NN104

Statistical Analysis Plan

RHAPSODY: Safety Evaluation of 3K3A-APC in Ischemic Stroke

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STATISTICAL ANALYSIS PLAN SIGNATURE PAGE

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SUMMARY OF CHANGES

SEPTEMBER 2017 - VERSION 1.0

- Initial version

OCTOBER 2017 - VERSION 1.1

- Modified language in section 3.2 describing outcome definition for key secondary endpoint assessing hemorrhage rates

DECEMBER 2017 - VERSION 2.0

- Modified language in sections 3.2, 6.3, and 8.2 to include revised definitions for key secondary endpoint assessing hemorrhage rates based on discussions at 10/2017 Iowa meeting and follow-up calls with NINDS to address issues with values obtained via central read. Modified language in section 10.1 to clarify specific requests for coding of certain AEs.

PREFACE

This statistical analysis plan (SAP) describes the planned analysis for the NeuroNEXT NN104 (RHAPSODY) study [National Institute of Neurological Disorders and Stroke (NINDS) grant # U01NS088312]. The planned analyses identified in this SAP are intended to support the completion of the Final Study Report (FSR) and will be included in regulatory submissions and/or future manuscripts. All interim analyses will involve only the primary study endpoint, and will be performed once each cohort has been enrolled and completed the study period for assessing dose-limiting toxicities. All final, planned analyses identified in this SAP will be performed only after the last subject has completed the full study period. Once all data have been cleaned and verified, a "locked" version of the data will be used for reporting the final study results. Key statistics and study results will be made available to the PPI and CCC following database lock and prior to completion of the final FSR.

1. STUDY DESIGN

This is a multicenter, prospective, randomized, placebo-controlled, double-blinded Phase 2 study intended to evaluate the safety, pharmacokinetics (PK), and preliminary efficacy of 3K3A-APC following administration of tPA, mechanical thrombectomy, or both in subjects with moderate to severe acute ischemic stroke. The study will utilize the continual reassessment method (CRM, O'Quigley et al, 1990). The original version of the CRM method proposed enrolling one subject at a time. However, others have shown that the performance of the CRM may be improved by enrolling cohorts instead of a single subject (Goodman et al, 1995). Correspondingly, this study will utilize a modified version of the CRM in order to establish a maximum tolerated dose (MTD).

The study will randomize approximately 115 subjects, which includes a planned 88 subjects in 22 cohort groups of four (each cohort will include one placebo and three study drug treated subjects). Additional placebo subjects who will be enrolled during safety review pauses will also be included. While placebo is not needed to determine the MTD, a placebo group has been included to conduct secondary analyses to examine for a reduction of pre-clinical tPA / mechanical thrombectomy-related hemorrhage and to obtain preliminary efficacy data that may be useful for the planning of future studies. It is predicted from pre-clinical studies that treatment may reduce thrombolysis-related hemorrhage.

Subjects will not be considered part of the intent-to-treat (ITT) cohort until they receive any amount of 3K3A-APC or placebo. For example, 'Early Responders' – defined as subjects whose symptoms resolve between initial randomization and initiation of Investigational Medicinal Product (IMP) infusion such that they are no longer eligible (repeat NIHSS < 5) – will be removed from the study and replaced. A Safety Review Committee (SRC) will review the safety data for each potential dose limiting toxicity (DLT), and make a final determination as to whether or not an incident should be classified as a DLT. At any time if enrollment is so rapid that two consecutive cohorts are awaiting DLT review, the IWRS will randomize any enrolled patients to placebo so as to preserve site-level blinding and trial enrollment momentum. These extra placebo treated subjects will enhance the power to detect a change in bleed rates for 3K3A-APC-treated subjects.

Four dose levels will be considered for this study: 120, 240, 360, and 540 µg/kg. Following completion of tPA infusion, or initiation of mechanical thrombectomy (arterial puncture), whichever is sooner, eligible adult subjects will receive 3K3A-APC or placebo 30 to 120 minutes later given as a 15 minute infusion. Subjects will receive another 15 minute infusion of study treatment every 12 hours (+/- 1 hour) for up to 5 total doses (or until discharge, whichever occurs first). It is preferable not to skip doses of study drug, and should a dose need to occur outside of the +/- 1 hour window (which will be reported as a protocol deviation), doses should never be given within 8 hours of one another.

For the purposes of this study, we assume an established background symptomatic intracerebral hemorrhage (SICH) rate of 3-6% (Wahlgreen et al, 2007; Hacke et al, 2008; Berkhemer et al, 2015; Campbell et al, 2015; Goyal et al, 2015; Saver et al, 2015). Correspondingly, this study aims to identify the highest dose with a DLT rate of 10% or less. Subjects will be enrolled to 3K3A-APC dose cohorts in groups of four (three to specified treatment dose and one to placebo). Subjects will generally be enrolled at the dose estimated from the model at that time, based on the assumed original dose-response model and all prior observed data to date, as the highest dose with a DLT rate of 10% or less. However, the initial cohort will start at the lowest dose (120 μg/kg) and the dose level may be escalated by no more than one dose between consecutive cohorts (there are no restrictions on dose level de-escalation). Intra-subject dose modification is not permitted during the study. After the final group of subjects is enrolled, the final MTD will be defined as the highest dose with an estimated toxicity probability less than or equal to the target toxicity level of 10%.

Each subject will be followed for 90 days in this study. Subjects will be considered for the study after beginning tPA administration, mechanical thrombectomy, or both for moderate to severe acute ischemic stroke. Eligible subjects will receive 3K3A-APC or placebo every 12 hours for up to 5 doses (approximately 3 days), or until discharge from this hospital – whichever occurs first. Subjects will be monitored for safety evaluations through Day 7. Subjects are expected to be seen for assessments on Days 7, 14, 30, and 90. MRI scans, including SWI sequences, will be obtained at Day 7 (or discharge), Day 30, and Day 90.

1.1 Primary Objective

Primary Objective: ***To evaluate the safety of multiple ascending intravenous (IV) doses of 3K3A-APC following recombinant tissue plasminogen activator (tPA) administration, mechanical thrombectomy, or both in subjects who have experienced moderate to severe acute ischemic stroke.***

It is hypothesized that, using the continual reassessment method, at least one of the proposed 3K3A-APC doses will be safe enough to carry forward to future clinical studies.

1.2 Secondary Objectives

Secondary Objective 1: ***To investigate the pharmacokinetic (PK) properties of 3K3A-APC following tPA, mechanical thrombectomy, or both in adults with acute ischemic stroke.***

Secondary Objective 2: ***To evaluate the effect of 3K3A-APC on the presence of tPA / mechanical thrombectomy-related bleeding (hemorrhage and microbleeds) in the brain as determined by MRI at Day 30.***

It is hypothesized, based on preclinical studies, that 3K3A-APC will reduce the expected recanalization therapy-related bleed rate.

2. PRIMARY ENDPOINT

The primary objective of the study is to evaluate the safety of multiple ascending intravenous (IV) doses of 3K3A-APC following tPA administration in subjects who have experienced moderate to severe acute hemispheric ischemic stroke. Safety will be assessed by defining dose limiting toxicities (DLTs), which will be graded using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), Version 4.03.

DLTs will be assessed from the first dose to 48 hours following the last dose of study treatment (unless specified below), and defined as any of the following AEs that have an attribution of “related” to study treatment (possibly, probably and definitely):

- An activated partial thromboplastin time (aPTT) that reaches 2x the upper limit of normal (ULN) at 1 hour post-dose. The ULN range is defined locally by the site laboratory.
- Symptomatic intracranial hemorrhage (SICH) defined as blood present on CT or MRI brain image that is associated with clinical worsening that meets the definition of neuroworsening (4 or more point increase on the NIHSS; see section 10.3 for definition) *and* in the opinion of the investigator represents a clinically significant change that can be attributed to the hemorrhage. Subarachnoid hemorrhage that occurs in subjects who receive mechanical thrombectomy will NOT be considered a DLT, and instead will be evaluated in an exploratory analysis upon study completion.
- Findings that meet all of the following three components (Hy’s Law):
    - ≥ 3x ULN of alanine aminotransferase (ALT) or aspartate aminotransferase (AST)
    - Serum total bilirubin (TBL) > 2x ULN, without initial findings of cholestasis [serum alkaline phosphatase (ALP) activity > 2x ULN]
    - And, no other reason can be found to explain the combination of increased aminotransferase (AT) enzymes and TBL, such as viral hepatitis A, B, or C, pre-existing or acute liver disease, or another drug capable of causing the observed injury.
- Any other bleeding event classified as serious by the Investigator, or any bleeding that required the administration of more than 2 units of packed red cells over any two consecutive days.
- Any Grade 3 laboratory value that, in the opinion of the investigator, is related to study treatment.

- Any adverse event that, in the opinion of the investigator, is related to study treatment and leads to cessation of further dosing.

The SRC will review the safety data for each potential DLT, and make a final determination as to whether or not an incident should be classified as a DLT. Reported DLTs that are considered possibly related to study drug but definitely related to another event will not be considered DLTs upon final adjudication. An example of such an event would be an elevated aPTT following the first dose in a subject who undergoes mechanical thrombectomy during which heparin is administered; the elevated aPTT can be attributed to the heparin and therefore should NOT be considered a DLT in this isolated instance. Another example would be the occurrence of hypofibrinogenemia in a subject who receives tPA. Low fibrinogen levels can be attributed to the tPA, and there is a documented rate of occurrence of 11% in subjects receiving tPA (Genentech, 2015). Furthermore, 3K3A-APC does not cause a reduction in the level of fibrinogen in plasma and therefore this finding should NOT be considered a DLT.

3. SECONDARY ENDPOINTS

3.1 Pharmacokinetic (PK) Properties

One secondary objective of this study is to investigate the PK properties of 3K3A-APC following tPA administration in stroke subjects. PK blood samples will be collected from approximately 40 subjects at a subset of study sites to ensure adequate sampling across all dose levels. Samples should be collected following one of the doses of 3K3A-APC at the following time points: end of infusion and 20, 40, 60, and 80 minutes after the end of infusion (5 total samples per subject). The investigator can decide which dose (dose 2-5) will be used for PK collection. Collection of samples outside of the requested nominal time will not be a protocol deviation, but it is essential that the actual time of collection be captured for analysis. Samples collected for PK will only be used for PK-related analysis and method development.

PK parameters following administration of 3K3A-APC in the study will be determined using concentration data obtained via the enzyme-immunocapture assay of 3K3A-APC amidolytic activity in plasma. PK parameters will be determined by fitting a compartmental model to each subject's data. Individual subject observed and model-predicted plasma concentration over time by treatment group will be plotted on linear and semi-logarithmic axes. Descriptive statistics of PK parameters will be provided for each dose level. Additionally, the pharmacokinetic-pharmacodynamic relationship between the change from baseline in aPTT and the concurrently measured 3K3A-APC plasma concentration will be evaluated 1 hour after the end of infusion.

3.2 Hemorrhage Rates

Another secondary objective of the study is to evaluate the effect of 3K3A-APC on the incidence of tPA / mechanical thrombectomy-related bleeding in the brain. Imaging will be used to assess the development of recanalization therapy-related hemorrhage seen on susceptibility-weighted magnetic resonance imaging (MRI). Each subject is expected to have two standard of care (SOC) imaging scans:

- Baseline Scan: Non-contrast CT or MRI (with prior approval), per local SOC
- 24-36 Hour Scan: Non-contrast enhanced head CT or MRI, per local SOC, 24-36 hours following tPA bolus administration if subject receives any IV tPA or 24-36 hours following mechanical thrombectomy if subject does not receive any IV tPA

These scans should be performed according to local SOC. Each site should use its standard stroke imaging protocol for CT scans. If MRI is performed as SOC at either time point, it is preferred that the MRI protocol for the study be used. We expect 80% of these 24-36 hour scans to be CT scans, and 20% to be MRI.

Additionally, each subject will have three trial-mandated scans:

- Day 7 / Discharge Scan: MRI: T1 and T2 weighted images, as well as DWI and susceptibility weighted imaging (SWI) sequences must be performed for all subjects on Day 7 or just prior to discharge, whichever occurs first. If the subject is discharged before Day 4, then it is expected that he/she return for Day 7 assessments or arrangements be made to collect this information from the treatment facility where the subject was transferred if the subject is unable to return.
- Day 30 Scan (+/- 5 Days): MRI: T1 and T2 weighted images, as well as DWI and SWI sequences must be performed for all subjects.

- Day 90 Scan (+/- 10 Days): MRI: T1 and T2 weighted images, as well as DWI and SWI sequences must be performed for all subjects. While MRI is preferred at Day 90, should a subject become unable to undergo MRI, a CT scan is allowed instead.

These trial-mandated scans should be performed on a 1.5T machine, with slice thickness between 2.5 and 7.5 mm, and will be used to evaluate secondary and exploratory outcomes in this study. The effect of 3K3A-APC on the presence of tPA/mechanical thrombectomy-related hemorrhage in the brain will be determined by 1.5T MRI at Day 30. Day 30 MRI scans will be collected and evaluated by a central radiologist for the presence of hemorrhage and by computerized assessment for microbleeds. Since patients will not necessarily have a baseline MRI, post-recanalization microbleeds (defined as SWI hypointensities less than 5mm in diameter) will be counted as tPA/mechanical thrombectomy-related only if found within the ischemic territory (Kimura et al, 2013). All other areas of SWI hypointensity larger than 5mm in diameter will be counted as tPA/mechanical thrombectomy-related regardless of the territory in which they are found.

The Central Reader's assessment of the scans will be recorded in the study database. The relevant form collects data on whether the scan is normal or not, presence of past strokes and/or edema, qualitative data for edema, quantitative data regarding hemorrhage location and volume, number and location of microbleeds, and stroke lesion volume. Only the questions that are pertinent to the type of scan (CT or MRI) will be enabled in the database, as some questions do not apply to all types of scans. The objectives of this central review are to:

- Qualitatively assess each subject's scan for:
    - Presence and location of hemorrhage (hematoma or petechiae) on all scans
    - Presence and location of microbleeds on MRI scans
    - Presence and location of edema on all scans
- Quantitatively assess each subject's scan for:
    - Volume of hemorrhage on all scans
    - Number of microbleeds on MRI scans
    - Infarct volume at Baseline and Day 90

Given the different imaging protocols used in this study, bleeding volume quantification will be retrieved from susceptibility sensitive sequences directly. All data will be processed using SPIN Software (Signal Processing in MRI, Magnetic Resonance Innovations, Detroit, MI). In order to accurately quantify bleeding volume, only hypointensities likely to be caused by bleeding will be deemed aberrant. These include: large bleeds, microbleeds, linear bleeding regions, iron deposition along vein paths, and pooled intraventricular blood. The processor will avoid blood vessels, calcifications, artifacts, and areas outside of normal brain parenchyma. In cases where Susceptibility Weighted Imaging (SWI) magnitude and phase are collected, Quantitative Susceptibility Mapping (QSM) will be used to confirm calcifications. However, given the nature of gradient echo imaging, some calcifications may be misrepresented when not accompanied by phase data. This will be mitigated by careful consideration of location and morphology on sequences available. Areas avoided due to the likelihood of calcifications will be: the falx, choroid plexus, pineal gland, globus pallidus, and parenchymal edges near bony structures (sinuses, ears). For instance, a small hypointensity located in the choroid plexus will not be considered aberrant. Some exceptions may be made based on accompanying sequences, especially when QSM or phase was available. A large bleed extending into the falx will be considered aberrant. A small hypointensity in the globus pallidus that is hyperintense on QSM will be considered aberrant.

For processing, two objects will be drawn for each scan. One will include all aberrant hypointensities outside of the infarct. Another will include all aberrant hypointensities within or reaching the infarct. A region of interest (ROI) containing normal appearing white matter will then be selected. The average intensity within this ROI will be obtained and halved. This value will be the threshold used for the bleeding volume quantification. The number of pixels lower than the threshold will be produced through SPIN software. The volumes of each thresholded object within the sequence will be calculated using the Cavalieri method with slice thickness including any gaps in data collection. To estimate the incidence of hemorrhage at Day 30, we define hemorrhage as hematoma + petechial bleeding and exclude microbleeds. Therefore, a Day 30 image will be considered "positive for hemorrhage" if the corresponding total hematoma volume by planimetry is larger than 0.06 mL (5 mm). However, this cutoff value is imperfect because a patient with two microbleeds totaling more than 0.06 mL should not be considered hemorrhage positive since each lesion would be <5 mm. In order to account for the uncertainty in the optimal definition of "hemorrhage positive", several alternative cut points will be implemented for sensitivity analysis:

- PRIMARY - Positive if total hematoma volume by planimetry (Question 7 on the Imaging Review Form) > 0.06mL (5 mm diameter)
- SENSITIVITY
    - Positive if total hematoma volume by planimetry > 0
    - Positive if total hematoma volume by planimetry > 0.5 mL (10 mm)
    - Positive if total hematoma volume by planimetry > 1.8 mL (15 mm)

The number of cerebral microbleeds (Question 8 on the Imaging Review Form) will be identified using the following parameters:

- Round or ovoid (rather than linear)
- Black on T2*-Weighted MRI
- Blooming on T2*-Weighted MRI
- Dipole effect in SWI phase
- Bright on SWIM
- Hypointense or not visualized on T1 and FLAIR
- Isolated (can be verified as isolated and not a vessel using SWI mIP)
- Devoid of signal hyperintensity on T1- or T2-Weighted sequences
- At least half surrounded by brain parenchyma
- Distinct from other potential mimics, such as iron/calcium deposit, bone, or vessel flow

A scan will be considered positive for microbleeds if a nonzero value is entered into the within stroke territory section (Question 8a on the Imaging Review Form).

4. ENROLLMENT & RANDOMIZATION

After beginning tPA or mechanical thrombectomy for moderate to severe acute hemispheric ischemic stroke, subjects or their legally authorized representatives will be presented with the study details and the informed consent form (ICF). All code stroke patients arriving at the site who receive tPA will be considered for this study. In addition, patients ineligible for tPA but considered candidates for mechanical thrombectomy alone, and who can begin intra-arterial therapy within 6 hours of symptom onset, will also be considered for this study. Written informed consent will be obtained from each study participant or his/her legally authorized representative before any study-specific procedures or assessments are performed. Once the consent form has been signed, the subject is considered enrolled in the study. Administration of tPA or initiation of mechanical thrombectomy should not be delayed due to study procedures. The consent process should start after the subject has started treatment with tPA or after the decision to begin mechanical thrombectomy has been made, whichever occurs sooner. Most pre-study procedures are performed in accordance with standard of care (SOC) for IV tPA administration and mechanical thrombectomy and therefore must be performed prior to informed consent. In accordance with good clinical practice, study specific procedures that do not coincide with SOC should only be performed after written informed consent is obtained (e.g., collection of blood samples for PK or antibody analysis).

Subjects who have signed consent (or whose representatives have provided surrogate consent), and meet all inclusion and exclusion criteria, can be randomized into the study. Subjects will be randomized using the NeuroNEXT Interactive Web Response System (IWRS) to either 3K3A-APC or placebo (in a 3:1 ratio). The Investigator, or designee, should randomize the subject in the IWRS to receive a unique subject number, which will also be linked to the allocated treatment assignment. Clinical staff will receive confirmation of the randomization, but remain blinded to the treatment assignment. Subjects who receive mechanical thrombectomy only should be randomized close to the start of the procedure (arterial puncture) to ensure that they continue to meet eligibility. There are 22 groups of four subjects planned, but fewer may be enrolled should the study meet either of the early stopping criteria (see section 7). If at any time enrollment is so rapid that there are two consecutive cohorts in the DLT review period, subjects will be assigned to placebo until the earlier of the two cohorts has been completely reviewed. The additional placebo subjects will be closely monitored and enrollment may be discontinued if the number enrolled exceed what was planned for the study.

The NeuroNEXT IWRS system will also serve to inform the unblinded study pharmacist at each site of the treatment assignment. Immediately after randomization, an email notification will be sent informing the unblinded site pharmacist(s) of the new subject, his/her weight, the treatment assignment, and the dose (if assigned to drug). The study pharmacist will then use this information to prepare the investigational treatment. An actual pre-dose weight for each subject is preferred, as all investigational doses will be based on this weight. If a subject's weight was estimated for the purposes of study drug dose calculation, then an actual weight must be obtained within 24 hours of Dose 1.

It is important to confirm eligibility again close to the first investigational dose as the NIHSS score may drop below 5 during the time between randomization and the first dose of study drug. For this reason, a repeat NIHSS should be assessed:

- Immediately prior to Dose 1 infusion if the subject received tPA treatment only, OR
- Immediately prior to draping the subject in preparation for mechanical thrombectomy if the subject is to receive mechanical thrombectomy alone or in combination with tPA.

Randomized subjects who experience significant neurologic improvement (defined as an NIHSS score < 5) prior to receipt of study drug will be termed 'Early Responders'. These subjects, and other randomized subjects who do not go on to receive study treatment for any reason, will not be included in the study, and will be replaced in the randomization sequence and CRM cohort. Any subject who receives any amount of study drug or placebo, however, will be considered "Dosed" and will be followed to the end of the study.

5. PRELIMINARY TABULATIONS

All subjects who provide informed consent will be accounted for in this study. Regularly generated enrollment reports will describe:

- Number of subjects consented, eligible, and randomized by site
- Ongoing study status of all randomized subjects
- Reasons for ineligibility
- Protocol deviations
- Early study completers

Subject data will also be summarized by treatment group (combined 3K3A-APC vs. placebo & individual dose groups vs. placebo) with respect to important demographic characteristics. Distribution of categorical variables will be tabulated by treatment group. Continuous variables will be summarized as mean, standard deviation, minimum, and maximum by treatment group and overall. Variables to be collected will include:

- Demographic Characteristics and Baseline Measurements
    - Gender
    - Race
    - Ethnicity
    - Age
    - Years of Education
    - Hand Preference
    - Height (cm)
    - Platelet Count
    - History of Diabetes
    - History of Hypertension
- Baseline Stroke Characteristics & Timeline
    - NIHSS Prior to Recanalization Therapy
    - NIHSS Eligibility
    - Recanalization Therapy
    - mRS
    - Time from Stroke Onset to First Therapy
        - If tPA Only, Time from Stroke Onset to tPA Initiation
        - If IAT Only, Time from Stroke Onset to First Skin Puncture

- If IAT & tPA, Time from Stroke Onset to tPA Initiation or First Skin Puncture (Whichever Occurred First)

6. ANALYSIS POPULATIONS

Since the primary objective aims to estimate a maximum tolerated dose, no formal testing will be involved. All analyses to address the key secondary objectives will be conducted at the 0.05 significance level. The analysis population of interest will differ depending on the objectives of any particular analysis.

6.1. Primary Analysis

Any given subject who receives only one dose of study drug and does not experience a DLT will not be included in the CRM calculation. Thus, the primary analysis involving the CRM will utilize a modified intent-to-treat (mITT) approach, with subjects considered evaluable for the model if they are a confirmed randomization (receive at least one dose of study treatment) and either:

- Receive two or more doses of study medication
- Have a confirmed DLT

6.2. Secondary PK Analysis Population

The PK population includes all subjects who have sufficient data for PK analysis, and who have not been excluded from analysis for protocol deviations (or other study-related events) that could impact the calculation or interpretation of the pharmacokinetic parameters (such as getting too much heparin or receiving a blood transfusion).

6.3. Secondary Bleed Rate (Hemorrhage & Microbleeds) Analysis

The secondary bleeding analysis will be based on the results of the Day 30 MRI scans. Correspondingly, the analysis population for this secondary analysis will include all subjects who had a Day 30 scan collected.

6.4. Safety Analysis

All safety analyses will be implemented using an ITT approach, defined as all subjects who are randomized and receive at least one dose of study medication.

7. PRIMARY ANALYSIS

The primary objective of the RHAPSODY study is to evaluate the safety of multiple ascending intravenous (IV) doses of 3K3A-APC following tPA administration, mechanical thrombectomy, or both in subjects who have experienced moderate to severe acute hemispheric ischemic stroke. Safety will be assessed by defining dose limiting toxicities (DLTs), which will be graded using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), Version 4.03. DLTs will be modeled using the continual reassessment method (CRM) to make the determination on which, if any, dose to bring forward to future clinical studies. The primary endpoint analysis will make use of Bayesian methods for estimating the probability of a DLT at each of the doses under investigation.

When designing a CRM, O'Quigley et al (1990) recommend using a single-parameter dose-response model, $\psi(x_i^*, a)$, which is the probability of a toxicity at dose x_i^* for some unknown parameter, a, which defines the shape of the dose-toxicity function. Here, the probability of a DLT at dose x_i^* can be estimated using $\psi(x_i^*, \hat{a})$. For this study, a hyperbolic tangent dose-response model will be used, in which:

$$\psi(x_i^*, a) = \left\{\frac{\tanh(x_i^*)+1}{2}\right\}^a.$$

The CRM requires specification of the prior probabilities of a DLT at each of the investigational doses of 3K3A-APC under consideration. Based on a best estimate from the literature and previous studies (Wahlgreen et al, 2007; Hacke et al, 2008; Berkhemer et al, 2015; Campbell et al, 2015; Goyal et al, 2015; Saver et al, 2015), the four dose levels will be assigned prior probabilities of toxicity as indicated in Table 1 below.

Table 1. Assumed Prior Probabilities of Toxicity at Four Dose Levels Considered for the Study

Dose Level (µg/kg)	120	240	360	540
Prior Probability of a Toxicity	5%	7%	9%	11%

Each study participant will be assessed for DLT occurrence. A subject may either experience a DLT or reach the end of the DLT observation period without experiencing a DLT. For subjects that reach the end of the observation period without experiencing a DLT, the independent medical monitor (IMM) will perform a final review to either confirm no DLT has occurred or to identify potential DLTs for consideration. If either a site or the IMM report a potential DLT, the event will be adjudicated by the safety review committee (SRC).

After each evaluable subject in a cohort is adjudicated by the SRC as having or not having a DLT, the CRM will be updated by an unblinded study statistician and independently verified by a second unblinded study statistician. Specifically, the posterior mean of a will be re-estimated using the prior probabilities from Table 1 and all evaluable data for each cohort of subjects. Subsequently, the posterior probabilities of observing a DLT at each 3K3A-APC dose, $(x_{120}, x_{240}, x_{360}, x_{540})$, can be calculated by solving the above equation for:

$$\psi(x_i^*, \hat{a}),\ i = 120, 240, 360, 540.$$

Based upon that estimate, the largest dose with estimated toxicity probability, $\psi(x_i^*, \hat{a})$, less than or equal to 10% will be allocated to the next cohort of randomized participants. If this dose is more than one level higher than the previous dose allocated, the dose will only be increased one level. Dose de-escalation is not subject to any restrictions. There are 22 cohorts of four subjects each planned, but fewer may be enrolled should the study meet any of the stopping criteria.

In summary, the CRM model will proceed as follows:

- Enroll the first 4 subjects into cohort 1.
    - Treat one of the four subjects (chosen randomly) with placebo.
    - Treat the other three subjects with the lowest dose: 120 µg/kg.
    - Observe the number of subjects (out of the three treated subjects) that have a DLT per the study DLT definition.
    - Determine the number of evaluable subjects in the cohort. Any given subject who receives only one dose of study drug and does not experience a DLT will not be considered “evaluable”, and will not be included in the CRM calculation (i.e., for subjects with no DLT observed, two or more doses will need to be administered to be included).
    - Based upon the observed information from the evaluable subjects in the cohort, refit the assumed dose-response curve.
- Initially (through version 7.1 of the protocol), the re-estimated dose-response curve using all cohorts enrolled to date was then used to determine the highest dose level of the four under consideration that has an estimated probability of toxicity less than or equal to 10%.
    - The next cohort of subjects is treated at the dose level specified above – unless the chosen dose level is more than one level higher than the current level. If so, treat the next cohort of subjects at the next dose level above the current level.
- Based on a DSMB recommendation, this process was changed as of version 8.0 of the protocol. The basic process proceeds as described above, but once all subjects in a given cohort (n) have been enrolled, data from all prior cohorts (cohort 1, cohort 2,…., cohort n-1) are used to determine the dose level of cohort n+1.
    - If enrollment is rapid such that both cohort (n-1) and cohort (n) are filled and awaiting DLT review, new subjects enrolled will be randomized to placebo until cohort (n-1) has been reviewed. (For example, if both cohort 13 and 14 are filled and awaiting review, subjects will be randomized to placebo until cohort 13 is closed and the model is rerun to determine the dose for cohort 15).
- Repeat the process.

At the conclusion of the study, the MTD will be selected using the rules specified above for the implementation of the CRM model. Specifically, the model will be run a final time using all observed data and the MTD will be defined as the highest dose with an estimated DLT rate of 10% or less based on that final model. The study will stop once the first of the following criteria have been met:

- The maximum number of cohorts (22) has been observed.

- If at any time after half of the cohorts (11) have been observed, two consecutive iterations suggest a 15% or higher toxicity rate at the lowest dose (stop for safety).
- If the study proceeds straight to the highest dose, and then observes 9 successive cohorts at the highest dose with no observed toxicity (stop and declare the highest dose the MTD).
    - Through simulation, it was determined that it would extremely unlikely to observe no DLTs after 9 successive cohorts if the true toxicity rate were 10%. Thus, after 9 cohorts we would have sufficiently determined that all doses under consideration are safe and early stopping would be justified.

Much of the process for implementing the selection of dose for each subsequent cohort of subjects has been automated. Once the DLT status for all evaluable subjects in a cohort is complete, the IWRS will notify both statisticians via email that a new cohort is available for analysis. At that point, the primary statisticians will independently complete the dose recommendation process. The statistician who submits the initial dose recommendation will be referred to as the initial statistician, while the statistician who submits the dose recommendation second will be referred to as the validation statistician. The initial statistician will enter the production database and save the table of DLTs for each cohort as a SAS table, to ensure that each dose recommendation can be reproduced at any time during or after the conduct of the study. In parallel fashion, both the initial and validation statisticians will run a SAS program that details the selection of the next dose and enter their selected dose for the next cohort of subjects into the study website. Once both statisticians enter the next dose recommendation and provided the two recommendations match, the randomization table will re-open and participants will be randomized to the newly recommended dose or placebo in a 3:1 ratio. If the two doses do not agree, the IWRS notifies the statisticians of a dose match error via email, and the process is repeated until a new dose recommendation is verified for the next cohort.

A list of backup statisticians will also be maintained. These individuals should be capable of serving in this capacity if either or both of the primary statisticians are unable to perform the tasks outlined below. It is the responsibility of the primary statisticians to place the backup statisticians 'on call' should the primary statisticians know of an upcoming period of unavailability.

Final CRM results will be displayed, by cohort, in the following format:

Table 2. CRM Cohort Toxicity Estimates

Cohort	Selected Dose (µg/kg)	# of Evaluable Subjects	# of DLTs	Toxicity Estimates			
				120 (µg/kg)	240 (µg/kg)	360 (µg/kg)	540 (µg/kg)
Priors							
1	120	X	X	XX.X%			
2	XXX	X	X	XX.X%			
…	…	…	…	…			
22	XXX	X	X	XX.X%			

8. PRE-SPECIFIED ANALYSES OF SECONDARY OUTCOMES

8.1 Analysis of PK Parameters

An enzyme capture immunoassay method has been developed and validated by Charles River Laboratories (Reno, Nevada) for determination of 3K3A-APC activity in plasma. Brief details are included below. Tables, listings, and graphs of pharmacokinetic data will be produced by the study pharmacokineticist (Bill Kramer). These results will not be included in the FSR produced by the NeuroNEXT DCC, but will be submitted in a separate report.

PK parameters following administration of 3K3A-APC will be determined using concentration data obtained via enzyme-immunocapture assay of 3K3A-APC amidolytic activity in plasma. Based upon analysis of PK data from the Phase I study, a one-compartment IV infusion model will be used and will be parameterized in terms of the primary parameters clearance (CL) and volume of distribution (V). Secondary parameters will include C_{max}, $AUC_{0\text{-}inf}$, and $t_{1/2}$ and will be calculated from the primary parameters. The actual times of the beginning and end of all 5 infusions and each blood sampling time relative to the start of the first dose will be used in the modeling.

Compartmental modeling will be done using Phoenix WinNonlin Version 6.2 or higher. All analysis data and final parameter estimates for both analyses will be in SAS datasets. SAS version 9.3 or higher will be used for

data management, and to produce individual subject listing and descriptive statistics for plasma concentrations and pharmacokinetic parameters and individual subject concentration vs. time graphs. In-text graphs will be produced using SigmaPlot Version 12.5 or higher.

Observed and model-predicted plasma concentrations will be plotted on linear and semi-logarithmic axes by subject. Descriptive statistics of plasma PK parameters will be provided for each dose cohort. PK parameters will be compared among doses using descriptive statistics and graphical displays.

The analysis for the PK population will be conducted according to actual dose received. The status of the subjects will be given in a summary table by treatment group:

- Number of subjects screened
- Number of subject randomized
- Number of subjects treated
- Number of subjects who completed the study
- Number of subjects who received planned number of doses
- Number of subjects in the PK population

For pharmacokinetic data, any concentrations that are "below lower limit of quantification (BLLQ)" will be considered 0 for the purposes of analyses.

Descriptive statistics will be reported for plasma concentrations, separately for each cohort and time point. Summary tables of pharmacokinetic tables will also be produced per treatment group for each cohort. The tables will display sample size, arithmetic mean, SD, and CV%, geometric mean and CV%, and minimum, median, and maximum. The analysis will also produce graphs showing mean +/- standard error plasma 3K3A-APC concentrations versus nominal time, per regimen and treatment group (linear and semi-logarithmic). Individual subject graphs of observed- and model-predicted plasma concentrations will also be prepared. The relationship between the change from baseline in aPTT and the concurrently measured 3K3A-APC plasma concentration 1 hour after the end of infusion will also be assessed.

Finally, a listing of individual blood sampling concentration data (PK) will be generated along with a listing of antibody development results.

8.2 Analysis of Hemorrhage Rates

The incidence of hemorrhage, overall and by type (as defined above), will be summarized in a table similar to the one below:

Table 3. Bleeding Rates

Type	Total (N = XX)	Treated (N = XX)	Placebo (N = XX)	P-Value
Hemorrhage (Volume > 0.06 mL)	XX (%)	XX (%)	XX (%)	X.XX
(Volume > 0 mL)	XX (%)	XX (%)	XX (%)	X.XX
(Volume > 0.5 mL)	XX (%)	XX (%)	XX (%)	X.XX
(Volume > 1.8 mL)	XX (%)	XX (%)	XX (%)	X.XX
Microbleeds	XX (%)	XX (%)	XX (%)	X.XX

To assess tPA/mechanical thrombectomy-related hemorrhage rates across the two groups, all treated subjects (regardless of dose) will be compared to placebo subjects using a Pearson chi-square test. This test will assess the null hypothesis that there is no relationship between treatment group assignment and hemorrhage rates versus the alternative hypothesis that the groups differ with respect to hemorrhage rates. If any of the expected cell counts are too small to justify the use of the Pearson chi-square test, Fisher's Exact test will be used instead.

If we fail to reject H_0, then we will conclude that there is no significant difference between the placebo and 3K3A-APC groups. If H_0 is rejected, then we will conclude that the two groups differ with respect to hemorrhage rates – with the direction of the difference indicating which group has an increased (decreased) risk of hemorrhage. For example, if H_0 is rejected and the hemorrhage rate is lower in the 3K3A-APC group, then we will conclude that participants in the 3K3A-APC group have a significantly lower rate of hemorrhage than participants in the placebo group.

An additional analysis will compare the total hemorrhage rates among placebo subjects and subjects treated at each dose level (assuming a sufficient number of subjects were treated at that level). Again, if the assumptions of the Pearson chi-square test are not met, then Fisher's exact test will be used instead.

All prior analyses will be repeated to compare the groups with respect to incidence of microbleeds at Day 30.

8.3 Exploratory Outcomes

A number of additional exploratory analyses are also planned, but will not be included as part of the FSR. Unless explicitly stated otherwise, the comparisons will be conducted first comparing all treated subjects (regardless of dose) versus all placebo subjects, and then comparing each dose individually. These exploratory analyses include, but are not limited to the analyses described below.

- This study will include additional bleeding outcome data of interest. While the sample size is likely too small to observe meaningful treatment effects, the data allow confirmation that outcomes in this trial resemble previously published trials. Corresponding, the following exploratory analyses will be considered:
    - Evaluating the effect of 3K3A-APC on the volume of tPA/mechanical thrombectomy related hemorrhage in the brain as determined by MRI at Day 30
    - Evaluating the effect of 3K3A-APC on the incidence of subarachnoid hemorrhage in subjects who receive mechanical thrombectomy
    - Evaluating the effect of 3K3A-APC on the incidence of hemorrhage at Day 7.
- To inform the design of any future phase II/III trial, the study will also include outcome data typically collected in all stroke trials, as well as sample collection to assess the immunogenic potential of 3K3A-APC. Correspondingly, additional exploratory analyses will include comparisons across groups for:
    - Day 7 National Institute of Health Stroke Scale (NIHSS) Scores
    - Change from Baseline NIHSS to Day 7 NIHSS
    - Day 90 Modified Rankin Scale (mRS)
    - Infarct Volume at 90 Days (MRI, or CT if unable to obtain MRI):
    - Day 90 Barthel Index (BI) Scores
    - The relationship between the 7-day NIHSS score and the 90-day mRS will be examined using a linear regression model. The degree of fit and the R-square will be determined, and the model will be used to assess the potential for using the 7-day outcome in subsequent studies.
    - Pre-Dose 1, Day 14, and Day 30 Anti-Drug Antibody Samples: The tendency of the drug to induce an immunogenic response in patients will be assessed.

9. SAMPLE SIZE CONSIDERATIONS

9.1 Primary Outcome

In order to assess the adequacy of the sample size, we conducted a simulation study to examine the ability of the study to identify the MTD under a range of assumed scenarios. The scenarios under consideration are summarized below in Table 4.

Table 4. Assumed True Toxicity Probabilities for Simulation Scenarios

Scenario	Description	Dose Level (μg/kg)			
		1 (120)	2 (240)	3 (360)	4 (540)
1	MTD = Dose 4	0.05	0.05	0.05	0.05
2	MTD = Dose 4	0.05	0.05	0.07	0.10
3	MTD = Dose 3	0.05	0.07	0.10	0.15
4	MTD = Dose 2	0.05	0.10	0.15	0.20
5	MTD = Dose 1	0.10	0.15	0.20	0.25
6	No MTD	0.15	0.20	0.25	0.30

The scenarios describe the full range of possibilities:

- Scenario 1 considers the case where all doses are safe (i.e., true toxicity probability is below the target threshold for each dose).

- Scenarios 2-5 consider the case where the true target toxicity level of 10% occurs at each successive dose level under consideration.
- Scenario 6 considers the case where no dose level is safe (i.e., true toxicity probability is above the target threshold for all doses).

The results of the simulation study are summarized in Table 5, and contrasted to the results from a pure 3+3 design (which was proposed in the original version of the protocol). Below, we provide a short summary of the findings from the simulation study:

- In scenario 1, both CRM approaches and the 3+3 method select the highest dose with high probability.
- In scenarios 2-5, both CRM approaches generally does a better job at selecting the "correct" dose as the MTD. The 3+3 method does slightly better at selecting the highest dose when it is the MTD (scenario 2). However, this comes at a cost of "overshooting" the MTD when the true MTD occurs at a lower dose – as high as 88% under scenario 5.
- In scenario 6, the CRM approaches correctly conclude that no dose satisfies the criteria of an MTD over half of the time, whereas the 3+3 has a small probability of declaring no MTD and seems to select any of the four doses in a near uniform manner.

Table 5. Results of Simulation Study

Scenario	Dose Level	% Recommended			# Subjects Allocated			%True Toxicity CRM
		CRM	CRM_{lag}	3+3	CRM	CRM_{lag}	3+3	
1	None	0%	1%	1%				
	120	2%	2%	2%	8.1	11.5	3.5	5%
	240	3%	4%	2%	5.3	8.9	3.4	5%
	360	4%	5%	3%	5.4	8.0	3.6	5%
	540	**91%**	**88%**	**91%**	**42.2**	**32.6**	**45.2**	**5%**
2	None	1%	1%	1%				
	120	4%	4%	2%	9.3	12.4	3.4	5%
	240	9%	9%	2%	8.3	10.8	3.4	5%
	360	19%	19%	10%	10.4	11.4	4.4	7%
	540	**68%**	**67%**	**86%**	**36.8**	**29.7**	**33.8**	**10%**
3	None	1%	1%	1%				
	120	10%	10%	2%	13.8	15.7	3.5	5%
	240	25%	25%	4%	14.0	14.9	3.6	7%
	360	**32%**	**31%**	**19%**	**14.2**	**13.9**	**5.0**	**10%**
	540	32%	33%	74%	23.6	20.9	23.1	15%
4	None	1%	2%	1%				
	120	27%	26%	2%	22.0	22.5	3.5	5%
	240	**42%**	**42%**	**8%**	**19.1**	**19.3**	**4.1**	**10%**
	360	21%	21%	29%	12.4	12.6	5.6	15%
	540	9%	10%	59%	12.1	11.1	15.5	20%
5	None	21%	20%	3%				
	120	**54%**	**54%**	**9%**	**37.4**	**36.9**	**4.0**	**10%**
	240	20%	20%	16%	12.5	13.9	4.4	15%
	360	5%	5%	32%	6.1	6.4	5.2	20%
	540	1%	1%	41%	4.8	3.8	9.4	25%
6	**None**	**61%**	**61%**	**6%**				
	120	34%	34%	19%	39.4	39.0	4.5	15%
	240	4%	4%	22%	6.2	7.7	4.5	20%
	360	1%	1%	29%	2.8	2.7	4.4	25%
	540	0%	0%	24%	1.9	1.3	5.2	30%

In summary, both the modified CRM and lagged CRM approaches generally have better performance than the 3+3 design with respect to successfully recommending the correct dose as the MTD (based on prior assumptions), and either would be well suited to achieve the goals of this study. The modified CRM design

being used here performs better than the more traditional approach at identifying the true MTD when it occurs at one of the intermediate doses. Clearly, better accuracy could be achieved by increasing the sample size for the study. However, when weighted against the costs of a larger study, the proposed sample size provides the right balance with respect to selecting the correct dose across all scenarios, and considering the probabilities of both "undershooting" and "overshooting" the assumed true MTD. Some efficiency is sacrificed by using the lagged version of the modified CRM. However, this minor loss of efficiency is completely offset by the operational advantages of requiring fewer "extra placebo subjects" to be enrolled.

9.2 Secondary Outcome (Bleed Rates)

We also assessed the power of the study to evaluate bleed rates between all treated subjects (collapsed across doses) versus placebo subjects. Previous experience (Derex et al, 2004; Kidwell et al, 2004; Fiehler et al, 2007; Cheng et al, 2013; Harada et al, 2013; Kimura et al, 2013; Shoamanesh et al, 2013) suggests that the MRI-detected bleed rate for tPA-treated subjects should be approximately 30%-40%. Furthermore, it is expected from pre-clinical studies that treatment with APC could lead to a rather dramatic decrease in bleed rates for 3K3A-APC treated subjects (Cheng et al, 2006; Wang et al, 2012; Wang et al, 2013). Pre-clinical data suggest that 3K3A-APC could reduce bleeding seen after IV tPA or after mechanical thrombectomy (Wang et al, 2012; Wang et al, 2013). Thus, it is expected that the study could observe a difference between the groups as substantial as a reduction from a 40% bleed rate among placebo subjects to a lack of observed bleeding in the treated group. However, to assess power over a range of conditions, we computed power for all combinations of the following conditions:

- Placebo group bleed rates of 20%, 30%, and 40%
- 3K3A-APC treated group bleed rates of 0.5%, 5%, and 10%
- Overall sample sizes of 48 (36 treated / 12 control) and 88 (66 treated / 22 control) – in order to assess power under the scenario where the trial proceeds to the end and under the scenario where the study stops early for lack of observing any toxicity at the highest dose

Table 6 provides a summary of these power calculations. In general, we find:

- *If the original assumptions are correct:* We would have very high power (>90%) in either scenario.
- *If the original assumption regarding the placebo rate is correct, but the treated rate is a bit higher:* We would have very good power (>85%) if the study proceeds to the end. We would have lower, but a still acceptable level of power (>64%) if the study stops due to a lack of observed toxicity after 12 cohorts.
- *If the original assumption regarding the treatment rate is correct, but the placebo rate is a bit lower:* We would have decent power (>66%) in either scenario, or very good power (>84%) in the scenario where the study proceeds to the end.
- *If both assumptions are wrong (placebo rate lower, treatment rate higher):* There is lower power overall, but still reasonable power in a number of settings (such as 30% placebo rate versus 10% treated rate).

In conclusion, it appears that the study has adequate power to detect differences in bleeding of interest, even if our assumptions are slightly optimistic. We believe that this sufficiently supports the adequacy of the comparison of bleeding rates.

Table 6. Power for Comparing Bleed Rates

Assumed Bleed Rate in Treated Group	Assumed Bleed Rate in Placebo Group					
	20%		30%		40%	
	N = 48	N = 88	N = 48	N = 88	N = 48	N = 88
0.5%	66%	84%	82%	95%	92%	99%
5%	39%	56%	63%	83%	80%	96%
10%	18%	26%	41%	61%	64%	85%

10. SAFETY MONITORING

Safety will be monitored by physical examinations, vital signs, clinical laboratory tests (i.e., chemistries, hematology, coagulation studies, and urinalysis), CT and MRI, ECGs, and adverse event (AE) assessment. Safety assessments will compare the groups on the basis of all AEs, changes in laboratory and vital signs values, and results of physical examinations. AE data will be listed individually, and will be descriptively summarized by body system, preferred terms within a body system, and treatment dose.

10.1 Adverse Events

For the purposes of this study, an adverse event (AE) is any untoward occurrence associated with the use of a drug in humans whether or not considered drug related. FDA, Office of Human Research Protection (OHRP), and NeuroNEXT CIRB requirements for reporting AEs will be followed. Adverse events are generally detected in two ways:

- Clinical – Symptoms reported by the subject or signs determined on examination
- Ancillary Tests – Abnormalities of vital signs, laboratory tests, and other diagnostic procedures (other than the outcome measures: the results of which are not being captured as AEs).

If discernible at the time of completing the AE source documentation, a specific disease or syndrome rather than individual associated signs and symptoms should be identified by the Site Investigator and recorded in the AE documentation. However, if an observed or reported sign, symptom, or clinically significant laboratory anomaly is not considered by the Site Investigator to be a component of a specific disease or syndrome, then it should be recorded as a separate AE in the source documentation. Clinically significant laboratory abnormalities, such as those that require intervention, are those that are identified as such by the Site Investigator.

Each clinical study site's Principal Investigator and research team (co-investigators, research nurse, clinical trial coordinator) are responsible for identifying and reporting AEs through the NeuroNEXT Online Adverse Event Reporting System. The AE definitions and reporting procedures used for this study comply with all applicable United States Food and Drug Administration (FDA) regulations and International Conference on Harmonization (ICH) guidelines. The Site Investigator will carefully monitor each subject throughout the study for possible AEs. All AEs will be documented on CRFs designed specifically for this purpose. It is important to report all AEs, especially those that result in permanent discontinuation of the investigational product being studied, whether serious or non-serious. Local site investigators are also required to fulfill all reporting requirements of their local institutions.

Subjects who sign consent and receive investigational treatment will be monitored for AEs from the time they sign consent through Day 7. Any AEs that occur prior to informed consent should be included in the subject's medical history. This study will utilize the CTCAE version 4.03 coding system for adverse event recording. AEs reported using CTCAE will be recoded into MedDRA terms by the DCC, with the exception that events of "Intracranial Hemorrhage", "Cerebral Hematoma", or "SICH" are coded as "Cerebral Hemorrhage" and events of "Infarct Extending" are coded as "Cerebrovascular Accident" (per request of PPI).

At each visit (including telephone interviews), the subject will be asked "Have you had any problems or symptoms since your last visit?" in order to determine the occurrence of AEs. If the subject reports an AE, the Investigator will determine:

- Type of event
- Date on onset and resolution (duration)
- Severity (mild, moderate, severe, life-threatening, results in death)
- Seriousness (does the AE meet the definition of an SAE)
- Causality, relation to investigational product and disease
- Action taken regarding investigational product
- Outcome

All clinical AEs are recorded in the AE data entry template in the subject's study binder. The site should fill out the AE data entry template and enter the information into the Online Adverse Event Reporting System (AERS) within 5 working days / 7 calendar days of the site learning of a new AE or receiving an update on an existing AE. Entries in the AE data entry template will include the following:

- Name and severity of the AE
- Date of onset
- Date of resolution
- Relationship to study drug
- Action taken

- Primary outcome of event

The severity of all AEs will be graded according to CTCAE, version 4.03. Any AE not listed in the CTCAE will be graded as follows:

- Grade 1 – Mild: Transient or mild discomfort; No limitation in activity; No medical intervention/therapy required
- Grade 2 – Moderate: Mild to moderate limitation in activity; Some assistance may be needed; No or minimal medical intervention/therapy required
- Grade 3 – Severe: Marked limitation in activity, some assistance usually required; Medical intervention/therapy required; Hospitalizations possible
- Grade 4 – Potentially Life-Threatening: Extreme limitation in activity; Significant assistance required; Significant medical intervention/therapy required; Hospitalization or hospice care probable
- Grade 5 – Death: The AE results in death

The relationship of the AE to the investigational product should be specified by the Site Investigator, using the following definitions:

- Definitely Related: The reaction follows a reasonable temporal sequence from administration of investigational product; Follows a known or expected response pattern to the investigational product; and is confirmed by improvement on stopping or reducing the dosage of the investigational product, and reappearance of the reaction on repeated exposure. (suspected ADR)
- Probably Related: The reaction follows a reasonably temporal sequence from administration of investigational product; Is confirmed by discontinuation of the investigational product or by re-challenge; and cannot be reasonably explained by the known characteristics of the subject's clinical state. (suspected ADR)
- Possibly Related: The reaction follows a reasonably temporal sequence from administration of the investigational product and follows a known response pattern to the suspected investigational product; Reaction could have been produced by the investigational product or could have been produced by the subject's clinical state or by other modes of therapy administered to the subject. (suspected ADR)
- Unlikely to be Related: The reaction has little or no temporal sequence from administration of the investigational product, and/or a more likely alternative etiology exists.
- Unrelated: Concomitant illness, accident, or event with no reasonable association with treatment.

For the purposes of this study, an AE is considered to be treatment-related if the attribution is possible, probable, or definite. All AEs should be followed until resolution or a new baseline is established, but no longer than study Day 90.

Headache has been identified as an AE that we would like to further characterize. Therefore, all headaches that start after signing of the ICF should be documented as AEs, and Investigators should also complete the Headache Record CRF. A new AE form, and new Headache Record should be completed for each new headache during the first seven days. Headaches that start prior to signing of the ICF, and do not worsen, should be reported only on the Medical History CRF and the Headache Record CRF should not be completed. If, however, a headache begins prior to consent and later worsens it should be reported as an AE and the Headache Record CRF should be completed.

10.2 Serious Adverse Events

The Site Investigator is initially responsible for classifying AEs as serious or non-serious. An AE is considered serious if it meets one or more of the following criteria:

- Results in death
- Is life-threatening (i.e., a subject is at immediate risk of death at the time the AE occurs, not an event where occurrence in a more serious form might have caused death)
- Requires inpatient hospitalization or prolongation of existing hospitalization
    - Hospitalization for an elective procedure (including elective PEG tube, g-tube, feeding tube placement) or a routinely scheduled treatment is not an SAE by this criterion because an elective or scheduled "procedure" or a "treatment" is not an untoward medical occurrence.

- Results in persistent or significant disability/incapacity
    - This serious criterion applies if the “disability” caused by the reported AE results in a substantial disruption of the subject’s ability to carry out normal life function.
- Results in a congenital anomaly / birth defect
- Necessitates medical or surgical intervention to preclude permanent impairment of a body function or permanent damage to a body structure
- Is another important medical event
    - Important medical events that may not result in death, are not life-threatening, or do not require hospitalization may also be considered SAEs when, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

An inpatient hospital admission in the absence of a precipitating, treatment-emergent, clinical adverse event may meet criteria for “seriousness” but is not an *adverse* experience, and will therefore not be considered an SAE. An example of this would include a social admission (subject admitted for other reasons than medical, e.g., lives far from the hospital, has no place to sleep).

A serious, suspected adverse drug reaction is an SAE that, in the opinion of the Site Investigator or Sponsor, suggests a reasonable possibility that the investigational product caused the event.

Serious adverse events (SAEs) will be collected and reported on subjects who receive investigational treatment from signing of ICF through study Day 30. All SAEs must be reported within 24 hours of the site being notified of the event. All SAEs should be followed until resolution (or resolution with sequelae), but no longer than Day 90.

The Protocol PI will appoint an Independent Medical Monitor (IMM). Upon entry of an SAE by a clinical site, the NeuroNEXT Online Adverse Event Reporting System (AERS) will immediately notify the IMM. The IMM will review the SAE report to assess seriousness, expectedness and relatedness to study treatment, and may request further information if necessary. An unexpected SAE is any SAE for which the specificity or severity is not consistent with the current Investigators Brochure or package insert or described in the protocol. An unexpected, suspected adverse drug reaction is any unexpected SAE that, in the opinion of the IMM is at least possibly related to study treatment. Such SAEs (unexpected and at least possibly treatment-related) require expedited reporting to the FDA. The DCC will prepare a MedWatch safety report for submission to the FDA. If warranted, the IMM will notify the DSMB chair.

10.3 Neuroworsening

For the purposes of this protocol, the incidence of “neuroworsening” is expected to be significant in this trial of acutely ill stroke patients. Per the protocol, neurological worsening is defined as a 4 or more point increase on the NIHSS, as compared to the most recent NIHSS, that lasts for more than 8 hours, is confirmed by a repeat NIHSS, follows administration of study drug, is resulting from edema, hemorrhage, hydrocephalus, and/or extending infarct, and is not felt to be attributable to non-neurological causes, such as iatrogenic sedation or medical co-morbidities. The cause of the neuroworsening will be captured as a serious adverse event, and must be declared by the site PI as hemorrhage, progressive cerebral edema, new infarct, or hydrocephalus. If occurring during the DLT reporting period, will be evaluated by the IMM as a potential DLT. For each case, sufficient imaging will be selected by the CSS Investigator, de-identified, and submitted to the IMM for review so that he can confirm the reason for neuroworsening. Refer to the ‘Imaging Plan’ for details. The Site Investigator will be asked to give primary and contributing causes of the worsening, and to gauge the depth of worsening by performing a NIHSS as close to the nadir as possible. The IMM will review all cases of neuroworsening, and will refer suspected cases of SICH to the SRC for adjudication. Refer to the ‘Safety Management Plan’ for further details.

10.4 Symptomatic Intracranial Hemorrhage (SICH)

All patients will have a follow-up CT scan at 24-36 hours following the tPA bolus, per local SOC and as deemed necessary by the investigator. If a CSS uses MRI as SOC at this time point, it is encouraged that the MRI protocol for this study be used. Symptomatic intracranial hemorrhage is defined as blood present on CT or

MRI brain images that is associated with clinical worsening that meets the definition of neuroworsening (4 or more point increase in NIHSS; see section 3.3.3) *and* in the opinion of the investigator represents a clinically significant change that can be attributed to the hemorrhage (Wahlgren et al, 2007). For each case, sufficient imaging will be selected by the CSS Investigator, de-identified and submitted to the IMM and SRC for review so that the SRC can adjudicate the possible DLT. Refer to the 'Imaging Plan' and 'Safety Management Plan' for further details.

10.5 Independent Medical Monitor

The PPI will appoint an Independent Medical Monitor (IMM). The IMM responsibilities will include independent review of safety data, including but not limited to review of all SAEs and DLTs in near real-time to help inform the CRM. At Day 8 for each enrolled subject, the IMM will also review a patient profile for each subject in order to thoroughly evaluate each subject for DLTs (including cases of neuroworsening). The patient profile for this Day 8 review will include a review of data from Days 1-7 for categories such as demographics, medical history and medications, vital signs, ECG, laboratory results, infusions, AEs, SAEs, site reported DLTs, neuroworsening, stroke scales, and protocol deviations. Should a DLT be reported or suspected, the IMM will notify the SRC which will be convened to review the AE(s). Any non-serious AEs that occur during the DLT period, but are not reported until after the Day 8 review will be evaluated in real-time by the IMM for safety, neuroworsening, and DLTs. Any non-serious AEs that occur after the DLT observation period can be reviewed on a quarterly basis or sooner at the discretion of the IMM.

The IMM will also assess 'key images' as part of the safety review of SICH (a DLT) and all cases of neuroworsening (an SAE). The IMM's assessment of each 'key image' will be captured in the database as part of the AE narrative for a given event. The objectives of the IMM review are:

- To confirm the presence of gross hemorrhage when an Investigator reports a DLT of SICH
- To confirm the reason for neuroworsening (hemorrhage, edema, extending infarct, or hydrocephalus) as reported by the Site Investigator

The IMM will also review all events that meet the regulatory definition of an SAE in real-time, upon notification via the NeuroNEXT Online Adverse Event Reporting System (AERS). In addition to performing real-time reviews of all SAEs (as described in section 10.2), the IMM will also receive aggregate, blinded reports of all AEs for review on a quarterly basis (or as requested). These aggregate reports will summarize all AEs by severity, attribution (expected or unexpected), and relationship to study drug procedures in a tabular form. The report will also provide summary information on all neuroworsening events, confirmed DLTs, and deaths. The IMM will be responsible for providing a written summary of this review to the DCC. Should any concerns arise due to observed trends, the IMM will send a written recommendation to the DCC requesting that the report be forwarded to Protocol PIs, the SRC, and/or the Data and Safety Monitoring Board (DSMB), as appropriate.

10.6 Safety Review Committee

An independent Safety Review Committee (SRC) will be formed and charged with reviewing and adjudicating DLTs in order to objectively inform the CRM. The SRC will be comprised of a committee chair, the IMM, and three qualified participants – as outlined in the 'Safety Review Committee Charter'. For every reported DLT, the SRC will review the event and make a final determination as to whether or not an incident should be classified as a DLT. The SRC's final adjudication (DLT = yes/no) will be recorded using the DLT Adjudication Form as part of the IMM Evaluation Module. Assignment of the dose for the following cohort will not occur until all four subjects in a cohort have completed the Day 8 assessment and all potential DLTs have been reviewed by the SRC. The SRC will be responsible for final determination of whether a particular AE will be classified as a DLT, but will not participate directly in dose selection. On a quarterly basis, the SRC will also review the same aggregate reports viewed by the IMM (see section 10.5).

If in the extraordinarily unlikely event the Central Reader identifies a new suspected DLT that was not previously recognized for that subject, the Central Reader will inform the PPI who will contact the DCC to initiate the AE/DLT review process so that the DLT can be adjudicated by the SRC.

10.7 Data and Safety Monitoring Board

The monitoring of subject safety and data quality will follow the NINDS Guidelines for Data and Safety Monitoring in Clinical Trials. A DSMB, appointed by NIH/NINDS, will meet at approximately six-month intervals (or as determined by the NINDS) to review partially unblinded study data provided by the study statistician. The

DSMB will periodically review and evaluate the accumulated data for participant safety, adverse events, study conduct, and study progress. The DSMB may suggest changes to the protocol or consent form to the Study Chair as a consequence of AEs. The DSMB may also make recommendations to NINDS concerning continuation, modification, or termination of the study. The frequency and format of DSMB meetings, reports, and guidelines for interim analysis will be agreed upon prior to study subject enrollment.

10.8 Safety Analyses

In addition to the determination of an MTD, additional safety assessments will involve a comparison of treatment-related AEs and SAEs across all 3K3A-APC treated subjects (regardless of dose) versus placebo. First, the percentage of subjects who experience any treatment-related AE or SAE in each group will be compared using a Fisher's exact test. If the null hypothesis is rejected, with a greater frequency observed in the 3K3A-APC group, we will conclude that 3K3A-APC was associated with a significantly greater frequency of treatment-related AEs. If the hypothesis is not rejected, we will conclude that the study does not provide sufficient evidence to conclude that 3K3A-APC was associated with a significantly greater frequency of treatment-related AEs. In addition to an overall comparison, this hypothesis will be repeated for the percentage of subjects having at least one treatment-related AE within each MedDRA system organ class (SOC). If there are significant differences between groups within any specific SOC, then additional tests will compare differences across groups for specific MedDRA preferred terms in order to further explore the cause of observed differences.

In addition to the comparison of percentages in the manner described above, the rates of treatment-related AEs in each group will be compared using the following Poisson regression model:

$$\log\left(\frac{Y_i}{T_i}\right) = \beta_0 + \beta_1 x_{1i} + \epsilon_i$$

where

- Y_i represents the number of treatment related SAEs experienced by the i^{th} subject.
- T_i represents the number of days between the date of randomization and the date of last follow-up for the i^{th} subject.
- x_i = 1 if i^{th} subject was randomized to one of the 3K3A-APC dose groups, and 0 if the subject was randomized to placebo group
- ϵ_i is random error for the i^{th} subject

To determine if the rate of treatment related SAEs differ across treatment group we will test the following hypothesis:

$$H_o: \beta_1 = 0 \text{ vs. } H_A: \beta_1 \neq 0$$

If the null hypothesis is rejected, the direction of β_1 will indicate the direction of the observed effect. Values of $\beta_1 > 0$ indicate an increased rate of treatment-related AEs associated with the 3K3A-APC group, while values of $\beta_1 < 0$ indicate a decreased rate of treatment-related AEs associated with the 3K3A-APC group.

Treatment-related SAEs will be analyzed in the same manner described above. Additional safety analyses will assess all treatment-emergent AEs, treatment-emergent SAEs, unanticipated SAEs, treatment-related & unanticipated SAEs, neuroworsening events, DLTs, and deaths in a similar manner. Finally, all safety analyses will be repeated for each pairwise comparison of subjects in each specific 3K3A-APC dose groups versus the placebo group.

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