixed on the images.
   2. Which sites do histology on a routine basis?
      1. AG: not sure it is part of the daily practice. Dr. Khan can send the sectioning to the Histo Core and site scans in the lab.
      2. U Iowa: uses a core. U Iowa did not PFA perfuse, brains were snap frozen.
      3. JHU: have not done Cresyl violet on MCAo brains. They are only fresh froze the tissue because they thought they would be doing westerns, PCRs. JHU did not PFA perfuse the brains. CC suggests using cryostat. JH can do thicker sections on cryostat.
      4. MGH: we can do it
      5. UTH: we do 90% of everything with the frozen sections. Any counterstain should work. The lesion we are going to decipher will be compared to the MRI. One potential thing we can do is look at the size of the ventricles which could be representative.
      6. Yale: we can do it, we have a cryostat, brains are pfa perfused and post-fixed cryoprotected
   3. Biggest issue could be if there is gross distortion between the sections but should be fixable
   4. Acute Lesion: After running D2 MRI with Stage 1 protocol, Sacrifice animals, do not perfuse. Perform TTC staining.
   5. Images of TTC can be sent in any format that can be put into imageJ.
5. **MRI Acquisition Stage 2**
   1. Stage 2 Pilot MRI Memo sent out on Friday 4/16
   2. LONI has created new Visit codes in IDA ‘Stage 2 Pilot BL, D2, D28’
   3. Sites should **email** [**spancc@usc.edu**](mailto:spancc@usc.edu) **when scans are uploaded into visit codes.**
      1. **In email to CC include Animal ID, Scan Date, Model/Species, Animal Age, Timepoint**
6. **Stage 2 Models**
   1. Obese/Hyperglycemic
   2. SHR
   3. NIA Aged Mice
      1. Reminder: Sites need to submit orders through NIA ordering system **no more than 30-days prior** to expected delivery date. Sites to order NIA mice according to spreadsheet submitted to NIA
7. **Finalize Stage 2 SOPs**
   1. SOP 40 Rat Middle Arterial Occlusion
      1. How many sites as a standard practice use Isoflurane and how many are using just O2 and nitric oxide?
         1. Yale: we use 70/30 with Isoflurane
         2. UTH: He used 4% Isoflurane with air not O2: nitric oxide.
         3. AG: uses isoflurane for rat around 3-4% but 70 Nitrous: 30O2
      2. JH and AG use 4.0 filament not 5.0 filament.
         1. The experimental protocol for rat MCAo lists suggested filament sizes as recommended by Doccol. However, what is most important is the size of the infarctions. Please try these filaments in surgeries and feedback to us from your experience. Sizes on SOP are from the Doccul. Company. Silicone coated sutures.
      3. CC would like to standardize on balanced anesthesia.
   2. SOP 52 Obesity Induced Hyperglycemic Model
   3. SOP 53 Aging Model
   4. SOP 54 Spontaneously Hypertensive Rat (SHR)
8. **Virtual Site Visits Completed**
9. **Miscellaneous**

**Summary**

1. **MRI Validation Reminders:** Sites should section entire brain.When site sends the scanned images Do Not have the lesion/hemispheric outlines permanently embedded on the images (This will be added to the protocol).
2. LONI has created **new Visit codes** in IDA for Run-in “Stage 2 Pilot BL, D2, D7, D28, D29”.
3. For every MRI scan or Behavior Video uploaded into “Stage 2 Pilot” Sites must **email the CC (**[**spancc@usc.edu**](mailto:spancc@usc.edu)**)**. Email to CC must include Animal ID, Scan Date, Model/Species, Animal Age, Timepoint
4. Sites will continue to share experiences with surgeries, scans, behaviors for 3 Stage 2 Models at the Friday Stage 2 Weekly Meetings. SOPs will be adjusted based on site feedback at these meetings.
5. Stage 1 Data analysis is ongoing