**SPAN 2 Embolic Clot Study (SPAN 2.EC.2) Statistical Analysis Plan**

# **Note**

This statistical analysis plan applies to the “second block” of the embolic model stage surgeries. This stage will be completed after the 4th and final stage of SPAN is completed in the spring of 2025. The surgeries for this plan will be completed between 6/23/25 and 8/20/25. The sample size was re-calculated and the data will be analyzed as a separate dataset from the “first” block of embolic model surgeries.

# **Introduction and Synopsis of the Study**

The Stroke Preclinical Assessment Network (SPAN) 2, is a randomized, controlled, blinded, multi-laboratory late-stage preclinical trial that seeks to bring rigor and transparency to the testing of putative cerebroprotectants. The embolic model has been suggested to be a model with better translation given that the occlusion is made with a natural substance (blood vs filament) and initiates inflammation and free radicals….

In the Embolic Clot study second block, six independent research laboratories will perform the embolic clot model of stroke surgery with either a 3 or 4cm clot, followed by either IV Tenecteplase (TNK) to cause clot lysis and reperfusion or IV saline in one animal model, young Sprague Dawley rats that includes equal numbers of males and females. No cerebroprotectants will be administered. In the Embolic Clot Study, the laboratories will adhere to a common protocol and each efficiently enroll 48 animals with excellent protocol adherence, full data completion and comprehensive animal tracking. By performing this protocol, we can validate the sensitivity of the clot model and help to determine if the model is appropriate for further use.

This statistical analysis plan (SAP) defines all pre-specified, planned analyses. If there are minor differences between the SPAN 2 Embolic Clot Experimental protocol and the SAP, the SAP will take precedence.

# **Treatment Arms**

All animals will receive either IV TNK to cause clot lysis and thus reperfusion or IV saline as a 1 minute bolus 120 minutes after the placement of either a 3 or 4cm clot.

NOTE: Since only one database project is used for both blocks, a variable is needed to differentiate these two cohorts. All data that will be used in this analysis plan will be from animals enrolled (enro\_enroll\_dt) in the REDCap database after 6/1/25. Data submitted to the statistical team will already be filtered by this enrollment date to simplify analysis.

**Feasibility**

**Enrolled Population**: Total animals received at sites who got ear tags and were entered into REDCap.

Definition:

enro\_animal\_id ≠ empty

Goal:

1. Feasibility

Descriptive tables by:

Total, Site, Sex, Animal Model

Variables:

site = redcap\_data\_access\_group

enro\_model

enro\_sex

**Ineligible for Randomization**: Subjects excluded prior to randomization. Definition:

rand\_conduct = no

Goal:

1. Feasibility

Descriptive tables by:

Total, Site, Sex

Variables:

site = redcap\_data\_access\_group

enro\_sex

rand\_conduct\_rsn

rand\_conduct\_rsn\_oth

**ITT Population**: Animals randomized and scheduled for surgery (submitted ITT for stroke surgery)

Definition:

rand\_conduct = yes  
  
Goal:

1. Feasibility

Descriptive tables by:

Total, Sex, Site, Clot length (rand\_clot\_length)

Variables:

site = redcap\_data\_access\_group

enro\_sex

txas\_reperfusion

rand\_clot\_length

**Exclusion from Treatment**: Randomized but unable to receive surgery.   
Definition:

rand\_conduct = yes AND srg\_conduct = no

Goal:

1. Feasibility

Descriptive tables by:

Total, Sex, Site, Reperfusion method (txas\_reperfusion), Clot length (rand\_clot\_length),

Variables:

site = redcap\_data\_access\_group

enro\_sex

srg\_conduct\_rsn

srg\_conduct\_rsn\_oth

txas\_reperfusion

rand\_clot\_length

**Procedural Dropouts**: Randomized, surgery began, but unable to complete occlusion and receive reperfusion vial. Will be a small group.

Definition:

rand\_conduct = yes AND srg\_conduct = yes AND srg\_reperfsn\_vial = blank (shows that the animal did not receive the reperfusion vial)

Goal:

1. Feasibility

Descriptive tables by:

Total, Sex, Site, Reperfusion method (txas\_reperfusion), Clot length (srg\_clot\_length; round to the nearest whole number)

Variables:

site = redcap\_data\_access\_group

enro\_sex

srg\_comments

txas\_reperfusion

srg\_clot\_length (round to the nearest integer number)

srg\_reperfsn\_vial

**Modified ITT Population (mITT):** Successful surgery (received the dose of TNK or saline)

Definition:

rand\_conduct = yes AND srg\_conduct = yes AND srg\_reperfsn\_vial ≠ blank

Goal:

1. Feasibility

Descriptive tables by:

Total, Sex, Site, Reperfusion method (txas\_reperfusion), Clot length (rand\_clot\_length)

Variables:

site = redcap\_data\_access\_group  
enro\_sex

srg\_animal\_age   
srg\_period = srg\_length\_surgery - subtract srg\_awake\_min   
srg\_exact\_occ  
srg\_concom\_meds\_sq  
srg\_concom\_meds\_lrs\_nacl  
srg\_comments

enro\_weight

srg\_weight

postop\_d1\_weight

postop\_d2\_weight

postop\_d3\_weight

postop\_d4\_weight

postop\_d5\_weight

postop\_d6\_weight

postop\_d7\_weight

postop\_d8\_weight - Note: Blank values for weights should not be treated as missing or zero values.

eos\_weight

srg\_nds\_score

postop\_d1\_nds\_score

postop\_d2\_nds\_score

eos\_nds\_score

eos\_day\_diff\_srg\_death

txas\_reperfusion

rand\_clot\_length

**Per Protocol:** mITT subjects who undergo successful surgery, received 75-125% dose of reperfusion vial.

Definition:

rand\_conduct = yes AND srg\_conduct = yes AND srg\_reperfsn\_vial ≠ blank AND:

srg\_reperfsn\_dose >=0.75(rand\_reperfusion\_dose ) but < =1.25(rand\_reperfusion\_dose)

Goal:

1. Feasibility

Descriptive tables by:

Total, Sex, Site, Reperfusion method (txas\_reperfusion\_actual), Clot length (srg\_clot\_length; round to the nearest whole number)

Variables:

site = redcap\_data\_access\_group  
enro\_sex

srg\_animal\_age   
srg\_period = srg\_length\_surgery - srg\_awake\_min

srg\_exact\_occ  
srg\_concom\_meds\_sq  
srg\_concom\_meds\_lrs\_nacl  
srg\_comments

enro\_weight

srg\_weight

postop\_d1\_weight

postop\_d2\_weight

postop\_d3\_weight

postop\_d4\_weight

postop\_d5\_weight

postop\_d6\_weight

postop\_d7\_weight

postop\_d8\_weight - Note: Blank values for weights should not be treated as missing or zero values.

eos\_weight

srg\_nds\_score

postop\_d1\_nds\_score

postop\_d2\_nds\_score

eos\_nds\_score

eos\_day\_diff\_srg\_death

txas\_reperfusion\_actual

srg\_clot\_length

**Loss to Follow-up Population:** Fully treated population that died before the experimental timepoint of 28 days post-op.

Definition:

Fully treated definition AND eos\_day\_diff\_srg\_death < 28

Goal:

1. Feasibility

Descriptive tables by:

Total, Sex, Site, Reperfusion method (txas\_reperfusion\_actual), Clot length (srg\_clot\_length; round to the nearest whole number)

Variables:

site = redcap\_data\_access\_group  
enro\_sex

srg\_animal\_age   
srg\_length\_surgery - Note: all rat surgeries should be awake- subtract srg\_awake\_min to get actual surgery period

srg\_exact\_occ  
srg\_concom\_meds\_sq  
srg\_concom\_meds\_lrs\_nacl  
srg\_comments

enro\_weight

srg\_weight

postop\_d1\_weight

postop\_d2\_weight

postop\_d3\_weight

postop\_d4\_weight

postop\_d5\_weight

postop\_d6\_weight

postop\_d7\_weight

postop\_d8\_weight - Note: Blank values for weights should not be treated as missing or zero values.

eos\_weight

srg\_nds\_score

postop\_d1\_nds\_score

postop\_d2\_nds\_score

eos\_nds\_score

eos\_day\_diff\_srg\_death

txas\_reperfusion\_actual

srg\_clot\_length (round to the nearest integer number)

**Full analysis population:** Fully treated population that survived to day 28 post operatively.

Definition:

Fully treated definition AND behav\_d30\_conduct = 1

Goal:

1. Feasibility

Descriptive tables by:

Total, Sex, Site, Reperfusion method (txas\_reperfusion\_actual), Clot length (srg\_clot\_length; round to the nearest whole number)

Variables:

site = redcap\_data\_access\_group  
enro\_sex

srg\_animal\_age   
srg\_length\_surgery - Note: all rat surgeries should be awake- subtract srg\_awake\_min to get actual surgery period

srg\_exact\_occ  
srg\_concom\_meds\_sq  
srg\_concom\_meds\_lrs\_nacl  
srg\_comments

enro\_weight

srg\_weight

postop\_d1\_weight

postop\_d2\_weight

postop\_d3\_weight

postop\_d4\_weight

postop\_d5\_weight

postop\_d6\_weight

postop\_d7\_weight

postop\_d8\_weight - Note: Blank values for weights should not be treated as missing or zero values.

eos\_weight

srg\_nds\_score

postop\_d1\_nds\_score

postop\_d2\_nds\_score

eos\_nds\_score

eos\_day\_diff\_srg\_death

txas\_reperfusion\_actual

srg\_clot\_length

**Compliance**

Population for Compliance: mITT and Fully Treated

Variables:

site = redcap\_data\_access\_group

enro\_sex

txas\_reperfusion

txas\_reperfusion\_actual

rand\_reperfusion\_dose

srg\_reperfsn\_dose

rand\_reperfusion\_vial

srg\_reperfsn\_vial

srg\_clot\_length

rand\_clot\_length

srg\_donor\_eartag

enro\_animal\_id

srg\_embolic\_draw\_sex

enro\_sex

srg\_actual\_surg\_dt

srg\_embolic\_draw\_dt

Definitions:

1. Compliance vial id (compliance\_vial)

Yes if: rand\_reperfusion\_vial = srg\_reperfsn\_vial

No if: rand\_reperfusion\_vial ≠ srg\_reperfsn\_vial

Note: A blank value should not be considered non-compliant.

1. Compliance TNK administration (compliance\_adm)

Yes if: txas\_reperfusion = txas\_reperfusion\_actual

No if: txas\_reperfusion ≠ txas\_reperfusion\_actual

Note: A blank value should not be considered non-compliant

1. Compliance TNK dose (compliance\_dose)

Yes if: srg\_reperfsn\_dose = >0.75(rand\_reperfusion\_dose ) but < 1.25(rand\_reperfusion\_dose)

No if: srg\_reperfsn\_dose ≠ <0.75(rand\_reperfusion\_dose ) but < 1.25(rand\_reperfusion\_dose)

Note: A blank value should not be considered non-compliant

1. Compliance of clot length (compliance\_clot; round to the nearest whole number)

Yes if: rand\_clot\_length = srg\_clot\_length

No: rand\_clot\_length ≠ srg\_clot\_length

Note: A blank value should not be considered non-compliant.

1. Compliance of using a different animal for the donor (compliance\_ID)

Yes if: srg\_donor\_eartag ≠ enro\_animal\_id)

No if: srg\_donor\_eartag =enro\_animal\_id)

Note: A blank value should not be considered non-compliant.

1. Compliance that the sex of the donor be different than the recipient (compliance\_sex)

Yes if: srg\_embolic\_draw\_sex ≠ enro\_sex

No if: srg\_embolic\_draw\_sex = enro\_sex

Note: A blank value should not be considered non-compliant.

1. Compliance that the clot not be drawn on the actual surgery date (compliance\_drawdate)

Yes if: srg\_actual\_surg\_dt ≠ srg\_embolic\_draw\_dt

No if: srg\_actual\_surg\_dt = srg\_embolic\_draw\_dt

Note: A blank value should not be considered non-compliant.

Goals:

1. Feasibility

Descriptive tables by:

Total, Site, Sex, Reperfusion method, Clot length

Endpoints:

compliance\_adm

compliance\_vial

compliance\_dose

compliance\_clot

compliance\_ID

compliance\_sex

compliance\_drawdate

Note: A blank value should not be considered non-compliant.

**Outcomes - Run on Modified ITT and Per Protocol Populations**

**Mortality**

Endpoints:

Overall survival within 30 days = eos\_day\_diff\_srg\_death

Goal:

Differences in TNK vs Saline and 3 and 4cm clot lengths within 30 days

Descriptive plots by:

Reperfusion method, Clot length within overall sample and stratified by Sex, Site

Regression models adjusted by:

Sex, Site

Variables:

site = redcap\_data\_access\_group

enro\_sex

txas\_reperfusion

txas\_reperfusion\_actual

rand\_clot\_length

srg\_clot\_length round to the nearest whole number

eos\_day\_diff\_srg\_death

**MRI**

Note: No Day 30 MRI

Endpoints:

Day 2 Total volume of the left hemisphere

Day 2 Total volume of the right hemisphere

Day 2 Fraction of csf to volume of the left hemisphere

Day 2 Fraction of lesion to volume of the left hemisphere

Day 2 Fraction of tissue to volume of the left hemisphere

Day 2 Fraction of csf to volume of the right hemisphere

Day 2 Fraction of lesion to volume of the right hemisphere

Day 2 Fraction of tissue to volume of the right hemisphere

p\_mri\_d2\_mid\_shift\_indx

Definitions:

Day 2 Total volume of the left hemisphere = p\_mri\_d2\_hem\_left\_tot = p\_mri\_d2\_hem\_left\_csf + p\_mri\_d2\_hem\_left\_ les + p\_mri\_d2\_hem\_left\_tis

Day 2 Total volume of the right hemisphere = p\_mri\_d2\_hem\_right\_tot = p\_mri\_d2\_hem\_right\_csf + p\_mri\_d2\_hem\_right\_les + p\_mri\_d2\_hem\_right\_tis

Day 2 Fraction of left csf to volume of the left hemisphere = p\_mri\_d2\_hem\_left\_csf / p\_mri\_d2\_hem\_left\_tot

Day 2 Fraction of left lesion to volume of the left hemisphere = p\_mri\_d2\_hem\_left\_les / p\_mri\_d2\_hem\_left\_tot

Day 2 Fraction of left tissue to volume of the left hemisphere = p\_mri\_d2\_hem\_left\_tis / p\_mri\_d2\_hem\_left\_tot

Day 2 Fraction of right csf to volume of the right hemisphere = p\_mri\_d2\_hem\_right\_csf / p\_mri\_d2\_hem\_right\_tot

Day 2 Fraction of right lesion to volume of the right hemisphere = p\_mri\_d2\_hem\_right\_les / p\_mri\_d2\_hem\_right\_tot

Day 2 Fraction of right tissue to volume of the right hemisphere = p\_mri\_d2\_hem\_right\_tis / p\_mri\_d2\_hem\_right\_tot

Day 2 Midline Shift Index = p\_mri\_d2\_mid\_shift\_indx

Variables:

p\_mri\_d2\_vol\_tot

p\_mri\_d2\_vol\_csf

p\_mri\_d2\_vol\_tis

p\_mri\_d2\_vol\_les

p\_mri\_d2\_fract\_csf

p\_mri\_d2\_fract\_tis

p\_mri\_d2\_fract\_les

p\_mri\_d2\_hem\_left\_csf

p\_mri\_d2\_hem\_left\_les

p\_mri\_d2\_hem\_left\_tis

p\_mri\_d2\_hem\_right\_csf

p\_mri\_d2\_hem\_right\_les

p\_mri\_d2\_hem\_right\_tis

p\_mri\_d2\_mid\_shift\_indx

p\_mri\_d2\_adc\_qa\_snr

p\_mri\_d2\_t2\_qa\_snr

Goal:

1. Feasibility

Descriptive tables by:

Total, Site, Sex, Reperfusion method, Clot length (round to the nearest whole number)

Variables:

site = redcap\_data\_access\_group

enro\_sex

mri\_d2\_conduct

mri\_d2\_conduct\_rsn

mri\_d2\_conduct\_rsn\_oth

mri\_d2\_upload\_ida  
p\_mri\_d2\_adc\_qa\_snr   
p\_mri\_d2\_t2\_qa\_snr

txas\_reperfusion

srg\_clot\_length

txas\_reperfusion\_actual

rand\_clot\_length

1. Differences in Reperfusion method and Clot length at Day 2 (edema)

Descriptive tables by:

Reperfusion method, Clot length within overall sample and stratified by Sex, Site

Regression models adjusted by:

Sex, Site, Clot Length (round to the nearest whole number)

Variables:

site = redcap\_data\_access\_group

enro\_sex

p\_mri\_d2\_fract\_csf

p\_mri\_d2\_fract\_tis

p\_mri\_d2\_fract\_les

Day 2 Fraction of left csf to volume of the left hemisphere

Day 2 Fraction of left lesion to volume of the left hemisphere

Day 2 Fraction of left tissue to volume of the left hemisphere

Day 2 Fraction of right csf to volume of the right hemisphere

Day 2 Fraction of right lesion to volume of the right hemisphere

Day 2 Fraction of right tissue to volume of the right hemisphere

p\_mri\_d2\_mid\_shift\_indx

txas\_reperfusion

txas\_reperfusion\_actual

rand\_clot\_length

srg\_clot\_length

**Behavioral Tests**

**Corner test**

Interpretation: Higher values indicate higher discrepancies between left and right sides corresponding to a more impaired animal. Lower values indicate smaller discrepancies between left and right sides corresponding to less impaired animals.

Endpoints:

1. Corner Index = Abs (left - right) / (left + right)

Timepoints: Baseline (bl), Day 7 (d7), Day 30 (d30)

Goal:

1. Feasibility

Descriptive tables by:

Total, Site, sex, Reperfusion method, Clot length (round to the nearest whole number)

Variables:

enro\_sex

site = redcap\_data\_access\_group

corner\_bl\_conduct  
corner\_bl\_conduct\_rsn  
corner\_d7\_conduct  
corner\_d7\_conduct\_rsn  
corner\_d30\_conduct  
corner\_d30\_conduct \_rsn

txas\_reperfusion

srg\_clot\_length

txas\_reperfusion\_actual

rand\_clot\_length

1. Differences among reperfusion method and clot length (round to the nearest whole number)

Descriptive tables by:

Reperfusion method, Clot length within overall sample and stratified by Sex, Site

Regression models adjusted by:

Sex, Site, Clot Length (round to the nearest whole number)

Variables:

enro\_sex

site = redcap\_data\_access\_group

crt\_bl\_right\_s9

crt\_bl\_left\_s9

crt\_d7\_right\_s9  
crt\_d7\_left\_s9

crt\_d30\_right\_s9

crt\_d30\_left\_s9

txas\_reperfusion

srg\_clot\_length

txas\_reperfusion\_actual

rand\_clot\_length

**Bederson Neurodeficit score**

Interpretation: The higher the neuroscore, the more impaired the animal is.

Endpoints:

Bederson Neurodeficit score at day 1

Bederson Neurodeficit score at day 2

Bederson Neurodeficit score at day 30

Time points: D1, D2, End of study (eos)

Goal:

1. Feasibility

Descriptive tables by:

Total, Sex, Reperfusion method, Clot length (round to the nearest whole number)

Variables:

site = redcap\_data\_access\_group

enro\_sex

postop\_d1\_nds\_score  
postop\_d2\_nds\_score

eos\_nds\_score

txas\_reperfusion

txas\_reperfusion\_actual

rand\_clot\_length

srg\_clot\_length

Definitions

bederson\_conduct = If postop\_d1\_nds\_score, postop\_d2\_nds\_score, eos\_nds\_score is empty, test was not performed. Else, yes.

1. Differences between reperfusion method and clot length

Descriptive tables by:

Reperfusion method, Clot length (round to the nearest whole number) within overall sample and stratified by Sex, Site

Regression models adjusted by:

Sex, Site, Clot Length

Variables:

site = redcap\_data\_access\_group

enro\_sex

postop\_d1\_nds\_score  
postop\_d2\_nds\_score

eos\_nds\_score

txas\_reperfusion

srg\_clot\_length

txas\_reperfusion\_actual

rand\_clot\_length

**Neurodeficit Battery test**

Interpretation: All scores 0 is not impaired. Higher score= more impaired. 99 = test could not be performed.

Endpoints:

1. Full SPAN. Max 27 points. = The sum of all 9 scores:

neuro\_d30\_spont

neuro\_d30\_circling

neuro\_d30\_symmetry

neuro\_d30\_outstretching

neuro\_d30\_trunk

neuro\_d30\_vibrissae

neuro\_d30\_face

neuro\_d30\_beam

neuro\_d30\_climb

1. Simple SPAN. Max 9 points = Any scores greater than 0 are converted to a value of 1. Do not convert scores of 99. The sum of any of those converted scores:

neuro\_d30\_spont

neuro\_d30\_circling

neuro\_d30\_symmetry

neuro\_d30\_outstretching

neuro\_d30\_trunk

neuro\_d30\_vibrissae

neuro\_d30\_face

neuro\_d30\_beam

neuro\_d30\_climb

1. Duke score. Max 7 points. Any scores from the following variables greater than 0 are converted to a value of 1. Do not convert scores of 99. The sum of any of those converted scores:

neuro\_d30\_circling

neuro\_d30\_symmetry

neuro\_d30\_trunk

neuro\_d30\_vibrissae

neuro\_d30\_face

neuro\_d30\_beam

neuro\_d30\_climb

1. MGH score. Max 21 points. The sum of the scores from the following variables:

neuro\_d30\_spont

neuro\_d30\_circling

neuro\_d30\_symmetry

neuro\_d30\_outstretching

neuro\_d30\_trunk

neuro\_d30\_vibrissae

neuro\_d30\_climb

|  |
| --- |
| Timepoints: D30 (only one time point was collected so there are no visit codes)  For animals with at most one missing item, scores will be calculated with available items and the total score will be re-scaled to be in the same range when all items are available.  Goals:   1. Feasibility   Yes if= behav\_d30\_conduct=1  Descriptive tables by:  Total, Site, Sex, Reperfusion method, Clot length (round to the nearest whole number)  Variables:  enro\_sex  site = redcap\_data\_access\_group  behav\_d30\_conduct  behav\_d30\_conduct\_rsn  txas\_reperfusion  srg\_clot\_length  txas\_reperfusion\_actual  rand\_clot\_length   1. Differences among reperfusion method and clot length (round to the nearest whole number)   Descriptive tables by:  Reperfusion method, Clot length (round to the nearest whole number) within overall sample and stratified by Sex, Site  Regression Models adjusted by:  Sex, Site, Clot length  Variables:  site = redcap\_data\_access\_group  enro\_sex  neuro\_d30\_spont  neuro\_d30\_circling  neuro\_d30\_symmetry  neuro\_d30\_outstretching  neuro\_d30\_trunk  neuro\_d30\_vibrissae  neuro\_d30\_face  neuro\_d30\_beam  neuro\_d30\_climb  txas\_reperfusion  srg\_clot\_length  txas\_reperfusion\_actual  rand\_clot\_length |

Statistical Analysis:

Study Design:

Descriptive analysis: Data will be summarized using descriptive measures such as mean with standard deviation, median with inter-quartile range, minimum and maximum for quantitative variables, and frequencies with percentages for qualitative variables. For all endpoints, violin-plots with box-plots will be used to display data by study arms. Overall survival within 30 days will be displayed by study arm with Kaplan-Meier plots.

Primary Analysis: We will fit a multivariable Probabilistic Index model with day 30 Full SPAN Neuro Deficit Score as the response variable and study arm (TNK and control) as main covariate adjusted by clot length, sex and site as fixed effects. Interaction will be tested between study arm and clot length. In the presence of interaction, study arms will be compared within each clot length. Otherwise, the interaction term will be removed from the model.

In the mITT and FT populations, animals with missing day 30 Full SPAN Neuro Deficit Score due death after surgery will have their outcomes imputed. Animals with missing day 30 Full SPAN Neuro Deficit Score due to other causes will be excluded. Probability index as effect size with 95% confidence interval will be reported.

Secondary Analyses: We will also compare clot lengths using the same multivariable model outlined in the primary analysis. In the presence of interaction, clot lengths will be compared within each study arm. In the absence of interaction, the interaction term will be dropped, and comparison between clot length stratified by study arm will be performed. When the interaction is significant, these stratified contrasts are primary. When not significant, they are supportive.

Furthermore, we will fit a multivariable Probabilistic Index model with secondary endpoints (Simplified SPAN Neuro Deficit Battery Score, Duke Neuro Deficit Battery Score, MGH Neuro Deficit Battery Score, Transformed Corner Test Index and Bederson Neuro Deficit Score) as the response variable and study arm (TNK and control) as main covariate adjusted by clot size, sex and site as fixed effects. Animals with missing endpoints due to death after surgery will have their outcomes imputed. Overall survival within 30 days will be compared between study arms and clot sizes using Proportional Hazard Cox regression model with study arm (TNK and control) as main covariate adjusted by and clot length (3cm and 4cm), sex as fixed effects and site as strata. For each model, interaction will be tested between study arm and clot size. In the presence of interaction, study arms will be compared within each clot size. Furthermore, clot lengths will be compared within each study arm. In the absence of interaction, the interaction term will be dropped, and study arm and clot size will be considered as additive effects. Comparison between study arms stratified analysis per clot size and comparison between clot size stratified by study arm will be also performed. When the interaction is significant, these stratified contrasts are primary. When not significant, they are supportive.

Probability index as effect size for PI models and hazard ratios for PH Cox model with 95% confidence intervals will be reported. Multiplicity correction will not be applied.

Missing data: For all endpoints, animals with missing data due death after surgery will have their outcomes imputed with the worst rank score and multiple imputation. Animals with missing endpoints due to other causes will be excluded. Worst score will be calculated considering different possible strata: (i) overall, (ii) animal model and sex and (iii) animal model, sex, study arm and clot size. Multiple imputation will be performed using multivariate imputation by chained equations (MICE) based on predictive-mean matching. The number of imputed datasets will be at least twenty. Predictors for multiple imputation will be treatment arm, animal model, sex, site, surgery weight, surgery animal age, surgery suture length and surgery suture width for endpoints at day 2/3. Additionally, early MRI fraction lesion will also be considered as a predictor for multiple imputation for endpoints at day 30. The plausibility of imputed values will be inspected by density plots of imputed and observed values. The outlined statistical analysis will be performed for each imputed dataset and results will be pooled according to Rubin’s rules. Similar analyses will be performed for all imaging endpoints.

All calculations will be performed using R, version 4.0 or higher.

Final version approved by email vote of the Steering Committee