

STUDY OFFICIAL TITLE: ZZ-3K3A-201: A multi-center, Phase 2 study using a continual reassessment method to determine the safety and tolerability of 3K3A-APC, a recombinant variant of human activated protein C (APC), in combination with tissue plasminogen activator (tPA), mechanical thrombectomy or both in moderate to severe acute ischemic stroke.

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FULL PROTOCOL TITLE

ZZ-3K3A-201: A multi-center, Phase 2 study using a continual reassessment method to determine the safety and tolerability of 3K3A-APC, a recombinant variant of human activated protein C (APC), in combination with tissue plasminogen activator (tPA), mechanical thrombectomy or both in moderate to severe acute ischemic stroke.

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RHAPSODY: Safety evaluation of 3K3A-APC in ischemic stroke

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INVESTIGATOR AGREEMENT

I have read the foregoing protocol, ZZ-3K3A-201, and agree to conduct the study as described herein. I will provide copies of this protocol and all pertinent information to the study personnel under my supervision. I will discuss this material with them and ensure they are fully informed regarding the investigational plan and the conduct of the study according to 21 CFR parts 50, 54, 56 and 812, ICH Good Clinical Practices Guidelines and Institutional Review Board (IRB) requirements.

NOTE: By signing the protocol, the Investigator agrees to keep all information provided by the NeuroNEXT Network in strict confidence and to request the same from his/her staff and the Institutional Review Board. Study documents provided by the NeuroNEXT Network will be stored appropriately to ensure their confidentiality. The Investigator should not disclose such information to others without authorization, except to the extent necessary to conduct the study.

Investigator Signature

Date

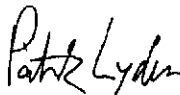
Print Investigator's Name

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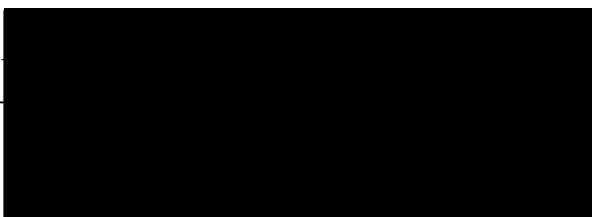
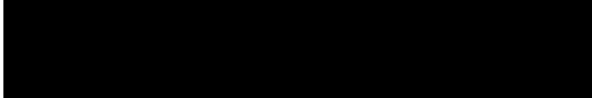
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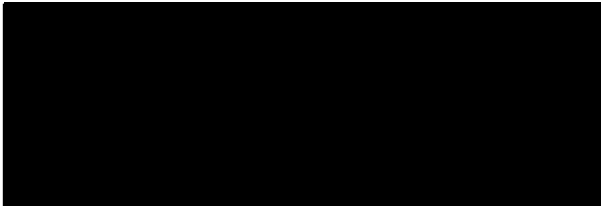
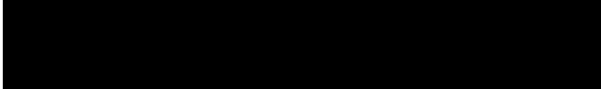
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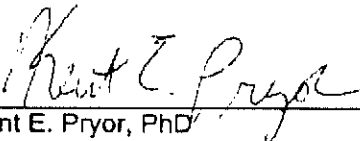
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NeuroNEXT Data Coordinating Center Approval:

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Sponsor Approval:

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LIST OF ACRONYMS, ABBREVIATIONS, AND DEFINITIONS OF TERMS

Abbreviation	Term
ACA	anterior cerebral artery
ADA	anti-drug antibody
AE	adverse event
AMI	acute myocardial infarction
APC	activated protein C
aPTT	activated partial thromboplastin time (same as PTT)
ASPECTS	Alberta stroke program early CT score
AUC _{0-inf}	area under the plasma concentration-time curve from time 0 extrapolated to infinity
AVM	arterio-venous malformation
BBB	blood brain barrier
βHCG	beta human chorionic gonadotropin
BI	Barthel Index
BMI	body mass index
C	centigrade
CBC	complete blood count
CCC	Clinical Coordination Center
CDE	Common Data Elements
CHO	Chinese hamster ovary
CL	total clearance
cm	centimeters
C _{max}	maximum observed plasma concentration
CFR	Code of Federal Regulations
CIRB	Central Institutional Review Board
Con Med	concomitant medication
CRF	Case Report Form
CRM	continual reassessment method
CS	clinically significant
C _{ss}	Steady-state concentration
CSS	clinical study site
CSS PI	clinical study site Principal Investigator
CT	computed tomography or computed tomography scan

Abbreviation	Term
CTCAE	Common Terminology Criteria for Adverse Events
DCC	Data Coordination Center
dL	deciliter
DLT	dose-limiting toxicity
DM	data management
DSMB	Data Safety Monitoring Board
DWI	diffusion-weighted imaging
ECG	electrocardiogram
ECL	electrochemoluminescence ligand
eCRF	electronic case report form
EDC	electronic data capture
EOI	end of infusion
EPCR	endothelial protein C receptor
ER	emergency room
FDA	Food and Drug Administration
g	grams
GCP	Good Clinical Practice
GFR	glomerular filtration rate
GRE	gradient recalled echo
HED	human equivalent dose
Hg	mercury
HIPAA	Health Insurance Portability and Accountability Act
IA	intra-arterial
ICA	internal carotid artery
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IMM	Independent Medical Monitor
IMP	Investigational Medicinal Product (refers to both active drug and placebo)
IND	Investigational New Drug
INR	International Normalized Ratio
IRB	Institutional Review Board
ITT	Intent-to-treat
IV	intravenous

Abbreviation	Term
IWRS	interactive web response system
kg	kilogram
L	liter
m	meter
M1	sphenoidal segment of middle cerebral artery
M2	insular segment of middle cerebral artery
mAOI	modified arterial occlusive lesion
MCA	middle cerebral artery
MCAO	middle cerebral artery occlusion
MedDRA	Medical Dictionary for Regulatory Activities
mEq	milliequivalent
mg	milligram
mL	milliliter
mm	millimeter
MRI	magnetic resonance imaging
mRS	modified Rankin Scale
MTD	maximum tolerated dose
MTL	minimum toxicity level
NCI	National Cancer Institute
NCS	Not clinically significant
NIHSS	National Institutes of Health Stroke Scale
NINDS	National Institute of Neurological Disorders and Stroke
NOAEL	no observed adverse effect level
PAR1	protease activated receptor 1
PE	physical examination
PK	pharmacokinetic
PPI	Protocol Principal Investigator
PSC	Protocol Steering Committee
PTT	partial thromboplastin time (see aPTT)
PT	prothrombin time
rt-PA	recombinant tissue plasminogen activator
rwT	recombinant wild-type
SAE	serious adverse event

Abbreviation	Term
SICH	symptomatic intracranial hemorrhage
SID	subject identification number
SOA	schedule of activities
SOC	standard of care
SRC	Safety Review Committee
SWI	susceptibility weighted imaging
$t_{1/2}$	elimination half-life
TBD	to be determined
TICI	thrombolysis in cerebral infarction
T_{max}	time at which the maximum plasma concentration was observed
tPA	recombinant tissue plasminogen activator
ULN	upper limit of normal
US	United States
USP	United States Pharmacopeia
VS	vital signs
V_z	volume of distribution
WNL	within normal limits
wt	wild-type
μg	microgram
λ_z	elimination rate constant

SYNOPSIS

Investigational Product

3K3A-APC, a Recombinant Variant of Human Activated Protein C (APC) in which 3 lysine residues (191-193) of the 37-loop are replaced by 3 alanine residues.

Study Title

ZZ-3K3A-201: A multi-center, Phase 2 study using a continual reassessment method to determine the safety and tolerability of 3K3A-APC, a Recombinant Variant of Human Activated Protein C (APC), in combination with tissue plasminogen activator (tPA), mechanical thrombectomy or both in moderate to severe acute ischemic stroke.

Objectives

Primary:

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

Secondary:

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

Exploratory:

- To evaluate the effect of 3K3A-APC on the volume of tPA/mechanical thrombectomy-related bleeding (hemorrhage and microbleeds) in the brain as determined by MRI at Day 30.
- To evaluate the effect of 3K3A-APC on incidence of subarachnoid hemorrhage in subjects who receive mechanical thrombectomy.
- To collect the 7-day National Institutes of Health Stroke Scale (NIHSS) scores as a predictor for 90-day modified Rankin Scale (mRS).
- To collect the 90-day mRS.
- To collect the 90-day Barthel Index (BI).
- To collect infarct volume at 90 days (MRI, or CT if unable to obtain MRI).
- To assess the immunogenic potential of 3K3A-APC

Design and Outcomes

Design:

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Version 8.1 Final
Version date 7-Oct-2016

This is a multicenter, prospective, randomized, controlled, double-blinded Phase 2 study intended to evaluate the safety, PK and preliminary efficacy of 3K3A-APC following administration of tPA or mechanical thrombectomy or both in subjects with moderate to severe acute ischemic stroke. Approximately 115 subjects will be randomized, which includes the planned 88 subjects in groups of four to either 3K3A-APC or placebo (in a 3:1 ratio) and additional placebo subjects who will be enrolled during safety review pauses. This study will utilize a modified version of the continual reassessment method (CRM) in order to establish a maximum tolerated dose (MTD)¹.

For the purposes of this study, we assume an established background symptomatic intracerebral hemorrhage (SICH) rate of 3-6%²⁻⁷. Correspondingly, the MTD will be defined as 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 assumed dose-response model and prior data to be closest to the MTD. However, the initial cohort will start at the lowest dose level (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%.

The design 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 study definition. 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 (i.e. two or more doses will need to be administered to be included).
 - Based upon the observed information from the three treated subjects, 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.)

The MTD will be defined as the dose that would be chosen from the CRM at the final step. 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 highest dose the MTD).

Outcomes and Criteria for Evaluation:

- Safety - monitored by physical examinations (PEs), vital signs (VS), clinical laboratory tests (i.e., chemistries, hematology, coagulation studies, and urinalysis), CT and MRI, ECGs and adverse event (AE) assessment.
 - Dose-limiting toxicities 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. Upper limit of normal range is defined locally by the site laboratory.
 - Symptomatic intracranial hemorrhage (SICH) 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 [9.4.1.3](#) 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):
 - ≥ 3 x ULN of alanine aminotransferase (ALT) or aspartate aminotransferase (AST),
 - Serum total bilirubin (TBL) >2 xULN, without initial findings of cholestasis (serum alkaline phosphatase (ALP) activity >2 x 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, preexisting 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. Refer to CTCAE v4.03 sections relevant to laboratory investigations.
- Any adverse event that, in the opinion of the Investigator, is related to study treatment and leads to cessation of further dosing.

NOTE: All suspected DLTs will be reviewed by a Safety Review Committee, and those 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 dose 1 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 tPA, and there is a documented rate of occurrence of 11% in subjects receiving tPA⁸. 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.

- PK analysis – blood samples will be collected from approximately 40 subjects at a sub-set of study sites 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.
- Incidence of tPA/mechanical thrombectomy-related Bleeding – Day 30 MRI scans will be collected and evaluated by a central radiologist for the presence of hemorrhage and microbleeds (as defined in section 8.2.2).
- Exploratory Outcomes - 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. While the sample size is too small to observe meaningful treatment effects, the data will allow confirmation that outcomes in this trial resemble previously published trials. The following will be collected:
 - Volume of bleeding (hemorrhage and microbleeds) in the brain as determined by MRI at Day 30
 - Incidence of subarachnoid hemorrhage in subjects who receive mechanical thrombectomy
 - Day 7 National Institutes of Health Stroke Scale (NIHSS) scores
 - Day 90 mRS
 - Day 90 BI
 - Infarct volume at 90 days (MRI, or CT if unable to obtain MRI)
 - Pre-dose 1, Day 14 and Day 30 anti-drug antibody samples

Interventions and Duration

Investigational or Reference Therapy, Dosage and Mode of Administration:

3K3A-APC will be diluted in 0.9% sodium chloride in water and administered as a 100 mL IV infusion over 15 minutes. Four dose levels of 3K3A-APC will be considered for this study: 120, 240, 360, and 540 µg/kg.

Matching placebo will be 0.9% sodium chloride in water, visually indistinguishable from the test product. Placebo, 100 mL, will be administered in the same manner as the active product.

Following completion of tPA infusion or initiation of mechanical thrombectomy (arterial puncture), whichever is sooner, eligible adult subjects will receive 3K3A-APC or matching placebo 30 to 120 minutes later given as a 15-minute infusion. Subjects will receive another 15-minute infusion of 3K3A-APC or placebo every 12 hours (+/- 1 hour) for up to 5 total infusions.

Study and Treatment Duration

Each subject will be followed for 90 days in this study. With an expected enrollment rate of 0.3 subjects/site/month in approximately 15 NeuroNEXT sites, it is anticipated that the study will take up to 28 months to enroll, which includes the observation window after each of the 22 cohorts to assess for DLTs. Subjects will be considered for the study after beginning tPA administration or 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 the hospital, whichever occurs first. Subjects will be monitored for safety evaluations through Day 7 and are expected to be seen on Days 7, 14, 30 and 90 for safety and outcome evaluations.

Sample Size and Population

Sample Size:

The study will enroll approximately 115 subjects, which includes the planned 88 subjects in groups of four (each cohort will include one placebo and three treated subjects) and additional placebo subjects who will be enrolled during safety review pauses. While placebo is not needed to determine the MTD, a placebo group has been included in order to conduct secondary analyses to examine for a reduction of tPA/mechanical thrombectomy-related bleeds by central read and to obtain preliminary efficacy data that may be useful for the planning of future studies.

Randomization Scheme:

Subjects will be randomized using an interactive web response system (IWRS) to either 3K3A-APC or placebo. There are 22 groups of four subjects planned, but fewer may be enrolled should the study meet either of the early stopping criteria. During the DLT review periods, subjects may be assigned to placebo. The additional placebo subjects will be closely monitored and their enrollment may be discontinued should the number enrolled exceed what was planned for the study. Subjects

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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,” subjects whose symptoms resolve between initial randomization and initiation of IMP infusion such that they are no longer eligible (repeat NIHSS <5), will be removed from the study and replaced.

Inclusion Criteria:

1. Age 18 to 90 years, inclusive
2. Acute ischemic stroke defined as focal, neurological deficit(s), secondary to a presumed vascular occlusive event
3. Able to receive IV tPA per local standard of care, OR, begin mechanical thrombectomy per local standard of care
4. National Institutes of Health Stroke Scale (NIHSS) score ≥ 5 at time of randomization
5. Signed informed consent by subject or authorized representative
6. Agreement to use effective birth control throughout the study (i.e., Day 90):
 - a. Males - barrier method of contraception plus a spermicide
 - b. Females of childbearing potential (i.e., not surgically sterile or post-menopausal defined as age > 51 years without menses for ≥ 2 years) – hormonal contraception or barrier method of contraception plus a spermicide
7. Willing (subject and/or caretaker) to commit to follow-up assessments
8. Mechanical thrombectomy subjects only: onset (last-seen-well) time to arterial puncture time < 6 hours

Exclusion Criteria:

Neurological

1. Rapid spontaneous improvement of neurological signs during screening
2. History of stroke or penetrating head injury within 90 days prior to enrollment
3. History of previous or current diagnosis of intracranial hemorrhage (i.e., intracerebral, epidural, subdural or subarachnoid) that represents—in the opinion of the investigator—a potential for re-hemorrhage if subjected to thrombolytic therapy or mechanical thrombectomy.
4. Moyamoya disease, cerebral arterio-venous malformation (AVM), or known unsecured aneurysm requiring intervention during the acute study period (Days 1 to 30)
5. Presence of other neurological or non-neurological co-morbidities (e.g., intracerebral neoplasm, metabolic encephalopathies, hemiplegic migraine, multiple sclerosis, convulsive disorder, monocular blindness) that, in the Investigator’s opinion, may lead, independently of the current stroke, to further deterioration in the subject’s neurological status during the trial period, or may render the study’s neurological assessments

inconclusive for the purpose of evaluating the effect of investigational product on the stroke

6. Presence of premorbid neurological deficits and functional limitations assessed by a retrospective Modified Rankin Scale (mRS) score of ≥ 2
7. Mechanical thrombectomy subjects only: baseline non-contrast computed tomography (CT) scan revealing a large core occlusion as defined by local protocol, for example an ASPECTS below a locally defined value or baseline CT perfusion data

Non-Neurological

8. Prolonged prothrombin time (INR >1.7)
9. Prolonged partial thromboplastin time (PTT) that exceeds the upper limit of normal (ULN)
10. Use of heparin within the 48 hours prior to enrollment, except to maintain catheter patency
11. Severe hypertension (systolic blood pressure [BP] > 185 mm Hg or diastolic BP > 110 mm Hg) or hypotension (systolic BP < 90 mm Hg), as measured by at least 2 consecutive supine measurements 10 minutes apart, that does not respond to simple treatment (e.g., 1 dose of labetalol or nicardipine infusion)
12. Estimated glomerular filtration rate (GFR) <35 mL/min
13. Blood glucose concentration < 50 mg/dL
14. Prior exposure to any exogenous form of APC (e.g., plasma-derived APC, 3K3A-APC, Xigris,[®] drotrecogin alfa [activated])

General

15. Weight > 129 kg
16. Unable to undergo MRI per local guidelines
17. Pregnancy or breastfeeding
18. Current abuse of alcohol or illicit drugs
19. Received treatment with an investigational drug or device within 30 days prior to enrollment
20. Any other condition that, in the opinion of the Investigator, may adversely affect the safety of the subject, the subject's ability to complete the study, or the outcome of the study

1 STUDY OBJECTIVES

1.1 Primary Objectives

- To evaluate the safety of multiple ascending intravenous (IV) doses of 3K3A-APC following tPA administration or mechanical thrombectomy or both in subjects who have experienced moderate to severe acute ischemic stroke.

1.2 Secondary Objectives

- To investigate the PK properties of 3K3A-APC following tPA or mechanical thrombectomy or both in adults with acute ischemic stroke.
- To evaluate the effect of 3K3A-APC on the presence of tPA/mechanical thrombectomy-related bleeding in the brain (hemorrhage and microbleeds) as determined by MRI at Day 30.

1.3 Exploratory Objectives

- To evaluate the effect of 3K3A-APC on the volume of tPA/mechanical thrombectomy-related bleeding (hemorrhage and microbleeds) in the brain as determined by MRI at Day 30.
- To evaluate the effect of 3K3A-APC on incidence of subarachnoid hemorrhage in subjects who receive mechanical thrombectomy.
- To collect the 7-day National Institutes of Health Stroke Scale (NIHSS) scores as a predictor for 90-day modified Rankin Scale (mRS).
- To collect the 90-day mRS
- To collect the 90-day Barthel Index (BI)
- To collect infarct volume at 90 days (MRI, or CT if unable to obtain MRI)
- To assess the immunogenic potential of 3K3A-APC

2 BACKGROUND

2.1 Ischemic Stroke

A stroke occurs when the blood supply to part of the brain is interrupted (ischemic stroke) or when a blood vessel in the brain bursts (hemorrhagic stroke), allowing blood into the spaces surrounding brain cells. Each year about 795,000 people in the United States (US) experience a new or recurrent stroke⁹. Of all strokes, 87% are ischemic and the remainder are hemorrhagic (75% intracerebral hemorrhage and 25% subarachnoid hemorrhage)¹⁰. Ischemic stroke is the third leading cause of death and the most common cause of disability in industrialized nations¹¹.

In ischemic strokes, an artery in the brain is occluded in one of 2 principal ways: thrombotic or embolic¹². A thrombotic stroke occurs when diseased or damaged cerebral arteries become blocked by the formation of a blood clot within the blood vessels supplying the brain. An embolic stroke is caused by a clot (or emboli) formed somewhere other than in the brain itself. Often

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originating in the heart, these emboli will travel the bloodstream until they become lodged and restrict the flow of blood to the brain.

2.2 Current Treatment for Stroke

Currently, the most commonly used approved treatment for acute stroke is thrombolytic therapy with recombinant tissue plasminogen activator (rtPA or tPA). tPA is approved for IV administration within 3 hours of onset of acute ischemic stroke in the US and for up to 4.5 hours following the stroke in Europe^{2,13}. Thrombolytic therapy with IV tPA up to 4.5 hours following stroke is recommended by the American Heart Association/American Stroke Association¹⁴. The primary adverse effect of tPA in clinical use is symptomatic intracranial hemorrhage (~ 6%); other risks include systemic bleeding, myocardial rupture (when used to treat acute myocardial infarction), and, in rare cases, anaphylaxis or angioedema¹³. Although tPA is widely available in the US, it is estimated that only 10 to 20% of stroke patients receive treatment with tPA^{15,16}, principally because they present for care > 3 hours after the onset of symptoms or are at increased risk for bleeding from concomitant medication use, or for other reasons.

Another effective treatment for stroke, albeit less frequently used, is mechanical thrombectomy in patients with documented large vessel occlusion⁴⁻⁷. As shown in Table 1, four large, well-controlled randomized clinical trials showed benefit of thrombectomy when added to IV tPA treatment. Large vessel occlusion is defined as thromboembolic blockage of the distal internal carotid artery (ICA), the M1 or proximal M2 portions of the middle cerebral artery (MCA), or the proximal anterior cerebral artery (ACA). In some of these trials, however, patients benefited who were ineligible for IV tPA and were treated with thrombectomy alone. In 3 prior trials, thrombectomy performed late (> 6 hours after stroke onset) did not appear to be successful¹⁷⁻¹⁹. These trials also used first generation devices whereas the 4 recent trials used second or third generation devices, so the prior negative results and more recent positive results could reflect improvements made in the thrombectomy technology. Regardless of the mechanism, it is clear that patients benefit when treated with latest generation devices very soon (< 6 hours) after stroke onset. Use of mechanical thrombectomy, with or without IV tPA treatment, is considered standard of care in patients with documented large vessel occlusion.

Table 1: Summary of Mechanical Thrombectomy Study Outcomes

KEY – Mechanical Thrombectomy Arm [tPA-only Control Arm]

Trial	% Achieving Reperfusion	mRS 0-2	SICH	Mortality
ESCAPE ⁴	72.4% [§] [31.2%] [¶]	53% [29.3%]	3.6% [2.7%]	10.4% [19.0%]

N=238		p < 0.001	p = 0.75	p = 0.04
EXTEND-I ⁵ N=70	89% [#] [34%] [#] p < 0.001	71% [40%] p = 0.01	0% [6%]	9% [20%]
MR CLEAN ⁶ N=500	58.7% [§] [57.5] [¶]	32.6% [19.1%] (95% CI, 5.9 - 21.2)	7.7% [6.4%]	21% [22%]
REVASCAT ²⁰ N=206	65.7% [§] [Not Reported]	43.7% [28.2%*] (95% CI, 1.1 – 4.0)	1.9% [1.9%*] p = 1.0	18.4% [15.5%*] p = 0.60
SWIFT PRIME ⁷ N=196	82.8% ^{§§} [40.4%] ^{§§} p < 0.0001	60.2% [35.5%] p = 0.0008	1.0% [3.1%] p = 0.37	9.2% [12.4%] p = 0.50

§ Defined as achieving TICI score of 2b or 3

¶ Defined as achieving mAOL score of 2 or 3

Defined as reperfusion >90% without SICH

§§ Defined as reperfusion ≥90%

*Note: 23 of 103 control pts did NOT rec IV-tPA

2.3 Study Drug: 3K3A-APC

3K3A-APC is a 405-residue protein expressed via recombinant technology in Chinese hamster ovary (CHO) cells. Its amino acid sequence differs from that of the wild-type human Activated Protein C (APC) and of the product drotrecogin alfa (activated) (Xigris®) in that 3 sequential lysine residues have been replaced with 3 sequential amino-acid substitutions (all lysine to alanine); the amino acid substitutions are K191A-K192A-K193A. This change retains the cytoprotective effects of native (wild-type) APC while significantly reducing its anticoagulant effects. Glycosylation of 3K3A-APC product differs from wild-type APC since it is expressed in CHO cells.

An important goal in the development of therapeutics intended for the treatment of acute stroke is to minimize bleeding risk while maximizing efficacy. 3K3A-APC is an APC variant that has been genetically engineered to maximize neuro- and cytoprotective activity and minimize anticoagulant activity. 3K3A-APC was developed by altering factor Va binding exosites (reducing anticoagulation) in APC without affecting exosites that recognize PAR1.

In *in vitro* assays, 3K3A-APC retains the cytoprotective activity of recombinant wild-type (rwt) APC but has < 10% of its anticoagulant activity (e.g. see Table 2 below²¹).

Table 2: Cytoprotective and Anticoagulant Activity of rwt-APC versus 3K3A-APC

APC Type	Anticoagulant Activity (% rwt) ¹	Cytoprotective Activity (% rwt) ²	Cytoprotective to Anticoagulant Ratio
rwt-APC	100	100	1.0
3K3A-APC	4.6	114	25

¹Based on the activated partial thromboplastin time (aPTT) dose-response

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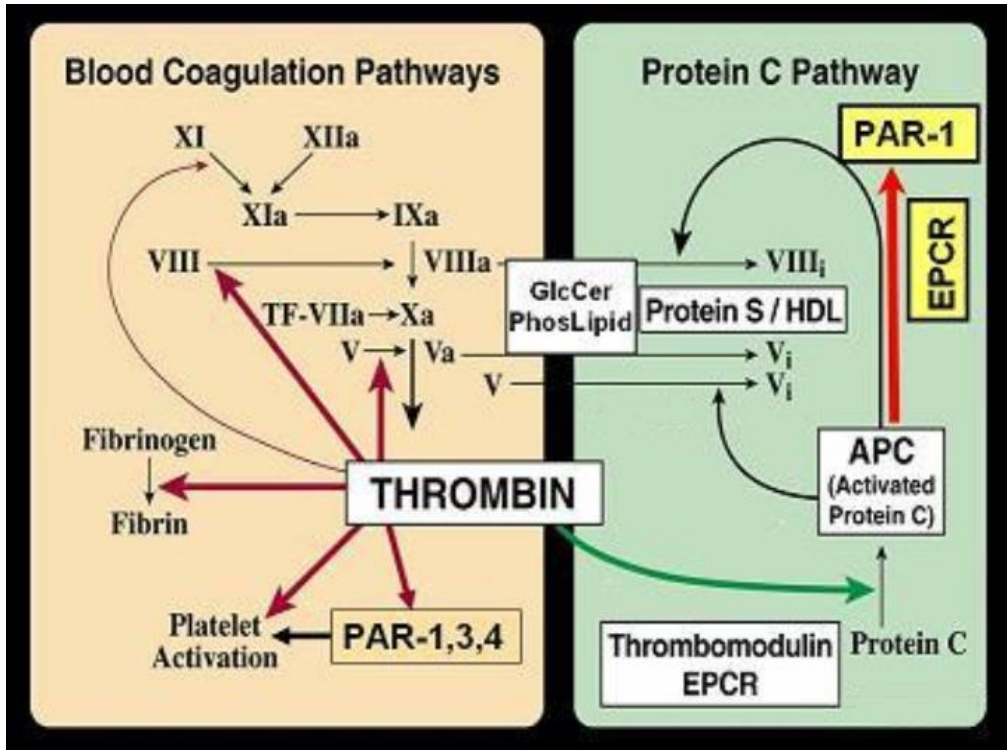
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²Derived from the concentrations of APC required for half-maximal inhibition of apoptosis induced by the protein kinase inhibitor, staurosporine

2.3.1 Rationale for APC in Treating Stroke

APC is an endogenous serine protease with systemic anticoagulant, anti-inflammatory, and anti-apoptotic activities²². APC's anticoagulant activity is independent of its cellular effects. Its anticoagulant activity is mediated by irreversible proteolytic degradation of factors Va and VIIIa with contributions by various cofactors, whereas its cytoprotective activities are mediated by proteolytic activation of protease activated receptor 1 (PAR1)²³. Both properties (anticoagulant and cytoprotective) may be useful in reversing the effects of an ischemic stroke (via thrombolysis) and in protecting the ischemic brain tissue from further damage. APC's cellular signaling results in cytoprotective alterations in gene expression profiles resulting in anti-inflammatory activity and anti-apoptotic activity²⁴⁻²⁸. APC is generated from zymogen protein C that is activated by thrombin on the surface of endothelial cells. The activation requires 2 membrane receptors, thrombomodulin and endothelial protein C receptor (EPCR) (Figure 2).

Figure 2: Blood Coagulation and Protein C Pathway



As noted above, APC acts directly on vascular and neuronal cells to exert multiple cytoprotective effects, mediated by its interaction with EPCR and the effector receptor, PAR-1²³. Specifically, APC protects neurons and brain endothelial cells from ischemic injury and cell death caused by a variety of apoptotic pathways^{24,29-31}. Early post-ischemic administration of APC within 4 hours of an ischemic insult is neuroprotective in rodent models of transient ischemia^{24,32} and embolic stroke³³. Delayed APC administration beginning at 6 hours following ischemic insult with continued bolus dosing 24, 48 and 72 hours following insult, is neuroprotective and mediates brain repair (i.e., neovascularization and neurogenesis), suggesting a significant extension of the therapeutic window for APC intervention in postischemic brain³⁴.

2.3.2 Rationale for use of APC following tPA or Mechanical Thrombectomy

The most frequently used FDA approved treatment for stroke is thrombolysis with recombinant human tPA¹³. Certain mechanical devices have received FDA approval for clot retrieval, and as shown above, mechanical thrombectomy has been shown to be effective in certain patients as a stroke therapy. All patients who meet tPA or mechanical thrombectomy treatment criteria and who arrive to the hospital acutely will receive tPA (within 4.5 hours of onset) or mechanical thrombectomy (within 6 hours of onset) or both, as standard of care. Neuroprotection appears to work best if delivered within 6 hours and in the setting of recanalization/reperfusion³⁵. Thus, the best way to study putative neuroprotectants is to combine them with recanalization treatments, which, if successful, results in reperfusion of ischemic tissue.

The combination of tPA and 3K3A-APC raises potential safety concerns. However, in all likelihood, there will be no tPA circulating by the time the first dose of 3K3A-APC begins. Modeled PK data for

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tPA administration in stroke are shown in Section 5.5.1. The terminal half-life of tPA is ~5 minutes and these modeled data predict that circulating levels of tPA are nearing 0 $\mu\text{g}/\text{mL}$ by 30 minutes following completion of the infusion. In this study, 3K3A-APC or placebo will be given 30-120 minutes following administration of tPA, and therefore little to no circulating tPA is expected to be present during infusion of the IMP.

Studies of wt-APC—which has greater anti-coagulant potential than 3K3A-APC—in combination with tPA provide safety reassurance. Despite having anticoagulant properties, wt-APC has been shown to reduce tPA-induced bleeding and neurotoxicity in preclinical models, probably due to the anti-inflammatory and anti-apoptotic activity previously discussed^{24,29,31,32,36}. There is, however, a theoretical concern that administration of 3K3A-APC following tPA could have deleterious effects on coagulation or clot lysis. The effects of tPA on the anticoagulant activity of 3K3A-APC have been studied using aPTT clotting assays in vitro; tPA at a concentration of 3 $\mu\text{g}/\text{mL}$ had little to no effect on the anticoagulant activity of either wt-APC or 3K3A-APC (Figure 3). Since we expect tPA levels to be virtually undetectable at the time of 3K3A-APC administration, we expect there will be no additional prolongation of aPTT in this study.

Figure 3: The effects of tPA on the anticoagulant activity of 3K3A-APC and wt-APC in aPTT assays

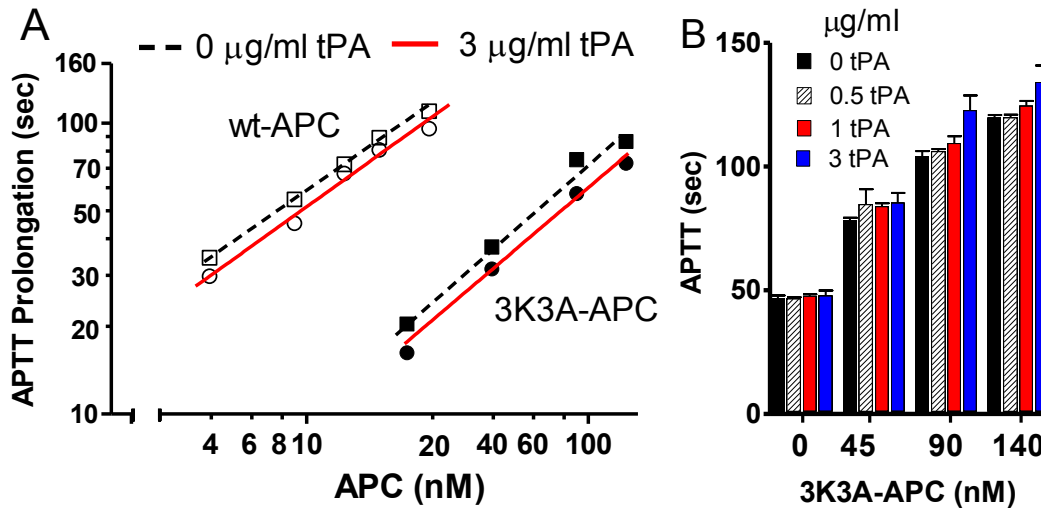


Figure Key: **(A)** Prolongation of the aPTT assay beyond the baseline reference clotting time of 47 sec was determined for plasma-derived wt-APC (open symbols) and for 3K3A-APC (solid symbols) when tPA was present at 0 (circles, solid lines) or 3.0 $\mu\text{g}/\text{mL}$ (squares, dashed lines). Data show difference between observed aPTT value and control value of 47 sec. **(B)** The influence of tPA on the anticoagulant activity of 3K3A-APC was determined at the indicated concentrations (0, 45, 90, 140 nM). The tPA concentrations ($\mu\text{g}/\text{mL}$) were as follows: 0 (black bar), 0.5 (hatched), 1.0 (red bar), 3.0 (blue bar). Data show observed aPTT values.

The effects of 3K3A-APC on the fibrinolytic activity of tPA has also been studied in vitro; no statistically significant effects on the ability of tPA to induce clot lysis were observed by either wt-APC or 3K3A-APC³⁷. Thus, we expect no impact on clot lysis in this study, either.

In summary, ischemic stroke patients who are eligible to receive tPA or mechanical thrombectomy or both are the most likely patient population to benefit from a neuroprotectant like 3K3A-APC, due to the need for reperfusion. Circulating tPA is cleared quickly from the body and levels are expected to be nearly undetectable by the time 3K3A-APC will be administered. Even so, in vitro studies show that there is minimal effect on 3K3A-APC coagulation and no significant effect on tPA clot lysis when both drugs are present at clinically relevant doses.

2.3.3 Recombinant Human APC (drotrecogin alfa [activated], Xigris®)

Xigris® [drotrecogin alfa (activated)] (Eli Lilly and Company, Indianapolis, IN) is a recombinant wild-type APC having the same amino acid sequence as the naturally occurring APC. Xigris® was approved in the US in 2001 (and in the EU in 2002) for treatment of severe sepsis. Although Xigris is no longer marketed because of lack of efficacy in a large post-marketing trial in patients with severe sepsis, bleeding was a dose-limiting side effect³⁸.

In the 1690-subject placebo-controlled study that was the basis for approval, the Xigris® arm had a higher proportion of patients with treatment-emergent adverse events of bleeding than did the placebo arm at all levels of severity, i.e., mild: 10.5% versus 7.7%; moderate: 5.5% versus 1.8%; and severe: 2.8% versus 1.3%. Serious bleeding events (e.g., gastrointestinal, intrathoracic or intracranial) were associated with Xigris® (3.5% versus 2.0% in placebo, $p = 0.06$), occurring more commonly in patients with predisposing conditions for bleeding, such as gastrointestinal ulceration, traumatic injury of a blood vessel or vascular organ, low platelet count or markedly abnormal parameters of coagulation (PT or aPTT)³⁹. The FDA-approved labeling for Xigris® (2008) reported an approximately 1% risk of intracerebral hemorrhage in sepsis patients during continuous infusion. There were no statistically significant differences between the two treatment groups in the percentage of patients who experienced at least one serious adverse event during the study drug infusion period (6.8% of Xigris-treated patients versus 6.5% of placebo-treated patients) or during the 28-day study period (12.5% of Xigris-treated patients versus 12.1% of placebo-treated patients).

Xigris® was recently under study in a Phase 1/2, multi-center, dose escalation safety and feasibility trial for treatment of acute ischemic stroke [Activated Protein C in Acute Stroke Trial (APCAST)]⁴⁰, however the study was terminated prematurely due to insufficient recruitment. As of September 2013, study results were not publically available.

2.4 Nonclinical Studies of 3K3A-APC

2.4.1 Efficacy in Animal Models of Stroke

3K3A-APC has been tested for neuroprotective activity in several mouse models of stroke. In a murine model of embolic stroke, an intact clot was placed at the origin of the middle cerebral artery, and functional neurological testing and histopathological analyses were conducted for 7 days following the stroke⁴¹. In this study, a single IV dose of 2.0 mg/kg of 3K3A-APC, administered 4

hours after stroke as a 50% bolus followed by 50% infusion over 30 minutes, significantly improved functional recovery and neurological scores, and reduced infarct and edema volume in the brain.

In murine models of middle cerebral artery occlusion (MCAO), the artery was occluded for 1 hour followed by 24 hours of reperfusion (transient MCAO model) or was permanently ligated (permanent MCAO model). Animals were assessed for neurological function, neuropathology, and hemoglobin content in the ischemic areas of the brain for up to 7 days after stroke. 3K3A-APC significantly improved neurological function and histopathology scores relative to vehicle control animals at single doses of 0.4 or 2.0 mg/kg, administered 5 minutes prior to transient MCAO, and at multiple doses of 1 mg/kg administered 12 hours and 1, 3, 5, and 7 days following permanent MCAO⁴². The improvement in neurological function and histopathology scores following 3K3A-APC monotherapy was superior to that observed with comparable doses of recombinant wild type APC (rw-APC). In addition, in both MCAO models, rw-APC treated animals showed increased hemoglobin content in the brain, indicative of bleeding, compared to vehicle controls or 3K3A-APC-treated mice.

3K3A-APC acts synergistically with tissue plasminogen activator (tPA) in both mouse and rat stroke models⁴³. Human recombinant tPA (10 mg/kg), alone or in combination with human recombinant 3K3A-APC (2 mg/kg), was administered intravenously 4 hours after proximal or distal transient MCAO in mice or embolic stroke in rats, followed by 3K3A-APC for 3 to 4 consecutive days after stroke. In this delayed treatment paradigm, tPA alone had no beneficial effects on infarct volume or behavior (neurological score, foot-fault, forelimb asymmetry, adhesive removal) compared with vehicle. In contrast to either therapy alone, the tPA plus 3K3A-APC combination significantly reduced infarct volume at 24 hours and at 7 days following proximal or distal transient MCAO in mice and at 7 days after embolic stroke in rats, by 65%, 63%, and 52%, respectively, ($P < 0.05$; determined by one-way analysis of variance followed by Tukey post hoc test) compared to vehicle control. Further, the combination significantly improved behavioral outcomes and eliminated tPA-related intracerebral microhemorrhages ($p < 0.01$ to 0.05).

These positive effects of 3K3A-APC extend to elderly animals and animals with comorbidities such as might be seen in the target patient population of this study. Murine recombinant 3K3A-APC (0.2 mg/kg) alone or with recombinant tPA (10 mg/kg) was given intravenously 4 hours after transient MCAO in aged female mice and 4 hours after embolic stroke in spontaneously hypertensive rats⁴⁴. 3K3A-APC was additionally administered within 3 to 7 days after stroke. Neuropathological analysis and neurological scores, foot-fault, forelimb asymmetry, and adhesive removal tests were performed within 7 and 28 days of stroke. In all models, tPA alone given 4 hours after stroke had no effects on the infarct volume or behavior. 3K3A-APC alone or with tPA reduced the infarct volume 7 days after the MCAO in aged female mice and embolic stroke in spontaneously hypertensive rat by 62% to 66% and 50% to 53%, respectively, significantly improved ($p < 0.05$) behavior, and eliminated tPA-induced intracerebral microhemorrhages.

In summary, 3K3A-APC appears to have less anticoagulant effect and provides greater neuroprotection than rw-APC in mouse models of stroke; when 3K3A-APC is combined with tPA, infarct volumes are reduced and intracerebral microhemorrhages are zero. At the same time, behavioral outcomes in both mouse and rat models of stroke are improved. 3K3A-APC also

extends the therapeutic window of tPA in ischemic stroke models in rodents, supporting further development of tPA and 3K3A-APC combination therapy for focal ischemic stroke in humans.

2.4.2 Toxicology Studies

Two 14-day Good Laboratory Practice (GLP) toxicology studies were performed in mice and monkeys. Doses of 0.4, 2.0, or 5.0 mg/kg were administered by IV bolus once daily to mice, and doses of 0.2, 1.0, or 5.0 mg/kg were administered by IV bolus once daily to monkeys. Endpoints included clinical signs, body weights, food intake, clinical chemistry and hematology, coagulation parameters, and histopathology. 3K3A-APC plasma concentrations were measured on Days 1 and 14 for toxicokinetic assessment. Sera were also obtained to evaluate the potential of 3K3A-APC to induce antibodies in both species.

No clinical signs of toxicity, no effects on hematology or clinical chemistry parameters, and no histopathological changes indicating target organ toxicity were observed. No drug-related effects on neurological, respiratory, or cardiovascular functions were observed in monkeys. However, dose-related increases in aPTT were observed on Days 1 and 14, with increases of approximately 1.5- and 4.5-fold control values at doses of 1 and 5 mg/kg of 3K3A-APC, respectively. PT was increased approximately 1.4-fold of control values at the highest dose (5.0 mg/kg) only. The elevated aPTT and PT values were lower 3 hours after injection and values returned to normal 24 hours post injection. The changes in coagulation parameters were not associated with clinical signs of bruising or bleeding in monkeys.

The no observed adverse effect level (NOAEL) of 3K3A-APC in the mouse and monkey toxicology studies was 5.0 and 0.2 mg/kg/day, respectively, which represent human equivalent doses (HEDs) of 0.40 and 0.06 mg/kg (400 and 60 µg/kg), respectively.

In a separate study, rwt-APC was administered IV in ascending doses to cynomolgus monkeys and PT and aPTT were measured 15 min, 3 hr and 24 hr after dosing. The magnitude of the anticoagulant effect (~ 1.4-fold increase from baseline) at 0.1 mg/kg was similar to the effect of 3K3A-APC at 1.0 mg/kg.

Antibodies to 3K3A-APC were detected in mice and monkeys treated with multiple doses of 3K3A-APC. No apparent relationship to dose was observed in either species. The generation of antibodies in these species is expected since 3K3A-APC is a protein of human origin.

2.5 Clinical Studies of 3K3A-APC

A Phase 1 study, ZZ-3K3A-001, was conducted in 64 healthy adult volunteers to characterize the safety and PK profile of single ascending and multiple ascending IV doses of 3K3A-APC. Intravenous administration of 3K3A-APC or matching placebo to cohorts of healthy adult volunteers at doses up to 720 µg/kg single-dose and 540 µg/kg multiple-dose (every 12 hours for 5 doses) did not result in any serious adverse events, severe adverse events or withdrawal from study due to an adverse event. After review of AEs (moderate headache, nausea and vomiting and mild vertigo) in 1 of the 2 subjects who received a single-dose of 720 µg/kg of 3K3A-APC, the SRC recommended reducing the dose to 540 µg/kg in subsequent subjects in the cohort. For that reason, 540 µg/kg was the highest dose evaluated in the multiple-dose cohorts. In summary, the

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evaluated safety measures indicate that 540 µg/kg is tolerated in single and multiple dosing in healthy volunteers.

The most common adverse events reported were mild or moderate; headache, hypertension, nausea and vomiting were reported in 54%, 8%, 8% and 4% of the 3K3A-APC treated subjects (n=50) respectively. Rates for the same events in placebo subjects (n=14) were 7%, 21%, 7% and 0%, respectively.

The majority of the drug-related AEs were headache; of subjects who received active drug across the four highest doses, headache occurred at least once in 3/10 (30%) subjects receiving 180 µg/kg, in 8/10 (80%) subjects receiving 360 µg/kg, in 9/10 (90%) subjects receiving 540 µg/kg and in 2/2 (100%) subjects receiving 720 µg/kg. Headache was reported in 1/14 (7%) subjects who received placebo. These data indicate that headache is related to 3K3A-APC exposure and appears to increase in frequency with dose. In no patient did headache lead to interruption of IMP or require treatment other than acetaminophen pain reliever.

Hypertension was reported with the second highest frequency; ten AEs of hypertension assessed as being related to the treatment occurred during the study. Four (4) of these AEs occurred in placebo-treated subjects (21% of all placebo-treated subjects), and 6 of these AEs occurred in 3K3A-APC-treated subjects (8% of all actively treated subjects). No association of hypertension with 3K3A-APC could be ascribed.

Increases in the coagulation parameters, aPTT and to a much lesser extent, PT, were observed following administration of 3K3A-APC; however, the magnitudes of the changes were not considered clinically significant. Elevations above the normal range for aPTT and PT were seen in 30% and 6% of 3K3A-APC-treated subjects (doses ranging 180 – 720 µg/kg only), respectively, and in 21% and 0% of placebo subjects, respectively. The highest aPTT level observed in a single subject was 1.51 times ULN (60.4 sec) at 1 hour following infusion #4 of 360 µg/kg. The highest observed PT in a single subject was 1.02 times ULN (16.1 sec) at 1 hour following infusion #2 of 540 µg/kg. The majority of elevated values returned to within normal limits by 2 hours following each dose. No clinical signs of bleeding or bruising were observed in the study subjects despite the mild prolongations in coagulation parameters.

Repeated evaluation of blood chemistry, hematology and coagulation yielded no AEs related to placebo or active treatment. Vital signs other than blood pressure (heart rate, body temperature and respiratory rate) also did not yield any abnormalities in both treatment groups. There were no clinically significant ECG or QTc changes noted in 3K3A-APC-treated subjects.

No subject tested positive for anti-3K3A-APC antibody formation following administration of 3K3A-APC.

Dose-related systemic exposure to 3K3A-APC (measured by a validated enzyme-immunocapture assay of 3K3A-APC amidolytic activity in plasma) was observed at dose levels of 30 µg/kg and greater. No evidence of accumulation of 3K3A-APC was observed in the multiple-dose cohorts.

Based on compartmental modeling, mean C_{max} ranged from 249 to 5,715 ng/mL (30 and 720 $\mu\text{g}/\text{kg}$, respectively); mean values for CL (11,693 to 18,701 mL/h) and V (4,873 to 6,971 mL) were independent of dose as was the $t_{1/2}$ with a mean of approximately 0.25 h (range 13 to 18 min).

In summary, 540 $\mu\text{g}/\text{kg}$ was tolerated in single- and multiple-dosing. The most common effects of 3K3A-APC observed in the single clinical study conducted were mild dose-related reversible increases in coagulation parameters (aPTT and PT) and mild and moderate dose-related, transient adverse events of headache, hypertension, nausea and vomiting.

2.6 Rationale for Dose Selection

3K3A-APC is delivered via intravenous infusion over 15 minutes. Brief intravenous infusions were selected for subjects because in all studies in rodents 3K3A-APC was administered using brief single systemic infusion^{41,42,45-47}. Short term dosing is preferred over longer infusions of drug because APC neuroprotection occurs predominantly via cytoprotective cell signaling mechanisms. Repeated administration of high dose 3K3A-APC has been remarkably successful in preclinical models, likely because this homeostatic enzyme (half-life circa 16 min) acts directly on cells to alter gene-expression profiles and to stabilize endothelial barrier function. 3K3A-APC engages therapeutically relevant signaling pathways that reduce inflammation, vascular leakage, and apoptosis³⁶. Low-dose, long-term infusions of this enzyme are less likely to reboot multiple cell protective mechanisms because initiation of cell signaling benefits from higher doses of agonist. Therefore, short term bolus dosing should achieve maximum benefits for changing the status of cell signaling networks. Furthermore, bolus dosing will eliminate the risk of zymogen factor V and VIII consumption that is associated with the continuous infusion of rwt-APC.

The proposed doses of 3K3A-APC for evaluation in the study are 120, 240, 360 and 540 $\mu\text{g}/\text{kg}$. These doses are based on the doses and administration that were tested in the Phase 1 clinical study in normal human volunteers during which single- and multiple-dose (q12 hours for 5 doses) infusions were well tolerated at doses up to 540 $\mu\text{g}/\text{kg}$. The proposed doses also cover a range that is expected to achieve circulating levels of 3K3A-APC that correlate with preclinical efficacy, or about 2000 ng/mL. In mouse studies, the dose of murine 3K3A-APC that exerts maximal protective effect in mice is 200 $\mu\text{g}/\text{kg}$ ⁴¹, which is similar to murine wt-APC²⁴. A clinical dose of 240 $\mu\text{g}/\text{kg}$ of 3K3A-APC is expected to achieve similar circulating levels.

3 STUDY DESIGN

3.1 Study Design and Enrollment:

This is a multicenter, prospective, randomized, controlled, double-blinded Phase 2 study intended to evaluate the safety, PK and preliminary efficacy of 3K3A-APC following administration of tPA or mechanical thrombectomy or both in subjects with moderate to severe acute ischemic stroke. Approximately 115 subjects will be enrolled, which includes the planned 88 subjects in groups of four to either 3K3A-APC or placebo (in a 3:1 ratio) and the additional placebo subjects who will be enrolled during safety review pauses. This study will utilize a modified version of the CRM in order to establish a MTD.

Following completion of tPA infusion or initiation of mechanical thrombectomy (arterial puncture), whichever is sooner, eligible adult subjects will receive study treatment 30 to 120 minutes later given as a 15-minute infusion. Subjects will receive another 15-minute infusion of 3K3A-APC every 12 hours (+/- 1 hour) for up to 5 total doses. The initial cohort dose of 3K3A-APC will be 120 µg/kg; the other dose levels that will be evaluated with the CRM are 240, 360, and 540 µg/kg. After the final group of subjects is enrolled, the final MTD will be defined as the highest dose with estimated toxicity probability less than or equal to the target toxicity level of 10%.

Enrollment will proceed as follows:

- Enroll the first 4 subjects in 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 study definition. 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 (i.e. two or more doses will need to be administered to be included).
 - Based upon the observed information from the three treated subjects, 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.)

The MTD will be defined as the dose that would be chosen from the CRM at the final step. 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 highest dose the MTD)

Through simulation it was determined that it would be 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 stopping early would be justified.

Non-serious adverse events (AEs) will be collected through actual Day 7, and SAEs collected through actual Day 30. More specifically, subjects will be evaluated for DLTs from administration of the first dose to 48 hours following the last dose of study treatment. Blood samples for PK will be collected from approximately 40 subjects at a sub-set of study sites following one of the 5 doses of 3K3A-APC at the following time points: end of infusion and 20, 40, 60 and 80 minutes after the end of infusion. Subjects must be seen for assessments on Days 7, 14, 30 and 90. MRI scans, including SWI sequences, will be obtained at 7 (or discharge) and 30 days after stroke.

3.1.1 Dose Limiting Toxicities:

Toxicities that require grading will use the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), Version 4.03.

Dose-limiting toxicities 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 aPTT following any dose that reaches 2 x the ULN at 1 hour post-dose. Upper limit of normal range is defined locally by the site laboratory.
- SICH 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 [9.4.1.3](#) 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.
- Laboratory findings that meet all of the following three components (Hy's Law):
 - ≥ 3 x ULN of ALT or AST,
 - In combination with a serum total bilirubin (TBL) >2 x ULN, without initial findings of cholestasis (serum ALP activity >2 x 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, preexisting 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. Refer to CTCAE v4.03 sections relevant to laboratory investigations.
- Any adverse event that, in the opinion of the Investigator, is related to study treatment and leads to cessation of further dosing.

NOTE: All suspected DLTs will be reviewed by a Safety Review Committee, and those 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 dose 1 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 tPA, and there is a documented rate of occurrence of 11% in subjects receiving tPA⁸. 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.

Should a subject experience a DLT, dosing of study treatment should be discontinued as soon as the Investigator becomes aware of the event.

3.1.2 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 as described in section [8.1.1](#). The SRC will be comprised of a committee chair, the IMM and three qualified participants, as outlined in the 'Safety Review Committee Charter.' Should a DLT be reported or suspected, the DCC will notify the SRC, which will be convened to review the AE(s). If a DLT is confirmed by the SRC, the NeuroNEXT statistics group will be informed so that the safety event can be fed into the CRM and the dose level determined automatically by the model for the next cohort needing a treatment level assignment. The SRC will be responsible for final determination of whether a particular event will be classified as a DLT, but will not participate directly in dose selection. Refer to 'Safety Review Committee Charter' for details.

3.1.3 Data Monitoring and Data 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 Data and Safety Monitoring Board (DSMB) appointed by the 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. This committee will monitor rates of adverse events and endpoints in the trial and will monitor the performance of the trial. The frequency and format of DSMB meetings, reports, and guidelines for interim analysis will be agreed upon prior to study subject enrollment. The PPI will appoint an IMM to review all adverse events, in a blinded fashion. The IMM will perform a review of Day 1-7 data (including non-serious AEs) on Day 8 to look for missed DLTs. During that same Day 8 review the IMM will evaluate for safety and for neuroworsening all AEs that have been reported. 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. In addition the IMM will review all events that meet the regulatory definition of a Serious Adverse Event (SAE), upon receipt of notification via the Electronic Data Capture (EDC) system, for safety, neuroworsening and DLTs. See the Safety Management Plan for more detail.

4 SELECTION AND ENROLLMENT OF SUBJECTS

4.1 Inclusion Criteria

1. Age 18 to 90 years, inclusive
2. Acute ischemic stroke defined as focal, neurological deficit(s), secondary to a presumed vascular occlusive event
3. Able to receive IV tPA per local standard of care, OR, begin mechanical thrombectomy per local standard of care.
4. National Institutes of Health Stroke Scale (NIHSS) score ≥ 5 at time of randomization
5. Signed informed consent by subject or authorized representative
6. Agreement to use effective birth control throughout the study (i.e., Day 90):
 - a. Males - barrier method of contraception plus a spermicide
 - b. Females of childbearing potential (i.e., not surgically sterile or post-menopausal defined as age > 51 years without menses for ≥ 2 years) – hormonal contraception or barrier method of contraception plus a spermicide
7. Willing (subject and/or caretaker) to commit to follow-up assessments
8. Mechanical thrombectomy subjects only: onset (last-seen-well) time to arterial puncture time < 6 hours

4.2 Exclusion Criteria

Neurologic

1. Rapid spontaneous improvement of neurological signs during screening
2. History of stroke or penetrating head injury within 90 days prior to enrollment
3. History of previous or current diagnosis of intracranial hemorrhage (i.e., intracerebral, epidural, subdural or subarachnoid), that represents—in the opinion of the investigator—a potential for re-hemorrhage if subjected to thrombolytic therapy or mechanical thrombectomy
4. Moyamoya disease, cerebral arterio-venous malformation (AVM), or known unsecured aneurysm requiring intervention during the acute study period (Days 1 to 30)
5. Presence of other neurological or non-neurological co-morbidities (e.g., intracerebral neoplasm, metabolic encephalopathies, hemiplegic migraine, multiple sclerosis, convulsive disorder, monocular blindness) that, in the Investigator's opinion, may lead,

- independently of the current stroke, to further deterioration in the subject's neurological status during the trial period, or may render the study's neurological assessments inconclusive for the purpose of evaluating the effect of investigational product on the stroke
6. Presence of premorbid neurological deficits and functional limitations assessed by a retrospective Modified Rankin Scale (mRS) score of ≥ 2
 7. Mechanical thrombectomy subjects only: baseline non-contrast CT scan revealing a large core occlusion as defined by local protocol, for example an ASPECTS below a locally defined value or baseline CT perfusion data

Non-Neurological

8. Prolonged prothrombin time (INR >1.7)
9. Prolonged partial thromboplastin time (PTT) that exceeds the upper limit of normal (ULN)
10. Use of heparin within the 48 hours prior to enrollment, except to maintain catheter patency
11. Severe hypertension (systolic blood pressure [BP] > 185 mm Hg or diastolic BP > 110 mm Hg) or hypotension (systolic BP < 90 mm Hg), as measured by at least 2 consecutive supine measurements 10 minutes apart, that does not respond to simple treatment (e.g., 1 dose of labetalol or nicardipine infusion)
12. Estimated glomerular filtration rate (GFR) <35 mL/min
13. Blood glucose concentration < 50 mg/dL
14. Prior exposure to any exogenous form of APC (e.g., plasma-derived APC, 3K3A-APC, Xigris,[®] drotrecogin alfa [activated])

General

15. Weight > 129 kg
16. Unable to undergo MRI per local guidelines
17. Pregnancy or breastfeeding
18. Current abuse of alcohol or illicit drugs
19. Received treatment with an investigational drug or device within 30 days prior to enrollment
20. Any other condition that, in the opinion of the Investigator, may adversely affect the safety of the subject, the subject's ability to complete the study, or the outcome of the study

4.3 Subject Discontinuation

4.3.1 Subject Withdrawal Criteria

Subjects may withdraw from the study at any time. The Site Investigator will document on the appropriate case report form (CRF) page the reason/circumstances for withdrawal.

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4.3.2 Discontinuation of Study Drug

A subject may be removed from further administration of study drug for the following medical or administrative reasons:

- AE/SAE related to study drug, or for another safety reason that, in the opinion of the Investigator, is in the best interest of the subject
- Subject was not eligible or becomes ineligible for study, or other major protocol deviation that poses a safety risk to the subject
- Subject experiences a DLT

Efforts will be made to follow all subjects who discontinue study drug for any reason. Such follow-up will include all relevant evaluations for safety including clinical assessments and collection of laboratory study results as set out in this protocol.

4.4 Study Enrollment Procedures

After beginning tPA or mechanical thrombectomy for moderate to severe acute ischemic stroke, subjects or their legally authorized representatives will be presented with the study details and the informed consent form (ICF) as described in Section [4.4.3](#).

4.4.1 Subject Recruitment and Retention

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. The Stroke Team will consider every treated patient for potential enrollment. During treatment, the study team will meet with the patient and family to emphasize the critical importance of long-term follow up. Every effort should be made by the study team to ensure subjects are seen for Day 7 (or discharge), 14, 30 and 90 follow-up visits (e.g., appointment reminder calls, ensuring transportation, etc.). Follow-up visits will be coordinated with standard return visits whenever possible to minimize patient inconvenience and promote cooperation with follow-up visits. If travel distances are significant, the site should contact the CCC for assistance. Study funds are available to reimburse patients for ambulance/special needs transportation. The CCC will work with the site to determine the reimbursement process and amounts. Sites may also seek to conduct follow-up visits at the subject's home, nursing home or other facility if the patient cannot travel. The NeuroNEXT Recruitment and Retention Committee will work with each site to identify recruitment and retention strategies that will be agreed upon and used throughout the study.

4.4.2 Reporting of Screening Data

Screening data documenting the reasons for ineligibility and reasons for nonparticipation of eligible subjects will be completed by the study coordinator and submitted to the NeuroNEXT Data Coordination Center via the EDC system. All code stroke patients who receive tPA or can begin mechanical thrombectomy < 6 hours of last known well time will be considered for this study. Using the EDC system, the stroke team study coordinator will record all patients considered for the study, and if not eligible, the reason(s) the patient was not enrolled, including specific inclusion or exclusion criteria. The coordinator will report as many criteria as applicable per patient. The

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screening data will be compiled and provided to the study team and the Protocol Steering Committee at an agreed upon frequency.

4.4.3 Informed Consent

Written informed consent will be obtained from each study participant or his/her legally authorized representative before any study-specific procedures or assessments are done and after the aims, methods, anticipated benefits, and potential hazards are explained. The site PI is ultimately responsible for the informed consent process and can delegate the ability to discuss and obtain informed consent from study participants to licensed sub-Investigators who are participating in the study. All such delegates will sign the “Delegation of Responsibility Log” and agree to adhere to the protocol. The participant’s willingness to participate in the study will be documented in writing in a consent form approved by the NeuroNEXT Central Institutional Review Board (CIRB), which will be signed by the participant or his/her legally authorized representative with the date of that signature indicated. The Investigator will keep the original consent forms and a copy will be given to the participant. It will also be explained to the participant that he/she is free to refuse entry into the study and free to withdraw from the study at any time without prejudice to future treatment. Written and/or oral information about the study in a language understandable by the participant will be given to all participants.

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). If, in the opinion of the Site Investigator, the subject’s mental status improves sufficiently to enable him/her to provide informed consent, the subject will be re-consented in person at the current or next study visit if consent was originally granted by another individual.

4.4.4 Randomization/Treatment Assignment

Subjects will be randomized using an IWRS to either 3K3A-APC or placebo (in a 3:1 ratio). Twenty-two groups of four subjects are planned, but fewer may be enrolled should the study meet either of the early stopping criteria (see section [8.1.1](#)). 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 reviewed. The additional placebo subjects will be closely monitored and their enrollment may be discontinued should the number enrolled exceed what was planned for the study.

The NeuroNEXT IWR system will also serve to inform the unblinded study pharmacist of the treatment assignment (refer to Section [5.3](#) for details on Study Drug Preparation).

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 if they were randomized to a cohort. For example, if a randomized subject experiences significant neurologic improvement (defined as a NIHSS score <5) prior to receipt of study drug he/she will be termed an 'Early Responder' and will not be included in the study. 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 STUDY DRUG

5.1 Description, Packaging, Supply and Labeling

5.1.1 3K3A-APC

3K3A-APC will be supplied by ZZ Biotech using the IWR system. Adequate drug supply for a single subject will automatically be supplied to each investigational site following collection of regulatory documents, site training and CIRB approval of the site. Automatic resupply will occur when the site confirms administration of the first dose of drug to a subject in the EDC system; however, the site pharmacist will check with the unblinded monitor to assure that re-supply requests have been noted.

3K3A-APC is supplied as a frozen, sterile, non-pyrogenic, citrate-buffered, hypertonic solution for intravenous infusion with a total extractable volume of 5.0 mL. Each vial contains 5.0 mg of 3K3A-APC drug substance, 25.8 mg of sodium citrate, 87.7 mg of sodium chloride, 1 mg of polysorbate-80 in 5 mL of water for injection. The vials are sealed with FluroTec® stoppers that are secured with Flip-Off® overseals. No preservatives or bacteriostatic agents are added.

Vials are labeled as shown in Figure 4 below.

Figure 4: 3K3A-APC Label

3K3A-APC Injection, 1.0 mg/mL, 5.0 mL

Lot # XXXXXXXX; Mfg Date: MMM YYYY

Storage: $\geq -90^{\circ}\text{C}$, $< -60^{\circ}\text{C}$, protected from light

For intravenous injection –Single use only

Instructions for Use: Refer to Protocol

Caution: New Drug—Limited by United States Law to Investigational Use.

ZZ Biotech, LLC, Houston, TX, [REDACTED]

5.1.2 Placebo

Placebo will be 0.9% sodium chloride in water in 100 mL infusion bags. Clinical sites will be responsible for supply of 0.9% sodium chloride infusion bags for administration of study drug and placebo.

5.2 Storage of Study Drug

3K3A-APC vials must be stored at $\leq -60^{\circ}\text{C}$ to $\geq -90^{\circ}\text{C}$. The study drug should be maintained in a secure, limited-access room and in an alarmed, temperature-controlled freezer. If the temperature exceeds -60°C for longer than 1 hour, or cumulatively 8 hours in a 30-day period, the Sponsor should be contacted. Temperature logs should be submitted to the assigned pharmacy monitor for interim remote monitoring and must be available during monitoring visits.

5.3 Preparation and Blinding of Study Drug

Subjects will be randomized using an IWRS to either 3K3A-APC or placebo (in a 3:1 ratio; however, during safety review pauses only placebo subjects will be enrolled). The treatment allocation will be made available to the designated unblinded site pharmacist by the IWRS. Each subject will receive up to 5 doses of 3K3A-APC or placebo according to the randomization schedule. Blinding will be controlled by administering the same volume to all subjects (i.e., the entire 100-mL infusion bag). Four possible dose levels of 3K3A-APC will be considered for evaluation in this study: 120, 240, 360 and 540 $\mu\text{g}/\text{kg}$.

Under sterile conditions, 3K3A-APC will be prepared as follows:

1. Calculate the volume of 3K3A-APC to be administered to the subject and remove the appropriate number of vials from the freezer.
2. Thaw the vials for approximately 15 minutes at room temperature, or until completely thawed. Accelerated heating methods should not be used.

3. Remove the calculated volume of 0.9% sodium chloride in water from the 100 mL infusion bag.
4. Remove the calculated volume of 3K3A-APC from the thawed vial(s).
5. Add the 3K3A-APC to the infusion bag.

Placebo will be administered as 0.9% sodium chloride in water, 100 mL.

Refer to the current '**Drug Storage, Handling and Administration Manual**' for further instructions and examples; the Drug Storage, Handling and Administration Manual will always supersede the study drug handling instructions in this protocol.

5.4 Administration of Study Drug

Study drug will be infused into a large bore peripheral arm vein at a rate of 400 mL/hr over 15 minutes for all dose levels. The first dose of study drug will be administered 30 to 120 minutes after tPA has completed or mechanical thrombectomy begins (arterial puncture), whichever is sooner. Subsequent doses of study drug should be given every 12 hours (+/- 1 hour) for 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. Please note that PK samples should be drawn from the opposite arm. See 'Drug Storage, Handling and Administration Manual' for further instructions on administration.

After the study drug infusion is complete, the IV line should be flushed with at least 20 mL of normal saline to ensure complete administration of the residual study drug. The calendar date, 24-hour clock time of the beginning and end of the infusion, and the volume infused should be recorded for capture in the CRF.

5.5 Accountability of Study Drug

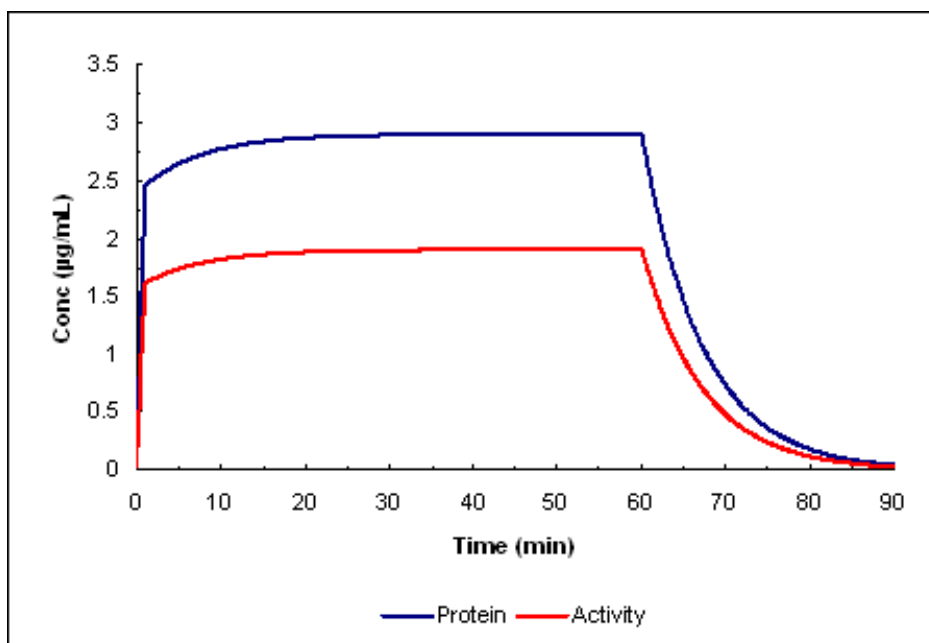
In accordance with local regulatory requirements, the Investigator, designated site staff, or head of the medical institution (where applicable) must document the amount of investigational product dispensed and/or administered to study subjects, the amount received from the central pharmacy, and the amount destroyed upon completion of the study. The local PI is ultimately responsible for ensuring product accountability records are maintained throughout the course of the study; however this responsibility should be delegated to the unblinded study pharmacist(s) for the purposes of maintaining the study blind throughout the study. The inventory will include details of 3K3A-APC received and dispensed to subjects, including the lot number and subject ID. All used and unused vials must be kept until reconciliation of delivery records with accountability logs by the monitor (unless alternative handling is approved by the CCC due to Institutional policy). After the monitor has performed accountability, the site will be instructed by the CCC or designee to either destroy the remaining study drug or return it to the Central Pharmacy or manufacturer. An accounting must be made of any drug deliberately or accidentally destroyed. Discrepancies between the amount of 3K3A-APC received and dispensed must be reconciled and documented.

5.5.1 Required Concomitant Medications/Interventions

Subjects will become eligible for this clinical study upon initiation of tPA or decision to begin mechanical thrombectomy, subject to fulfillment of the study inclusion and exclusion criteria. The administration of tPA should follow the American Heart Association's national accepted published guidelines¹⁴. Use of mechanical thrombectomy should follow the local Institutional protocol, and should only include the use of FDA approved thrombectomy devices.

The PK of tPA in stroke patients has not been published; however, the PK can be extrapolated from data widely available in acute myocardial infarction (AMI). The tPA label for AMI states that the CL is ~475 mL/min and the $t_{1/2}$ is ~5 min⁸, therefore V is ~3,400 mL. Assuming a one-compartment model and a 90 mg dose (with 10%, 9 mg, given over the 1st minute and the remaining 81 mg given from 1 to 59 minutes for a total dosing time of 60 minutes), the simulated concentration-time profile is shown in Figure 5.

Figure 5: Simulated Concentration-Time Profile of tPA in Stroke



The steady-state concentration (C_{ss}), 2.9 µg/mL, is consistent with data in the literature for a regimen in AMI resulting in a C_{ss} of 3.2 ± 0.84 µg/mL based on an immunochemical assay (measurement of protein, not activity). As expected, activity was lower: 2.1 ± 0.23 µg/mL. An estimate of true variability can be based on published data, where the %CV of the steady-state protein and activity were 26% and 11%, respectively⁴⁸. Assuming a similar variability for the stroke regimen, the steady-state protein and activity would be 2.9 ± 0.75 µg/mL and 1.9 ± 0.21 µg/mL, respectively. If a 95% confidence interval is assumed, the upper limits would be ~4.5 and ~2.3 µg/mL, respectively. Steady-state concentration is achieved by 20 to 25 minutes.

5.5.2 Prohibited Medications and Interventions

Medications:

The use of anticoagulants such as vitamin K antagonists, factor Xa inhibitors and thrombin inhibitors are prohibited from the time of randomization to 4 hours following the last dose of 3K3A-APC. Anti-platelet medication must be started in all patients unless clinically contraindicated, e.g., hemorrhagic transformation, within 24 hours of admission. Note: the use of unfractionated or low molecular-weight heparin in accordance with standard of care for the prevention of DVT, or heparin used to maintain catheter patency, is allowed. Heparin used during mechanical thrombectomy should not exceed 8,000 USP Heparin units unless clinically required for patient safety. Should a subject receive a prohibited medication or undergo a prohibited procedure during the dosing period (Days 1 to 3), the PPI will be consulted as to whether to continue treating the subject with remaining doses or discontinue the subject from the study drug.

Interventions:

No empiric or experimental treatment will be allowed during the study period (90 days). Elective procedures should generally be avoided during the study period, unless required for patient safety.

6 STUDY PROCEDURES

6.1 Schedule of Activities

Procedure	Screen/ Pre- Dose 1	Study Day 1 ⁵	Study Day 2 ⁵	Study Day 3 ⁵	Study Day 4 ⁶	Study Days 5- 6 ⁶	Study Day 7, or Discharge +/-3 days	Study Day 14 +/-4 days	Study Day 30 +/-5 days	Study Day 90 +/-10 days
tPA Administration or Mechanical Thrombectomy ¹	SOC									
Informed Consent ²	X									
Inclusion/Exclusion	X									
Medical History	SOC									
Weight ³	X	X ³								
Acute Blood Pressure Monitoring ⁴	SOC	SOC								
Vital Signs ⁷	SOC	X	X	X	SOC	SOC	X		X	X
Physical Exam	SOC		SOC	SOC	SOC	SOC	X		X	X
Neurologic Exam: NIHSS ⁸	X		X	X	X	X	X		X	X
Modified Rankin Scale	X								X	X
Barthel Index									X	X
Brain Imaging (CT or MRI) ⁹	SOC		SOC							
Brain Imaging (MRI) ¹⁰							X		X	X
Serum Chemistry ¹¹	SOC / X		X	X	X					
CBC ¹²	SOC		X	X	X					

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Procedure	Screen/ Pre- Dose 1	Study Day 1 ⁵	Study Day 2 ⁵	Study Day 3 ⁵	Study Day 4 ⁶	Study Days 5- 6 ⁶	Study Day 7, or Discharge +/-3 days	Study Day 14 +/-4 days	Study Day 30 +/-5 days	Study Day 90 +/-10 days
Coagulation Studies ¹³	SOC	X	X	X						
Urinalysis – Dipstick ¹⁴	X		X	X	X					
Pregnancy Test ¹⁵	X									
ECG	SOC	X ¹⁶								
3KA-APC Plasma Antibody	X							X	X	
Blood for PK		X ¹⁷								
Study Drug Administration ¹⁸		X	X	X						
AE Assessment		X	X	X	X	X	X ²⁰		X ^{19,20} SAEs only	
Con Med Assessment		X	X	X	X	X	X ²⁰		X ^{19,20}	
DLT Assessment Confirmation							X			
Discharge Medications							X			

¹**tPA or Mechanical Thrombectomy:** Subjects will be eligible to consent for this study following initiation of tPA administration or the decision to begin mechanical thrombectomy, whichever occurs sooner.

²**Informed consent:** Informed consent (and re-consent, as applicable) must be obtained by the CSS PI or a qualified designee (licensed physician investigator) listed on the FDA Form 1572. If informed consent was originally provided by a legally authorized representative, and, in the opinion of the CSS PI, the subject's mental status improves sufficiently to enable him/her to provide informed consent, the subject must be re-consented in person at the current or next study visit. Re-consenting once the subject regains capacity is an expected part of Good Clinical Practice (GCP).

³**Weight:** Every effort should be made to obtain an actual pre-dose weight for each subject, 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. For those sites participating in the PK study, an actual weight must also be recorded on the day of PK sampling.

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⁴**Acute Blood Pressure Monitoring:** SOC tPA blood pressure monitoring requires that for the first 24 hours after starting treatment, collection is: every 15 minutes for 2 hours after starting the infusion, then every 30 minutes for 6 hours, then every hour for 18 hours. The eCRF will capture one hourly time point for the first 24 hours. For subjects who undergo mechanical thrombectomy only, one hourly time point for the first 24 hours must be captured.

⁵**Days 1-3:** If a subject is discharged prior to receiving all 5 doses of study drug, then further dosing will be halted and the subject should be seen for Day 7 assessments.

⁶**Day 4-6 Assessments:** Only required if the subject remains inpatient.

⁷**Vital Signs:** Heart rate, respiratory rate, blood pressure, oxygen saturation and temperature should be collected daily during the first 6 days (SOC), and on the Day 7, 30 and 90 visits. In addition, following each 3K3A-APC infusion, vital signs should be collected just prior to and at end of infusion (EOI) and every hour for 4 hours (+/- 30 minutes). Temperature at EOI is not required.

⁸**NIHSS/Days 4-6:** If a subject remains inpatient on Days 4-6, a NIHSS should be collected daily, and any other available data will be collected in the eCRF.

⁹**Brain Imaging:** As per local standard of care, CT or T1 MRI must be performed at baseline and 24-36 hours following tPA bolus administration. Note that MRI may be used in place of CT for the baseline scan only if the site receives prior approval from the CCC.

¹⁰**Brain Imaging:** 1.5T MRI examination to include—at minimum—T1 and T2 weighted images, as well as DWI and SWI sequences must be performed for all subjects on Day 7 (or just prior to discharge, whichever occurs first), Day 30 and Day 90. Changes to the protocol-specified MRI criteria that receive prior approval by the PPI will not be considered protocol deviations. While MRI is preferred at Day 90, should a subject become unable to undergo MRI, a CT scan should be performed.

¹¹**Chemistry:** Perform liver function, glucose and electrolytes.

¹²**CBC:** Perform a complete blood count with platelets; a differential must be performed if the white blood cell count is elevated (above the site's ULN).

¹³**Coagulation:** Perform aPTT, PT, INR and fibrinogen. For each 3K3A-APC dose, samples should be drawn and processed pre-dose (window varies; see below) and 1 hour post-dose (+/- 15 minutes). For doses 2-5 the pre-dose window is within 60 minutes prior to the start of the infusion. For dose 1 however, note that some of these pre-dose assessments are required for screening and they should all be prior to tPA (in those subjects who receive tPA). Therefore, the pre-dose draw for dose 1 may be more than 60 minutes prior to the infusion. If the pre-dose samples for dose 1 are drawn after tPA they should not be drawn until at least 20 minutes has passed since tPA finished so as to minimize tPA's effect on the laboratory results.

¹⁴**Urinalysis:** Microscopic urinalysis must be performed if dipstick is positive for RBCs.

¹⁵**Pregnancy Test:** Serum or urine pregnancy test for women of child-bearing potential only.

- ¹⁶ **ECG:** One post-dose ECG should be collected at 'end of infusion' (Cmax) (+ 5 minutes). The Investigator can decide which dose, 1-5, will be used for ECG collection.
- ¹⁷ **PK Samples:** PK samples will be collected from subjects at a subset of participating sites; samples will be collected following one of the doses of 3K3A-APC at the following time points: end of infusion, 20, 40, 60 and 80 minutes post-dose. The Investigator can decide which dose, 2-5, will be used for PK collection.
- ¹⁸ **Study Drug Administration:** 3K3A-APC (or placebo) will be administered as a 15-minute infusion every 12 hours for 5 doses, or until discharge. The first dose of study drug (or placebo) should be started 30 to 120 minutes following completion of tPA or initiation of mechanical thrombectomy (arterial puncture), whichever occurs sooner. Subsequent doses should be given every 12 hours (+/- 1 hour) for 5 total doses (or until discharge). Dose 1 must be confirmed in the data system within 2 hours of infusion time.
- ¹⁹ **SAE and Con Med Collection after Day 7:** SAEs will be collected and reported out to *actual* study Day 30. Should a SAE be reported between Day 7 and Day 30, then all Con Meds through Day 30 need to be reported in the eCRF.
- ²⁰ **Ensuring Complete Data Collection:** Should the Day 7 or Day 30 visit occur on the early side of the allowed window, a phone call is required and should be documented to ensure that all AEs are captured through actual Day 7, and SAEs through actual Day 30, along with the required concomitant medications.

6.2 Schedule of Activities: Study Drug Infusion Detail

Procedure	Dose 1			Doses 2-5 ⁵		
	Pre	During	Post	Pre	During	Post
tPA Administration or Mechanical Thrombectomy ¹	SOC					
Informed Consent ²	X					
Inclusion/Exclusion	X					
Medical History	SOC					
Weight ³	X		X	X ³ 1x, PK day only		
Acute Blood Pressure Monitoring ⁴	SOC	SOC	SOC			
Vital Signs ⁶	SOC/ X		X	X		X
Physical Exam	SOC			SOC 1/day		
Neurologic Exam: NIHSS ⁷	X			X ⁷ 1/day unless clinically indicated		
Modified Rankin Scale	X					
Barthel Index						
Brain Imaging (CT or MRI) ⁸	SOC			SOC 1x only, 24-36 hrs post tPA		
Serum Chemistry ⁹	SOC / X			X 1/day		
CBC ¹⁰	SOC			X 1/day		
Coagulation Studies ¹¹	SOC		X	X		X
Urinalysis – Dipstick ¹²	X			X 1/day		
Pregnancy Test ¹³	X					
ECG	SOC		X ¹⁴ 1 dose only			X ¹⁴ 1 dose only
3KA-APC Plasma Antibody	X					
Blood for PK						X ¹⁵ 1 dose only

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Procedure	Dose 1			Doses 2-5 ⁵		
	Pre	During	Post	Pre	During	Post
Study Drug Administration ¹⁶		X			X	
AE Assessment		X			X	
Con Med Assessment		X			X	

¹ **tPA or Mechanical Thrombectomy:** Subjects will be eligible to consent for this study following initiation of tPA administration or the decision to begin mechanical thrombectomy, whichever occurs sooner.

² **Informed consent:** Informed consent (and re-consent, as applicable) must be obtained by the CSS PI or a qualified designee (licensed physician investigator) listed on the FDA Form 1572. If informed consent was originally provided by a legally authorized representative, and, in the opinion of the CSS PI, the subject's mental status improves sufficiently to enable him/her to provide informed consent, the subject must be re-consented in person at the current or next study visit. Re-consenting once the subject regains capacity is an expected part of Good Clinical Practice (GCP)

³ **Weight:** Every effort should be made to obtain an actual pre-dose weight for each subject, 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. For those sites participating in the PK study, an actual weight must also be recorded on the day of PK sampling.

⁴ **Acute Blood Pressure Monitoring:** SOC tPA blood pressure monitoring requires that for the first 24 hours after starting treatment, collection is: every 15 minutes for 2 hours after starting the infusion, then every 30 minutes for 6 hours, then every hour for 18 hours. The eCRF will capture one hourly time point for the first 24 hours. For subjects who undergo mechanical thrombectomy only, one hourly time point for the first 24 hours must be captured.

⁵ **Day 3:** If a subject is discharged prior to receiving all 5 doses of study drug, then further dosing will be halted and the subject should be seen for Day 7 assessments.

⁶ **Vital Signs:** Heart rate, respiratory rate, blood pressure, oxygen saturation and temperature should be collected daily during the first 6 days (SOC). In addition, following each 3K3A-APC infusion, vital signs should be collected just prior to and at end of infusion (EOI) and every hour for 4 hours (+/- 30 minutes). Temperature at EOI is not required.

⁷ **NIHSS:** A NIHSS should be collected daily, or more frequently when clinically indicated.

⁸ **Brain Imaging:** As per local standard of care, CT or T1 MRI must be performed at baseline and 24-36 hours following tPA bolus administration. Note that MRI may be used in place of CT for the baseline scan only if the site receives prior approval from the CCC.

⁹ **Chemistry:** Perform liver function, glucose and electrolytes.

¹⁰ **CBC:** Perform a complete blood count with platelets; a differential must be performed if the white blood cell count is elevated (above the site's ULN).

¹¹ **Coagulation:** Perform aPTT, PT, INR and fibrinogen. For each 3K3A-APC dose, samples should be drawn and processed pre-dose (window varies; see below) and 1 hour post-dose (+/- 15 minutes). For doses 2-5 the pre-dose window is within 60 minutes prior to the start of the infusion. For dose 1 however, note that some of these pre-dose assessments are required for screening and they should all be prior to tPA (in those subjects who receive tPA). Therefore, the pre-dose draw for dose 1 may be more than 60 minutes prior to the infusion. If the pre-dose samples for dose 1 are drawn after tPA they should not be drawn until at least 20 minutes has passed since tPA finished so as to minimize tPA's effect on the laboratory results.

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- ¹² **Urinalysis:** Microscopic urinalysis must be performed if dipstick is positive for RBCs.
- ¹³ **Pregnancy Test:** Serum or urine pregnancy test for women of child-bearing potential only.
- ¹⁴ **ECG:** One post-dose ECG should be collected at 'end of infusion' (C_{max}) (+ 5 minutes). The Investigator can decide which dose, 1-5, will be used for ECG collection.
- ¹⁵ **PK Samples:** PK samples will be collected from subjects at a subset of participating sites; samples will be collected following one of the doses of 3K3A-APC at the following time points: end of infusion, 20, 40, 60 and 80 minutes post-dose. The Investigator can decide which dose, 2-5, will be used for PK collection.
- ¹⁶ **Study Drug Administration:** 3K3A-APC (or placebo) will be administered as a 15-minute infusion every 12 hours for 5 doses, or until discharge. The first dose of study drug (or placebo) should be started 30 to 120 minutes following completion of tPA or initiation of mechanical thrombectomy (arterial puncture), whichever occurs sooner. Subsequent doses should be given every 12 hours (+/- 1 hour) for 5 total doses (or until discharge). Dose 1 must be confirmed in the data system within 2 hours of infusion time.

6.3 Timing of Study Activities

6.3.1 Screening/Pre-Randomization Evaluations/procedures

Screening procedures are described in Section [4.4](#).

6.3.1.1.1 Admission/Standard of Care Procedures

The following procedures will be performed upon arrival of the stroke team (those marked with an asterisk* will be collected in accordance with standard of care and are expected to be performed prior to tPA, mechanical thrombectomy and informed consent):

- Medical history and PE*
- Assessment of Baseline Medications (all medications taken within 3 days prior to tPA administration)*
- Vital signs (blood pressure, heart rate, temperature, respiratory rate, oxygen saturation)*
- Body weight*
- Non-contrast enhanced head CT* or MRI (if preapproved)
- Neurological examination: NIHSS*
- 12-lead electrocardiogram (ECG)*

Laboratory Tests:

- CBC (complete blood count and platelets)*
- Chemistries (liver function, glucose and electrolytes)*
- Coagulation (PT, aPTT, INR, fibrinogen)*
- Urinalysis (dipstick or microscopic, per SOC)*

Patients who meet the criteria for tPA administration or mechanical thrombectomy or both (see Section 5.5.1) will be considered for participation in this protocol. The administration of tPA should follow the American Heart Association's national accepted published guidelines¹⁴. Use of mechanical thrombectomy should follow the local Institutional protocol, and should only include the use of FDA-approved thrombectomy devices. Note that MRI may be used in place of CT for standard of care imaging prior to tPA/mechanical thrombectomy only if the site receives prior approval from the CCC.

The AHA Guidelines recommend assessing PTT, INR, and fibrinogen prior to administering tPA¹⁴. Eligibility for enrollment in this study is based on PTT and INR levels. So as to have an accurate reflection of the subject's true baseline, the pre-dose 1 blood draws for coagulation assessments should occur as SOC prior to tPA for patients receiving tPA. If that is not done for some reason, then the pre-dose 1 blood draw should occur at least 20 minutes after tPA completion, in order to minimize tPA's effect on the levels.

6.3.1.2 Informed Consent

Once the patient begins tPA administration or the decision to begin mechanical thrombectomy is made, whichever occurs sooner, he/she can be presented with the consent form and evaluated for study enrollment. Refer to Section [4.4.3](#) for details on the consenting process.

6.3.1.3 Slot Assignment and Randomization

Following consent of the subject, or his/her legally authorized representative, the following assessments should be performed:

- Estimated pre-stroke mRS (to determine study eligibility)
- A pregnancy test in women of child bearing potential (serum or urine, per Investigator's discretion) will be required to qualify the eligibility of female patients.
- 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.

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; randomization will be managed electronically via the IWRS. The Investigator, or designee, should randomize the subject in the IWRS to receive the unique subject number, which will also be linked to the allocated treatment assignment (blinded to clinical staff). Subjects who will receive mechanical thrombectomy only should be randomized close to the start of the procedure (arterial puncture) to ensure that they continue to meet eligibility.

The unblinded pharmacist(s) will receive the treatment assignment from the IWRS that will be used to prepare the investigational treatment as described in Section [5.3](#).

It is important to confirm eligibility again close to investigational dose 1 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.

If the new NIHSS score has improved such that the subject is no longer eligible (repeat NIHSS<5) do not administer study drug. Submit the termination form and other required documents so that this subject can be replaced in the cohort. Refer to the NN104 'Manual of Operations' for more detail. Randomized subjects who experience significant neurologic improvement (defined as a NIHSS score <5) prior to receipt of study drug will be termed 'Early Responders' and will not be included in the study. 'Early Responders' will not be followed, and will be replaced in the CRM. Any subject who receives any amount of study drug will be considered "Dosed" and will be followed to the end of the study.

6.3.2 On-Study Evaluations/procedures

6.3.2.1.1 Study-Specific Baseline Procedures

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Once a subject has been randomized into the study, the following will be performed prior to receipt of the investigational treatment:

- Chemistry (liver function, if not obtained during SOC pre-tPA work-up)
- Blood for serum antibody sample ('pre-dose')
- 'Pre-dose' vital signs (blood pressure, heart rate, temperature, respiratory rate, oxygen saturation)

6.3.2.1.2 Study-Specific Post-Treatment Procedures

Investigational Dose #1:

The first investigational dose of study drug should be started 30 to 120 minutes following completion of tPA or initiation of mechanical thrombectomy (arterial puncture), whichever occurs sooner.

After administration of 100 mL of investigational treatment, the following should be performed:

- Vital signs (blood pressure, heart rate, temperature, respiratory rate, oxygen saturation) should be collected at end of infusion (EOI) and approximately every hour (+/- 30 minutes) for 4 hours, then per local SOC. Temperature at EOI is not required.

NOTE: the NINDS tPA protocol should guide vital sign collection following tPA administration; therefore, actual collection may be more frequent than what will be captured for this study protocol. The eCRF will capture one hourly time point for the first 24 hours. For subjects who undergo mechanical thrombectomy only, one hourly time point for the first 24 hours must be captured.

- Blood for coagulation (PT, aPTT, INR, fibrinogen) should be collected 1 hour after EOI (+/- 15 minutes).
- One post-dose ECG should be collected at EOI (C_{max}) (+ 5 minutes). The Investigator can decide which dose, 1-5, can be used for ECG collection. Since the ECG collection is flexible, it should be planned well in advance so that it is collected as close to EOI as possible. Should the ECG window be missed, it is preferred to defer the collection to the next planned investigational dose.

Investigational Doses #2-5:

Subjects should receive 100 mL of investigational treatment every 12 hours (+/- 1 hour) for up to 5 total doses (refer to section [5.4](#)). Subjects will only receive investigational treatment for as long as they remain as an inpatient. The following should be performed:

- Vital signs should be collected just prior to infusion, at EOI and approximately every hour (+/- 30 minutes) for 4 hours, then per local SOC
- Blood for coagulation (PT, aPTT, INR, fibrinogen) should be collected from the arm opposite the infusion:
 - Prior to each infusion (within 60 minutes of infusion)

- 1 hour after EOI (+/- 15 minutes)
- If not collected previously, one post-dose ECG should be collected at EOI (C_{max}) (+ 5 minutes). The Investigator can decide which dose, 1-5, can be used for ECG collection. Since the ECG collection is flexible, it should be planned well in advance so that it is collected as close to EOI as possible. Should the ECG window be missed, it is preferred to defer the collection to the next planned investigational dose.

Investigational Dose #2-5 - Pharmacokinetics (performed at a sub-set of sites):

Blood for plasma PK samples must be collected from the arm opposite the infusion:

- EOI (“post-dose: 0”)
- 20 minutes after EOI (“post-dose: 20m”)
- 40 minutes after EOI (“post-dose: 40m”)
- 60 minutes after EOI (“post-dose: 60m”)
- 80 minutes after EOI (“post-dose: 80m”)

The Investigator can decide which dose, 2-5, will be used for PK collection. **PK samples should only be collected following one dose of study drug.**

6.3.2.1.3 Study-Specific Daily Procedures, Days 2-6

Study Day 1 will be defined as the first 24-hour period during which Infusion #1 is given. For example, if admission occurs at 11:55 PM and the first investigational infusion occurs at 1:00 AM, then Day 1 will begin at 12:00 AM, and admission would be considered Day 0. Every subject enrolled will have a different investigational treatment schedule based on the time of tPA or mechanical thrombectomy and the subsequent first dose of 3K3A-APC or placebo. Procedures that center around the administration of investigational product may not fall on a specific study day, and therefore are outlined in section [6.3.2.2](#). The following procedures should be performed daily, or as indicated:

Daily

- PE (only notable changes from baseline must be recorded)
- Assessment of AEs
- Assessment of Concomitant Medications
- Vital signs, and as indicated in section [6.3.2.2](#) around investigational treatment
- Neurological examination: NIHSS

Laboratory Tests:

- CBC (complete blood count and platelets), Days 2-4 only; a differential must be performed if the white blood cell count is elevated.
- Chemistries (liver function, glucose and electrolytes), Days 2-4 only

- Urinalysis (dipstick or microscopic), Days 2-4 only; microscopic urinalysis must be performed if dipstick is positive for RBCs.

Once Only

- Non-contrast enhanced head CT or T1 MRI, per SOC, 24-36 hours following tPA bolus administration.

NOTE: If a CSS uses MRI as SOC at this time point, it is encouraged that the MRI protocol for this study be used. Refer to the 'Imaging Plan' for details.

6.3.2.1.4 Study-Specific Procedures, Day 7 or Discharge (+/- 3 day window)

- PE (only notable changes from baseline must be recorded)
- 1.5T MRI: T1 and T2 weighted images, as well as DWI and SWI sequences
- Assessment of AEs (through *actual* Day 7 only)
- Assessment of Concomitant Medications (through *actual* Day 7 only)

NOTE: Should the Day 7 visit occur on the early side of the allowed window, a phone call is required and should be documented to ensure all AEs are captured through actual Day 7 along with the required concomitant medications. Concomitant medications must also be collected through Day 30 only when a SAE occurs on or before actual Day 30.

- Assessment of Discharge Medications (medications that the subject is sent home with when discharged from the hospital)
- Vital signs (blood pressure, heart rate, temperature, respiratory rate, oxygen saturation)
- Neurological examination: NIHSS

6.3.2.1.5 Study-Specific Procedures, Day 14 (+/- 4 day window)

- Blood for serum antibody sample ('post-dose D14')

NOTE: Every effort should be made to ensure the subject is seen on Day 14 in order to collect the antibody sample. The CSS coordinator should contact the CCC to discuss alternative options available to sites for subjects who are unable to return to the CSS.

6.3.2.2 Study-Specific Procedures, Day 30 (+/- 5 day window)

- PE (only notable changes from baseline must be recorded)
- 1.5T MRI: T1 and T2 weighted images, as well as DWI and SWI sequences
- Assessment of SAEs (through *actual* Day 30 only)
- If a SAE is reported, assessment of Concomitant Medications

NOTE: Should the Day 30 visit occur on the early side of the allowed window, a phone call is required and should be documented to ensure all SAEs are captured through actual Day 30 along with the required concomitant medications.

- Vital signs (blood pressure, heart rate, temperature, respiratory rate, oxygen saturation)
- Neurological examination: NIHSS
- mRS
- BI
- Blood for serum antibody sample ('post-dose D30')

6.3.2.3 Study-Specific Procedures, Day 90 (+/- 10 day window)

- PE (only notable changes from baseline must be recorded)
- Vital signs (blood pressure, heart rate, temperature, respiratory rate, oxygen saturation)
- Neurological examination: NIHSS
- mRS
- BI
- 1.5T MRI: T1 and T2 weighted images, as well as DWI and SWI sequences

NOTE: While MRI is preferred at Day 90, should a subject become unable to undergo MRI, a CT scan should be performed.

6.4 Special Instructions and Definitions of Evaluations

6.4.1 Protocol Deviations and Violations

The site monitor is responsible for collection of all protocol deviations that occur during the course of the study to ensure proper reporting in the clinical study report.

Generally, any activity that is not performed as outlined in the protocol should be categorized as a 'Protocol Deviation'. Missed visits and any procedures not performed (not attempted) for reasons other than illness, injury or progressive disability (i.e., subject is physically unable to perform test) will be reported as protocol deviations. Procedures or visits not performed due to illness, injury or disability, including procedures that were attempted but failed (i.e., blood samples unable to be drawn after multiple attempts, or weight unable to be obtained due to subject immobility) will not be reported as protocol deviations. Deviations fall into two categories, 'major' and 'minor', and are defined as follows:

Major: Any deviation that impacts safety, welfare, or rights of a study subject, or impacts the primary outcome of the study (safety assessments). Examples include: Informed Consent issues affecting a subject's rights, Inclusion/Exclusion violations, Missed Assessments affecting safety endpoints, Study Drug Administration that affects a study endpoint or the subject's welfare. A major deviation is also termed a protocol violation.

Minor: Any deviation that does not fall within the above category. Examples include: Out of Window or Missed study assessments that do not affect a study endpoint or the subject's welfare.

6.4.2 Medical History

The subject's medical history should include the standard intake history as well as all AEs occurring after signing of the informed consent form, but before the first dose of study drug.

6.4.3 Treatment History

All treatments, including administration of tPA or use of thrombectomy devices, will be recorded in the subject's medical record (source documentation) and in the eCRF. Baseline medications will be recorded 3 days prior to tPA or mechanical thrombectomy through just prior to first administration of study drug.

6.4.4 Concomitant Medications/Treatments

All medications taken following the first administration of study drug through *actual* Day 7 will be recorded. If a subject experiences a SAE between Day 7 and Day 30, then concomitant medications should also be captured through *actual* Day 30.

6.4.5 Discharge Medications

Discharge medications will be recorded in the medical record and captured in the eCRF and should include all medications that the subject is sent home with when discharged from the hospital.

6.4.6 Protocol Amendments and Study Termination

All revisions and/or amendments to this protocol must be approved in writing by the Sponsor and the CIRB. The Investigator will not make any changes to the conduct of the study or the protocol without first obtaining written approval from the Sponsor and the CIRB, except where necessary to eliminate an apparent immediate hazard to a study subject.

The Sponsor and NeuroNEXT Network reserve the right to discontinue the study at a clinical study site(s) for safety or administrative reasons at any time. Should the study be terminated and/or the clinical study site closed for any reason, all documentation and study drug pertaining to the study must be returned to the Sponsor or its representative.

6.4.7 Special Blood Sample Collection and Handling

6.4.7.1 Pharmacokinetic Sample Collection and Handling (Plasma)

PK samples will be collected from approximately 40 subjects at a sub-set 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: EOI and 20, 40, 60, 80 minutes after the EOI (5 total samples per subject). The Investigator can decide which 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.**

Collection:

- Approximately 4 mL of blood will be collected into Sponsor-provided tubes containing sodium citrate and the serine protease inhibitor benzamidine.
- After blood collection, invert gently 8-10 times; samples will be held at room temperature pending centrifugation.

Processing:

- To separate plasma, blood samples will be centrifuged at room temperature within 10 – 30 minutes at 3000 rpm (2000-4000 x g) for 10 minutes.
- The plasma will be removed from the blood, split into 2 equal aliquots, and stored in 2 mL labeled Cryotube™ containers.
- Plasma will immediately be placed in a temperature-controlled (with recordings) and alarmed $\leq -70^{\circ}\text{C}$ freezer.

Storage:

- Samples will be stored in a $\leq -70^{\circ}\text{C}$ freezer at the Investigational site.

Shipping:

- Frozen samples will be batch shipped on dry ice to the central laboratory at the University of Rochester for storage. Pre-printed air bills and temperature-validated packaging will be provided by the central laboratory. Primary and back-up aliquots should always be shipped separately in order to mitigate against loss. Refer to the 'Laboratory Manual' for additional detail on the shipping frequency of samples.

Analysis:

- PK analysis will occur at [REDACTED]. An enzyme capture immunoassay method has been developed and validated by [REDACTED] for determination of 3K3A-APC activity in plasma. Samples collected for PK will only be used for PK-related analysis and method development.

6.4.7.2 Blood Sample Collection and Handling for Antibody Testing (Serum)

Blood samples for anti-3K3A-APC antibody analysis will be collected from all subjects just prior to receiving the first dose of investigational treatment and again at the Day 14 and Day 30 visits (3 total samples per subject).

Collection:

- Approximately 2-3 mL of blood will be collected into standard clot tubes that do not contain anticoagulant.
- After blood collection, samples will be held at room temperature for at least 20 minutes to allow clotting prior to centrifugation. **However please note that samples must be processed within 60 minutes of collection time and divided serum must be stored in a $\leq -70^{\circ}\text{C}$ freezer within 2 hours of collection time.**

Processing:

- To separate serum, blood samples will be centrifuged at 1500 - 2200 rpm (1500 x g) at $2^{\circ}\text{-}8^{\circ}\text{C}$ for 10-15 minutes.
- The serum will be removed from the blood, split into 2 equal aliquots, and stored in 2 mL labeled Cryotube™ containers.

- Serum will be immediately placed in a temperature-controlled (with recordings) and alarmed $\leq -70^{\circ}\text{C}$ freezer within 2 hours of collection.

NOTE: Subjects unable to return to the study site may have blood drawn at an outside medical facility or at home. Serum collected outside a medical facility may be placed in a wet ice slurry for 2-3 minutes and centrifuged at room temperature at 1500 - 2200 rpm (1500 x g) for 10-15 minutes. After aliquoting, the serum must be placed on dry ice for transport or shipping to the study site.

Storage:

- Samples will be stored in a $\leq -70^{\circ}\text{C}$ freezer at the Investigational site.

Shipping:

- Frozen samples will be batch shipped on dry ice to the central laboratory at the University of Rochester for storage. Pre-printed air bills and temperature-validated packaging will be provided by the central laboratory. Primary and back-up aliquots should always be shipped separately in order to mitigate against loss. Refer to the 'Laboratory Manual' for additional detail on the shipping frequency of samples.

Analysis:

- Antibody analysis will occur at [REDACTED]. A qualitative enzyme-linked immunosorbent assay (ELISA) method has been developed and validated by [REDACTED] for anti-3K3A-APC antibody detection. Samples collected for anti-drug antibody analysis will only be used for antibody-related analysis and method development.

6.4.8 Questionnaire Certification

Investigators and designated study staff will be trained and certified to use the NIHSS and the mRS, using a recognized certifying agency.

6.4.9 Imaging Central Review

A central reader will review all scans for each subject who receives at least one infusion of study drug. Five scans per subject are expected, including two standard of care scans and three study mandated scans. Please refer to the 'Imaging Plan' for details.

7 MANAGEMENT OF ADVERSE EXPERIENCES

Expected AEs due to stroke can be estimated from the Virtual International Stroke Trials Archive (VISTA)⁴⁹. In the VISTA dataset, 5775 placebo-treated patients were analyzed. One-hundred-thirty-two (132) of 756 types of AEs accounted for 82.7% of reported events. Thirty-five (35) of the most common types of SAEs are listed below:

Stroke in evolution	11.2% ¹
Pneumonia	5.6%
Ischemic cerebral infarction	4.8%
Brain edema	4.3%
Cardiac failure	3.4%

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Myocardial infarction	2.9%
Cerebral hemorrhage	2.5%
Aspiration pneumonia	2.5%
Respiratory failure	2.2%
Hemorrhagic transformation	2.2%
Pulmonary embolism	2.0%
Atrial fibrillation	1.8%
Urinary tract infection	1.7%
Pulmonary edema	1.5%
Cerebrovascular disorder	1.5%
Cardiac arrest	1.5%
Carotid artery disease	1.4%
Sepsis	1.3%
Angina pectoris	1.2%
Hypotension	1.1%
Coma	1.1%
Pyrexia	1.1%
Renal failure	1.0%
Headache	0.9%
ICP increased	0.9%
Respiratory tract infection	0.9%
Bradycardia	0.8%
Transient Ischemic Attack	0.8%
Hypertension	0.8%
Hemorrhagic Cerebral Infarction	0.7%
Somnolence	0.7%
Cerebral Incarceration	0.6%
Deep vein thrombosis	0.6%
Syncope	0.5%
Depression	0.5%

¹Shown as percentage of total SAE occurrence; 53 types of SAEs in total were identified.

Bacterial pneumonia is one of the most common causes of death in acute stroke patients. Reduction of bulbar reflexes, drowsiness, dysphagia, and subsequent aspiration are considered to be major contributors to the high incidence of bacterial pneumonia after stroke.⁵⁰ Recent studies in patients with moderate to severe stroke found pneumonia rates between 10.7 and 21%.⁵¹⁻⁵³ Whether or not the increase in pneumonia rate affects 3-month outcome after stroke is controversial.⁵⁴ Symptomatic ICH occurs in about 6% of patients treated with tPA¹³.

8 STATISTICAL CONSIDERATIONS

The NeuroNEXT Data Coordinating Center will develop a Statistical Analysis Plan (SAP) in collaboration with the protocol principal Investigator and protocol steering committee.

8.1 General Design Issues

8.1.1 Summary of Study Design

This is a multicenter, prospective, randomized, controlled, double-blinded Phase 2 study intended to evaluate the safety, PK, and preliminary efficacy of 3K3A-APC following administration of tPA, or mechanical thrombectomy or both, in patients with moderate to severe acute ischemic stroke. The original version of the CRM method proposed enrolling one subject at a time⁵⁵. However, others have shown that the performance of the CRM may be improved by enrolling cohorts instead of a single subject¹. Correspondingly, this study will utilize a modified version of the CRM in order to establish a MTD.

The study will enroll approximately 115 subjects, which includes the planned 88 subjects in groups of four (each cohort will include one placebo and three treated subjects) and the additional placebo subjects who may be enrolled during safety review pauses. While placebo is not needed to determine the MTD, a placebo group has been included in order to conduct secondary analyses to examine for a reduction of pre-clinical tPA/mechanical thrombectomy-related bleeds by central read (it is predicted from studies that treatment reduces thrombolysis-related petechial 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,' subjects whose symptoms resolve between initial randomization and initiation of IMP infusion such that they are no longer eligible (repeat NIHSS <5), will be removed from the study and replaced. 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.

Following completion of tPA infusion or initiation of mechanical thrombectomy (arterial puncture), whichever occurs 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 3K3A-APC every 12 hours (+/- 1 hour) for up to 5 total infusions. Four dose levels will be considered for this study: 120, 240, 360, and 540 µg/kg. For the purposes of this study, we assume an established background symptomatic intracerebral hemorrhage (SICH) rate of 3-6%²⁻⁷. Correspondingly, the MTD will be defined as 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

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dose and one to placebo). Subjects will generally be enrolled at the dose estimated from the assumed dose-response model and prior data to be closest to the MTD. However, the initial cohort will start at the lowest dose level (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.

Prior to starting the CRM, based on a best estimate from the literature and previous studies, the four dose levels will be assigned prior probabilities as indicated in Table 3 below.

Table 3. Assumed prior probabilities of toxicity at four dose levels considered for the study:

Dose Level	120	240	360	540
Prior Probability of Toxicity	5%	7%	9%	11%

When designing a CRM, O’Quigley, Pepe and Fisher recommend using a single-parameter dose-response model $\psi(x_i^*, a)$, which is probability of toxicity at dose x_i^* for some unknown parameter, a , which defines the shape of the dose-toxicity function. For this study, a hyperbolic tangent dose-response model will be used, in which

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

After each group of subjects has been treated, and their status observed as to whether or not each had a DLT, the posterior mean of a will be calculated. Based upon that estimate, \hat{a} , the highest dose with estimated toxicity probability, $\psi(x_i^*, a)$ less than or equal to 10% will be allocated to the next group of subjects needing treatment assignment. If this dose is more than one level higher than the previous dose allocated to a cohort, the dose will only be increased one level. After the final group of subjects is enrolled, the final MTD will be defined as the highest dose with estimated toxicity probability less than or equal to the target toxicity level of 10%.

Hence, the design 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 study definition. 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 (i.e. two or more doses will need to be administered to be included).
 - Based upon the observed information from the three treated subjects, 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.)

The MTD will be defined as the dose that would be chosen from the CRM at the final step. 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 highest dose the MTD)

Through simulation it was determined that it would be 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 stopping early would be justified.

Non-serious adverse events (AEs) will be collected through actual Day 7. More specifically, subjects will be evaluated for DLTs from administration of the first dose to 48 hours following the last dose of study treatment. Blood samples for PK will be collected from approximately 40 subjects at a sub-set of study sites 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. Subjects must be seen for assessments on Days 7, 14, 30 and 90. MRI scans, including SWI sequences, will be obtained at 7, 30 and 90 days after stroke.

A 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. Assignment of the dose for a cohort will not occur until all potential DLTs have been reviewed by the SRC..

8.1.2 Randomization and Blinding

The NeuroNEXT IWR system will be used to randomize subjects according to the scheme pre-defined by the DCC and will also serve to inform the unblinded study pharmacist of the treatment assignment. Subjects will be randomized to either 3K3A-APC or placebo. There are 22 groups of four subjects planned, but fewer may be enrolled should the study meet either of the early stopping criteria. Additionally, if two consecutive cohorts are in the DLT review period, subjects will be

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assigned to placebo. The additional placebo subjects will be closely monitored and their enrollment may be discontinued should the number enrolled exceed what was planned for the study. Subjects will not be considered part of the ITT cohort until they receive any amount of 3K3A-APC or placebo. For example, 'Early Responders,' subjects whose symptoms resolve between initial randomization and initiation of IMP infusion such that they are no longer eligible (repeat NIHSS <5), will be removed from the study and replaced. 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 (i.e. two or more doses will need to be administered to be included).

8.2 Outcomes

8.2.1 Primary Outcome (Including Definition)

The primary objective of the study is to evaluate the safety of multiple ascending intravenous (IV) doses of 3K3A-APC following tPA administration or mechanical thrombectomy or both in subjects who have experienced moderate to severe acute 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 3K3A-APC (unless specified below), and will be 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 images that is associated with clinical worsening that meets the definition of neuroworsening (4 or more point increase in NIHSS; see section 9.4.1.3 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.
- Laboratory findings that meet all of the following three components (Hy's Law):
 - $\geq 3x$ 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. Refer to CTCAE v4.03 sections relevant to laboratory investigations.
- Any adverse event that in the opinion of the Investigator is related to study treatment and leads to cessation of further dosing.

NOTE: All suspected DLTs will be reviewed by a Safety Review Committee, and those 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 dose 1 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 tPA, and there is a documented rate of occurrence of 11% in subjects receiving tPA⁸. 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.

8.2.2 Secondary Outcome(s)

Secondary Outcome #1 (PK Properties): One secondary objective of this study is to investigate the PK properties of 3K3A-APC following tPA administration in stroke subjects. 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. The mean +/- standard error plasma concentration over time by treatment group will be plotted on linear and semi-logarithmic axes. 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 end of infusion.

Secondary Outcome #2 (Bleed Rates): Another secondary objective of the study is 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 1.5T MRI at Day 30. Each patient will have a MRI examination to include—at minimum—T1 and T2 weighted images, as well as diffusion weighted imaging (DWI) and susceptibility weighted imaging (SWI) sequences. Changes to the protocol-specified MRI criteria that receive prior approval by the PPI will not be considered protocol deviations. Since patients will not necessarily have a baseline MRI, post-recanalization microbleeds—defined as hypointensities less than 5mm in diameter seen on SWI—will be counted as tPA/mechanical thrombectomy-related only if found within the ischemic territory⁵⁶. All other areas of hypointensity on SWI larger than 5mm diameter will be counted as tPA/mechanical thrombectomy-related regardless of the territory in which they are found.

Exploratory Outcomes: This 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. While the sample size is too small to observe meaningful treatment effects, the data allow confirmation that outcomes in this trial resemble previously published trials. The following will be collected:

- Volume of bleeding (hemorrhage and microbleeds) in the brain as determined by MRI at Day 30
- Incidence of subarachnoid hemorrhage in subjects who receive mechanical thrombectomy
- 7-day National Institutes of Health Stroke Scale (NIHSS) scores
- 90-day modified Rankin Scale (mRS)
- 90-day Barthel Index (BI)
- Infarct volume at 90 days (MRI, or CT if unable to obtain MRI)
- Pre-Dose 1, Day 14 and Day 30 anti-drug antibody samples

8.3 Sample Size and Accrual

8.3.1 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. 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 probably is above the target threshold for all doses).

Table 4. Assumed True Toxicity Probabilities for Simulation Scenarios

Scenario	Description	Dose Level			
		120	240	360	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 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 do 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 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 modified CRM approaches generally have better performance with respect to successfully recommending the correct dose as the MTD (based on prior assumptions), and are well suited to achieve the goals of this study. The modified CRM being used here performs no 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 weighed 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

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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” be enrolled.

8.3.2 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 suggests that the MRI-detected bleed rate for tPA-treated subjects should be approximately 30%-40%⁵⁶⁻⁶². Furthermore, it is expected from pre-clinical studies that treatment with APC could lead to a rather dramatic decrease in bleed rates for 3K3A-treated subjects^{31,44,63}. Pre-clinical data suggest that 3K3A-APC could reduce bleeding seen after IV tPA or after mechanical thrombectomy^{44,63}. 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%, & 40%
- 3K3A-APC treated group bleed rates of 0.5%, 5%, & 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, and 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.

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Table 6. Power for Comparing Bleed Rates (Assumes Chi-Square Test, Significance Level = 0.05, and 75% of Subjects Assigned to Treatment)

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%

8.4 Data Monitoring

All aspects of the study will be monitored by qualified individuals designated by the sponsor. Monitoring will be conducted according to Good Clinical Practice and applicable government regulations. The Investigator agrees to provide monitors access to the clinical supplies, dispensing and storage areas, and to the clinical files of the study subjects, and, if requested, agrees to assist the monitors. The site will provide the monitor adequate working space and full access to the medical record. If the site uses an electronic medical record, the monitor will be provided a temporary password and access to one patient chart, or, the site coordinator will be available throughout the monitoring visit to provide access. The site PI will be available for an exit visit with the monitor.

Safety monitoring will include careful assessment and appropriate reporting of adverse events. Medical monitoring will include contemporaneous assessment of serious adverse events.

The monitoring of subject safety and data quality will follow the NINDS Guidelines for Data and Safety Monitoring in Clinical Trials. A Data and Safety Monitoring Board (DSMB) appointed by the 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. This committee will monitor rates of adverse events and endpoints in the trial and will monitor the performance of the trial. The frequency and format of DSMB meetings, reports, and guidelines for interim analysis will be agreed upon prior to study subject enrollment during the first meeting of the DSMB with the representatives of the DCC. The DSMB will review the data seen by the SRC during their deliberations of dose escalation steps.

The Protocol PI will appoint an Independent Medical Monitor (IMM) to review all adverse events in a blinded real-time fashion in order to monitor for AEs that could meet the definition of a DLT. Should a DLT be reported or suspected, the IMM will notify the SRC, which will be convened to review the AE(s). In addition, the IMM will review all events that meet the regulatory definition of a Serious Adverse Event, upon receipt of notification via the Electronic Data Capture (EDC).

An adverse event (AE) is any untoward medical 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. Subjects who receive investigational treatment will be monitored for AEs from the time they sign consent until 7 days for AEs and 30 days for SAEs following permanent discontinuation of study drug. At that point, all ongoing AEs will be followed to resolution, or until Day 90, but no new AEs will be recorded. The IMM/DSMB will review cumulative AEs; the frequency of this review will be determined by the IMM/DSMB in conjunction with the Protocol PI.

Each Clinical Study Site Principal Investigator (CSS PI) and research team (co-Investigators, research nurse, clinical trial coordinator) are responsible for identifying and reporting AEs. The CSS PI is responsible for determining the relationship of the event to the study drug/study procedures. Aggregate reports blinded by treatment group, detailed by severity, attribution (expected or unexpected), and relationship to the study drug/study procedures, will be available from the DCC for review by the IMM. A separate report detailing protocol compliance will also be available monthly from the DCC for review by the Protocol PI, who will provide feedback to individual sites as needed. The Protocol Steering Committee (PSC) will advise the Protocol PI as to whether the protocol or informed consent document requires revision based on these reports.

8.5 Data Analyses

8.5.1 Primary Objective

Determine Maximum Tolerated Dose: The MTD will be selected using the rules specified above for the implementation of the CRM model. The MTD will be defined as the dose that would be chosen from the CRM model at the final step. Additional safety assessments will compare the groups on the basis of all AEs, changes in laboratory and vital signs values, and results of physical examinations. AEs will be classified with regard to seriousness, severity, duration, and relatedness of the event. AE data will be listed individually and will be descriptively summarized by body system, preferred terms within a body system, and treatment dose.

8.5.2 Secondary Objectives

Secondary Outcome #1 (PK Parameters): 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-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 first dose will be used in the modeling. All modeling will be done using WinNonlin.

Secondary Outcome #2 (Bleed Rates): To assess tPA/mechanical thrombectomy-related bleed rates across the two groups, all treated subjects (regardless of dose) will be compared to all placebo subjects using a Pearson chi-square test. An additional analysis will compare the bleed rates among placebo subjects and subjects treated at each dose level (assuming a sufficient number of subjects were treated at that level).

Exploratory Outcomes: (Stroke Outcome and Immunogenicity Data): To inform the design of any future Phase III trial, the following exploratory outcomes will be compared at the end of the study. Unless explicitly stated otherwise, the comparisons will be conducted first comparing all treated subjects (regardless of dose) versus all placebo subjects, and then comparing all subjects receiving the dose selected as the MTD versus all placebo subjects.

- The mean bleeding (hemorrhage and microbleeds) volume at Day 30 will be compared using a linear regression model. Median and categorical volumes (based upon quartiles) will be compared.

- Rates of subarachnoid hemorrhage in subjects who receive mechanical thrombectomy will be compared; all mechanical thrombectomy 3K3A-APC-treated subjects (regardless of dose) will be compared to all placebo subjects using a Pearson chi-square test.
- The 7-day NIHSS mean scores will be compared using a Kruskal-Wallis test.
- The change from Baseline NIHSS to 7-day NIHSS will be compared using a linear regression model, with baseline NIHSS score as a covariate.
- The 90-day mRS will be compared using a Pearson chi-square test.
- The mean infarct volume at 90 days (MRI, or CT if unable to obtain MRI) will be compared using a linear regression model. Median and categorical infarct volumes (based upon quartiles) will be compared.
- The 90-day BI will be compared using a Kruskal-Wallis test.
- 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.
- The tendency of the drug to induce an immunogenic response in patients will be assessed.

9 DATA COLLECTION, SITE MONITORING, AND ADVERSE EXPERIENCE REPORTING

9.1 Data Management

Site personnel will collect, transcribe, correct, and transmit the data onto source documents, CRFs, and other forms used to report, track and record clinical research data. The DCC will monitor clinical sites to ensure compliance with data management requirements and Good Clinical Practices. The DCC is responsible for developing, testing, and managing clinical data management activities, as required, at the study sites, the CCC, and at the DCC.

The general NINDS Common Data Elements (CDE) will be used to construct data collection forms. All study data will be collected via systems created in collaboration with the DCC and will comply with all applicable guidelines regarding patient confidentiality and data integrity.

9.1.1 Enrollment Registration

Participants will be considered screened after signing the informed consent form for this study. Eligible screened participants will be 'randomized' once registered in the NeuroNEXT IWRS and considered 'dosed' only after receipt of study drug. Enrollment of participants on this protocol will employ an interactive data system in which the clinical study site will attest to the participant's eligibility as per protocol criteria after obtaining informed consent. NeuroNEXT CIRB approval for the protocol must be on file at the DCC before accrual can occur from the clinical study site. The participant is not considered validly enrolled until the first study infusion begins. If a participant becomes ineligible between randomization and the onset of study drug infusion, he/she will be considered a randomization failure and will be replaced.

The DCC will use a system of coded identifiers to protect participant confidentiality. When the participant is registered to participate in the study using the DCC-provided web-based registration, the system will assign a participant ID number. The unique ID code will include a protocol ID, a site ID, and a unique participant ID. To confirm the correct participant ID, the data entry system will require a second entry of the unique participant ID and compare for consistency. In this fashion, no personal identifiers would be accessible to the DCC and the data will be collected on the correctly identified subject.

9.1.2 Data Entry

Data entry will occur at the enrolling clinical study sites. Data quality assurance and analyses will be performed by the DCC. The DCC, located at The University of Iowa, will coordinate all data and statistical services for the study, as well as on-site monitoring for all participating clinical study sites.

Data collection for this study will be accomplished with online electronic case report forms. Using encrypted communication links, online forms will be developed that contain the requisite data fields.

9.2 Role of Data Management

Data Management (DM) is the development, execution and supervision of plans, policies, programs, and practices that control, protect, deliver, and enhance the value of data and information assets.

All data will be managed in compliance with NeuroNEXT policies, and applicable Sponsor and regulatory requirements. The DCC will instruct site personnel to collect, transcribe, correct, and transmit the data onto source documents, CRFs, and other forms used to report, track and record clinical research data. The DCC will monitor clinical sites to ensure compliance with data management requirements and Good Clinical Practices. The DCC is responsible for developing, testing, and managing clinical data management activities, as required, at the clinical study sites (CSS), the CCC, and at the DCC.

The DCC is responsible for all aspects of clinical data management, and for properly instructing key study personnel (including the CCC, the CSS, and DCC staff) on how to collect, transcribe, correct and transmit the data onto CRFs or other data collection forms and logs.

The DCC is responsible for establishing procedures to ensure that clinical data management activities occur as required at the CCC, the CSS, and at the DCC.

9.3 Quality Assurance

By signing this protocol, the Sponsor and Investigator agree to be responsible for implementing and maintaining quality control and quality assurance systems with written standard operating procedures (SOPs) to ensure that studies are conducted and data are generated, documented, and reported in compliance with the protocol, accepted standards of GCP, and all applicable federal, state, and local laws, rules and regulations relating to the conduct of the clinical study.

All participating clinical sites have been pre-qualified by the NeuroNEXT management team according to NeuroNEXT SOPs.

9.3.1 Development of Monitoring Plan

On-site monitoring visits will be conducted by DCC monitors according to a pre-defined Monitoring Plan. The Monitoring Plan will detail the frequency of on-site visits, the study data to be monitored, the review of any regulatory files, drug and supplies accountability (if applicable), documentation of the on-site visit, and the resolution process for data errors that are discovered during the visits. All participating clinical study sites will be monitored at least once after study initiation and all sites will have a close-out visit for each protocol. One on-site blinded monitoring visit is anticipated for each clinical study site per year. Additionally, one on-site unblinded pharmacy visit is anticipated for each site per year. All subjects will be monitored for inclusion and exclusion criteria, informed consent procedures, and adverse events. A certain percentage of data is also monitored/ source data verified against the data entered into the study database. The monitoring plan will include flexibility to revise the frequency of visits or data monitored depending on clinical study site or study related issues.

9.3.2 Site Monitoring Visits

On-site blinded monitoring visits will be conducted by DCC monitors according to a pre-defined Monitoring Plan for each protocol. The goal of on-site monitoring is to analyze (review) the data as it is collected, to check the validity and integrity of the data, to verify source documentation, to ensure protection of human subjects, and to ensure protocol compliance with federal regulations. During the monitoring visit, the monitor assesses the overall status of the study, staff, and facilities to determine whether the study is being conducted per protocol and in compliance with regulatory requirements. The monitor also conducts a CRF review that includes checks of all adverse event documentation, verifies the presence of all critical correspondence and records related to investigational products and clinical supplies (if applicable), and determines if protocol violations have occurred and are documented properly. After the monitoring visit, the monitor documents the results of the monitoring visit and completes a post-visit monitoring letter that conveys any issues discovered during the visit and the need for data corrections, if appropriate. Unblinded pharmacy monitoring visits will be conducted by independent monitors to ensure proper drug storage, handling and administration. Drug and supplies accountability may also be monitored during the site visit. The DCC will work closely with the unblinded pharmacy monitors to monitor and document drug distribution from the manufacturers to the clinical study sites (CSS). Each CSS will be provided with a drug accountability log, which will be reviewed by the independent monitors and reconciled with distribution logs. At the study closeout visit, the monitors confirm that appropriate data have been reviewed, source documentation has been verified, and all required documents are present in the Study Regulatory File.

9.3.3 Laboratory Data Flow

Safety Monitoring Labs: The DCC will provide clinical study sites with online forms and/or electronic data exchange mechanisms, depending on their capabilities and needs, in order to enter, update, and obtain relevant data. The CSS will be responsible for the entry of laboratory data into the electronic data capture (EDC) system.

9.4 Adverse Experience Reporting

The adverse event (AE) definitions and reporting procedures provided in this protocol 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 adverse events. 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.

AEs should be collected and reported on subjects who receive investigational treatment from signing of the ICF through study Day 7, and should be followed until resolution or a new baseline is established, but no longer than study Day 90. Any AEs that occur prior to informed consent should be included in the subject's medical history. SAEs will be collected and reported on subjects who receive investigational treatment from signing of the ICF through study Day 30, and should be followed until resolution (or resolution with sequelae), but no longer than study Day 90.

Each clinical study site's Principal Investigator and research team are responsible for identifying adverse events and reporting them through the DCC Online Adverse Event Reporting System. Investigators are also responsible for complying with NeuroNEXT CIRB's reporting requirements for all safety reports. Copies of each report and documentation of IRB notification and receipt will be kept in the Investigator's study file.

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.

On-line Adverse Event Reporting System

Upon entry of a serious adverse event by a site Investigator, the DCC Online Adverse Event Reporting System will immediately notify the Independent Medical Monitor (IMM).

- Within **24 hours** (of learning of the event), Investigators must report any Serious Adverse Event (SAE). Investigators should report all other AEs within **5 working days/7 calendar days** (of learning of the event).

Serious adverse events: The site Investigator determines causality (definitely not related, unlikely related, possibly related, probably related, definitely related) of the adverse event. The IMM will review the SAE report. The IMM may request further information if necessary. The Online Adverse Event Reporting System maintains audit trails and stores data (and data updated) and communication related to any adverse event in the study. The Sponsor in conjunction with the IMM may determine that the Serious Adverse Event requires 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. The DSMB may suggest changes to the protocol or consent form to the Study Chair as a consequence of adverse events.

Non-serious adverse events: Non-serious adverse events that are reported to or observed by the Investigator or a member of his/her research team should be submitted to the DCC in a timely fashion (within 5 working days/7 calendar days). See the 'Site Manual of Operations' for more detail. The events will be presented in tabular form and given to the IMM on a quarterly basis or as requested. Local site Investigators are also required to fulfill all reporting requirements of their local institutions.

The DCC will prepare aggregate reports of all adverse events (serious/not serious, expected/unexpected and relationship to study drug) for the IMM and the DSMB on a quarterly basis or as requested. In addition, all adverse events will be coded using the MedDRA system. A separate report detailing protocol compliance will also be available from the DCC for DSMB and/or site review monthly or as requested. The research team will then evaluate whether the protocol or informed consent document requires revision based on the reports.

9.4.1 Definitions of Adverse Events, Suspected Adverse Drug Reactions & Serious Adverse Events

9.4.1.1 Adverse Event and Suspected Adverse Drug Reactions

An adverse event (AE) is any untoward occurrence associated with the use of a drug in humans whether or not considered drug related.

A pretreatment AE is any untoward medical occurrence that occurs after consent signed and prior to study drug administration.

Adverse drug reactions (ADR) are all noxious and unintended responses to a medicinal product related to any dose. The phrase "responses to a medicinal product" means that a causal relationship between a medicinal product and an adverse event is at least a reasonable possibility, i.e., the relationship cannot be ruled out. Therefore, a subset of AEs can be classified as suspected ADRs, if there is a causal relationship to the medicinal product.

Examples of adverse events include: new conditions, worsening of pre-existing conditions, clinically significant abnormal physical examination signs (i.e. skin rash, peripheral edema, etc.), or clinically significant abnormal test results (i.e. lab values or vital signs), with the exception of outcome measure results, which are not being recorded as adverse events in this trial (they are being collected, but analyzed separately). Stable chronic conditions (i.e., diabetes, arthritis) that are present prior to the start of the study and do not worsen during the trial are NOT considered adverse events. Chronic conditions that occur more frequently (for intermittent conditions) or with greater severity, would be considered as worsened and therefore would be recorded as adverse events.

Adverse events are generally detected in two ways:

Clinical → symptoms reported by the subject or signs detected 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 source 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.

An unexpected adverse event is any adverse event, the specificity or severity of which 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 adverse event that, in the opinion of the Site Investigator or Sponsor, there is a reasonable possibility that the investigational product caused the event.

9.4.1.2 Serious Adverse Events

A serious adverse event (SAE) is defined as an adverse event that meets any of the following criteria:

1. Results in death.
2. Is life threatening: that is, poses an immediate risk of death as the event occurred.
 - a. This serious criterion applies if the study subject, in the view of the Site Investigator or Sponsor, is at immediate risk of death from the AE as it occurs. It does not apply if an AE hypothetically might have caused death if it were more severe.
3. Requires inpatient hospitalization or prolongation of existing hospitalization.
 - a. 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.
4. Results in persistent or significant disability or incapacity.
 - a. 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 functions.
5. Results in congenital anomaly or birth defect in the offspring of the subject (whether the subject is male or female).
6. Necessitates medical or surgical intervention to preclude permanent impairment of a body function or permanent damage to a body structure.
7. 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.

The Site Investigator is responsible for classifying adverse events as serious or non-serious.

9.4.1.3 Neuroworsening

For the purposes of this protocol neurologic worsening is defined as a 4 or more points 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-neurologic causes, such as iatrogenic sedation or medical co-morbidities. The cause of the neuroworsening will be captured as a serious adverse event and, 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 Investigator will be asked to give the 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 details.

If a subject's neurological status deteriorates (whether gradually or rapidly) once the DLT observation period has expired the event will be reported as an AE/SAE as applicable but will not be considered neuroworsening according to the definition listed above.

9.4.1.4 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 [9.4.1.3](#) for definition) *and* in the opinion of the Investigator represents a clinically significant change that can be attributed to the hemorrhage³. 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 details.

9.4.1.5 Assessment and Recording of Adverse Events

This study will utilize the CTCAE version 4.03 coding system for adverse event recording. Adverse events reported using CTCAE will be recoded into MedDRA terms by the DCC.

Assessment of Adverse Events

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 adverse events. If the subject reports an adverse event, the Investigator will determine:

1. Type of event
2. Date of onset and resolution (duration)
3. Severity (mild, moderate, severe, life-threatening, results in death)
4. Seriousness (does the event meet the above definition for an SAE)
5. Causality, relation to investigational product and disease
6. Action taken regarding investigational product
7. Outcome

Severity of Adverse Events

The severity of AEs and SAEs 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.

Relatedness of Adverse Event to Investigational Product

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

1. Not Related: Concomitant illness, accident or event with no reasonable association with treatment.
2. Unlikely: The reaction has little or no temporal sequence from administration of the investigational product, and/or a more likely alternative etiology exists.
3. 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; the 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)
4. 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)
5. Definitely Related: The reaction follows a reasonable temporal sequence from administration of investigational product; that follows a known or expected response pattern to the investigational product; and that is confirmed by improvement on stopping or reducing the

dosage of the investigational product, and reappearance of the reaction on repeated exposure (suspected ADR).

Recording of Adverse Events

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

Please Note: Serious Adverse Events (SAEs) must be reported to the NeuroNEXT Data Coordinating Center within 24 hours of the site learning of the SAE.

Entries in the AE data entry template (and into the online Adverse Event Reporting System) will include the following: name and severity of the event, the date of onset, the date of resolution, relationship to investigational product, action taken, and primary outcome of event.

Adverse Events and Serious Adverse Events - Reportable Events

The following are considered reportable events and must be reported to the NeuroNEXT Data Coordinating Center within 24 hours of the site being notified of the event.

- All events that meet the above criteria for Serious Adverse Events (SAEs)

All occurrences of Serious Adverse Events (SAEs) must be reported within 24 hours of discovery of the event. All other Adverse Events (AEs) should be reported *within 5 working days/7 calendar days (of discovery of the event)*.

Adverse Event Data Management System (AEDAMS)

Upon entry of a serious adverse event by a clinical site, the DCC Online Adverse Event Reporting System will immediately notify the IMM. If warranted, the IMM will notify the DSMB chair.

Serious adverse events: The site Investigator determines causality (definitely not related, probably not related, possibly related, probably related, definitely related) of the adverse event. The IMM will review the SAE report. The IMM may request further information if necessary. The DSMB may suggest changes to the protocol or consent form to the Project PI as a consequence of adverse events. The Online Adverse Event Reporting System maintains audit trails and stores data (and data updated) and communication related to any adverse event in the study.

Non-serious adverse events: Non-serious adverse events that are reported to or observed by the Investigator or a member of his/her research team should be submitted to the DCC within 5 working days/7 calendar days. The events will be presented by DCC in tabular form and given to the IMM on a monthly basis or as requested. Local site Investigators are also required to fulfill all reporting requirements of their local institutions.

The DCC will prepare aggregate reports of all adverse events (serious/not serious and expected, unexpected) for the DSMB.

9.4.1.6 Breaking the Study Blind

Every effort should be made to preserve the double-blind treatment assignment of study subjects in the randomized phase of the study. In the majority of cases, the Investigator should simply assume the patient is on active treatment and treat accordingly. Should EMERGENCY unblinding

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need to occur, the clinical staff (or pharmacist) must contact the NN104 study Hotline for permission.

Hotline (24/7): [REDACTED]

The DCC will be immediately notified of the broken blind. Refer to the 'Site Manual of Operations' for details.

10 HUMAN SUBJECTS

Documented approval from the NeuroNEXT CIRB will be obtained for all participating centers prior to clinical trial start, according to ICH GCP, local laws, regulations and organization. When necessary, an extension, amendment or renewal of the CIRB approval must be obtained.

Evidence of training in responsible conduct of research shall be on file for each CSS PI and co-Investigator.

10.1 Central Institutional Review Board (CIRB) Review and Informed Consent

This protocol and the informed consent document and any subsequent modifications will be reviewed and approved by the NeuroNEXT CIRB responsible for oversight of the study. A signed consent form, approved by the NeuroNEXT CIRB, will be obtained from the subject. For subjects who cannot provide consent for themselves, an authorized representative defined by the local regulations must sign the consent form; additionally, the subject's assent must also be obtained if he or she is able to understand the nature, significance, and risks associated with the study. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the subject, and/or authorized representative, and this fact will be documented in the subject's record. Should the subject regain capacity and consent was originally provided by someone else, the subject will be re-consented himself/herself.

10.2 Subject Confidentiality

All laboratory specimens, evaluation forms, reports, and other records that leave the clinical study site will be identified only by the study specific Subject Identification Number (SID) to maintain subject confidentiality. All records will be kept in a locked file cabinet. All computer entry and networking programs will be done using study specific SIDs only. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by CIRB, the FDA, the NINDS, the OHRP, the Sponsor, or the Sponsor's designee.

10.3 Study Modification/Discontinuation

The study may be modified or discontinued at any time by the CIRB, the NINDS, the sponsor, the OHRP, the FDA, or other government agencies as part of their duties to ensure that research subjects are protected.

11 PUBLICATION OF RESEARCH FINDINGS

Publication of the results of this trial will be governed by the policies of the NeuroNEXT Network and procedures developed by the NeuroNEXT Data Sharing and Publication Committee. Any

presentation, abstract, or manuscript will be made available for review by the Sponsor and the NINDS prior to submission.

12 REFERENCES

1. Goodman SN, Zahurak ML, Piantadosi S. Some practical improvements in the continual reassessment method for phase I studies. *Stat Med*. June 15 1995;14(11):1149-1161.
2. Hacke W, Kaste M, Bluhmki E, et al for the ECASS Investigators. Thrombolysis with Alteplase 3 to 4.5 Hours after Acute Ischemic Stroke. *The New England Journal of Medicine*. 2008;359(13):1317-1329.
3. Wahlgren N, Ahmed N, Davalos A, Ford GA, Grond M, Hacke W, et al. Thrombolysis with alteplase for acute ischaemic stroke in the Safe Implementation of Thrombolysis in Stroke-Monitoring Study (SITS-MOST): an observational study. *Lancet*. 2007;369:275-282.
4. Goyal M, Demchuk AM, Menon BK et al. Randomized Assessment of Rapid Endovascular Treatment of Ischemic Stroke. *N Engl J Med*. 2015.
5. Campbell BC, Mitchell PJ, Kleinig TJ et al. Endovascular Therapy for Ischemic Stroke with Perfusion-Imaging Selection. *N Engl J Med*.
6. Berkhemer OA, Fransen PS, Beumer D et al. A randomized trial of intraarterial treatment for acute ischemic stroke. *N Engl J Med*. Jan 1 2015;372(1):11-20.
7. Saver J, SOLITAIRE FR with the intention for thrombectomy as primary endovascular treatment for acute ischemic stroke. Feb 11 2015.
8. Activase®(Alteplase) Prescribing Information. Genetech, Inc.; February 2015.
9. Lloyd-Jones D, Adams RJ, Brown TM, et al, American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics--2010 update: a report from the American Heart Association. *Circulation*. 2010;121(7):e46-e215.
10. *American Heart Association. Heart Disease and Stroke Statistics – 2010 Update*. Dallas: American Heart Association;2010.
11. Thom T, Haase N, Rosamond W, et al. Heart disease and stroke statistics--2006 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation*. 2006;113(6):e85-151.
12. Stroke National Institute of Neurological Disorders and. Stroke: Hope Through Research. 2010; http://www.ninds.nih.gov/disorders/stroke/detail_stroke.htm.
13. Group NINDS rt-PA Stroke Study. Tissue plasminogen activator for acute ischemic stroke. *N Engl J Med*. 1995;333(24):1581-1587.
14. Jauch EC, Saver JL, Adams HP Jr, et al. Guidelines for the early management of patients with acute ischemic stroke: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke*. 2013;44(3):870-947.
15. Steiner A, Lyden P. Evolution of the thrombolytic treatment window for acute ischemic stroke. *Curr Neurol Neurosci Rep*. January 2010;10(1):29-33.
16. Grotta JC, Burgin WS, El-Mitwalli A, et al. Intravenous tissue-type plasminogen activator therapy for ischemic stroke: Houston experience 1996 to 2000. *Arch Neurol*. 2001;58(12):2009-2013.
17. Kidwell CS, Jahan R, Alger JR. Design and rationale of the Mechanical Retrieval and Recanalization of Stroke Clots Using Embolectomy (MR RESCUE) Trial. *Int J Stroke*. 2014;9(1):110-116.
18. Ciccone A, Valvassori L, Nichelatti M. Endovascular treatment for acute ischemic stroke. *N Engl J Med*. 2013;368(10):904-913.

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19. Broderick J, Palesch YY, Demchuk AM. Endovascular therapy after intravenous t-PA versus t-PA alone for stroke. *N Engl J Med*. 2013;368(10):893-903.
20. Jovin TG, Chamorro A, Cobo E, de Miguel MA, et al. Thrombectomy within 8 hours after symptom onset in ischemic stroke. *N Engl J Med*. 2015;372(24):2296-2306.
21. Mosnier LO, Gale AJ, Yegneswaran S, Griffin JH. Activated protein C variants with normal cytoprotective but reduced anticoagulant activity. *Blood*. 2004;104(6):1740-1744.
22. Mosnier LO, Yang XV, Griffin JH. Activated protein C mutant with minimal anticoagulant activity, normal cytoprotective activity, and preservation of thrombin activable fibrinolysis inhibitor-dependent cytoprotective functions. *J Biol Chem*. 2007;282(45):33022-33033.
23. Mosnier LO, Zlokovic BV, Griffin JH. The cytoprotective protein C pathway. *Blood*. 2007;109(8):3161-3172.
24. Cheng T, Liu D, Griffin JH, Fernández JA, Castellino F, Rosen ED, Fukudome K, Zlokovic BV. Activated protein C blocks p53-mediated apoptosis in ischemic human brain endothelium and is neuroprotective. *Nat Med*. 2003;9(3):338-342.
25. Dömötör E, Benzakour O, Griffin JH, Yule D, Fukudome K, Zlokovic BV. Activated protein C alters cytosolic calcium flux in human brain endothelium via binding to endothelial protein C receptor and activation of protease activated receptor-1. *Blood*. 2003;101(12):4797-4780.
26. Joyce DE, Gelbert L, Ciaccia A, DeHoff B, Grinnell BW. Gene expression profile of antithrombotic protein c defines new mechanisms modulating inflammation and apoptosis. *J Biol Chem*. 2001;276(14):11199-11203.
27. Mosnier LO, Griffin JH. Inhibition of staurosporine-induced apoptosis of endothelial cells by activated protein C requires protease-activated receptor-1 and endothelial cell protein C receptor. *Biochem J*. 2003;373(Pt. 1):65-70.
28. Riewald M, Petrovan RJ, Donner A, Mueller BM, Ruf W. Activation of endothelial cell protease activated receptor 1 by the protein C pathway. *Science*. 2002;296(5574):1880-1882.
29. Liu D, Cheng T, Guo H, Fernandez JA, Griffin JH, Song X, Zlokovic BV. Tissue plasminogen activator neurovascular toxicity is controlled by activated protein C. *Nat Med*. 2004;10:1379-1383.
30. Guo H, Liu D, Gelbard H, Cheng T, Insalaco R, Fernández JA, Griffin JH, Zlokovic BV. Activated protein C prevents neuronal apoptosis via protease activated receptors 1 and 3. *Neuron*. 2004;41(4):563-572.
31. Cheng T, Petraglia AL, Li Z, Thiyagarajan M, Zhong Z, Wu Z, Liu D, Maggirwar SB, Deane R, Fernandez JA, LaRue B, Griffin JH, Chopp M, Zlokovic BV. Tissue plasminogen activator neurovascular toxicity is controlled by activated protein C. *Nat Med*. 2006;12:278-285.
32. Shibata M, Kumar SR, Amar A, Fernandez JA, Hofman F, Griffin JH, Zlokovic BV. Anti-inflammatory, antithrombotic, and neuroprotective effects of activated protein C in a murine model of focal ischemic stroke. *Circulation*. 2001;103:1799-1805.
33. Zlokovic BV, Zhang C, Liu D, Fernandez J, Griffin JH, Chopp M. Functional recovery after embolic stroke in rodents by activated protein C. *Ann Neurol*. 2005;58(3):474-477.
34. Thiyagarajan M, Fernandez JA, Lane SM, Griffin JH, Zlokovic BV. Activated protein C promotes neovascularization and neurogenesis in postischemic brain via protease-activated receptor 1. *J Neurosci*. November 26 2008;28(48):12788-12797.
35. Sutherland BA, Minnerup J, Balami JS, Arba F., Buchan AM and Kleinschnitz C. Neuroprotection for ischaemic stroke: Translation from the bench to the bedside. *Intern J Stroke*. July 2012;7:407-418.
36. Zlokovic BV, Griffin JH. Cytoprotective protein C pathways and implications for stroke and neurological disorders. *Trends Neurosci*. 2011;34(4):198-209.

ZZ-3K3A-201

Version 8.1 Final

Version date 7-Oct-2016

37. Fernández JA, Mosnier LO, Davis TP, Zlokovic BV and Griffin JH. Influence of the 3K3A-activated protein C variant on the in vitro fibrinolytic activity of tPA *J Thrombos Haemostas (submitted; acceptance pending)*.
38. Xigris® Prescribing Information. Eli Lilly; 2008.
39. Bernard GR, Vincent JL, Laterre PF, et al. Efficacy and safety of recombinant human activated protein C for severe sepsis. *N Engl J Med*. 2001;344(10):699-709.
40. Activated Protein C in Acute Stroke Trial (APCAST). 2010; <http://www.clinicaltrials.gov/ct2/show/NCT00533546?term=apcast&rank=1>.
41. Guo H, Wang Y, Singh I, Liu D, Fernández JA, Griffin JH, Chow N, Zlokovic BV. Species-dependent neuroprotection by activated protein C mutants with reduced anticoagulant activity. *J Neurochem*. 2009;109(1):116-124.
42. Wang Y, Thiyagarajan M, Chow N, Singh I, Guo H, Davis TP, Zlokovic BV. Differential neuroprotection and risk for bleeding from activated protein C with varying degrees of anticoagulant activity. *Stroke*. 2009;40(5):1864-1869.
43. Wang Y, Zhang Z, Chow N, Davis TP, Griffin JH, Copp M, Zlokovic BV. An Activated Protein C Analog With Reduced Anticoagulant Activity Extends the Therapeutic Window of Tissue Plasminogen Activator for Ischemic Stroke in Rodents. *Stroke*. 2012;43.
44. Wang Y, Zhao Z, Chow N, Rajput PS, Griffin J, Lyden P, et al. An activated protein C analog protects from ischemic stroke and extends the therapeutic window of tPA in aged female mice and hypertensive rats. *Stroke*. 2013 44(12):3529-3536.
45. Guo H, Singh I, Wang Y, Deane R, Barrett T, Fernández JA, Chow N, Griffin JH, Zlokovic BV. Neuroprotective activities of activated protein C mutant with reduced anticoagulant activity. *Eur J Neurosci*. 2009;29(6):1119-1130.
46. Walker CT, Marky AH, Petraglia AL, Ali T, Chow N, Zlokovic BV. Activated protein C analog with reduced anticoagulant activity improves functional recovery and reduces bleeding risk following controlled cortical impact. *Brain Res*. 2010;1347:125-131.
47. Zhong Z, Ilieva H, Hallagan L, Bell R, Singh I, Paquette N, Thiyagarajan M, Deane R, Fernandez JA, Lane S, Zlokovic AB, Liu T, Griffin JH, Chow N, Castellino FJ, Stojanovic K, Cleveland DW, Zlokovic BV. Activated protein C therapy slows ALS-like disease in mice by transcriptionally inhibiting SOD1 in motor neurons and microglia cells. *J Clin Invest*. 2009;119(11):3437-3449.
48. Tanswell P, Tebbe U, Neuhaus KL, Glasle-Schwarz L, Wojcik J, and Seifried E. Pharmacokinetics and fibrin specificity of alteplase during accelerated infusions in acute myocardial infarction. *J Am Coll Cardiol*. 1992;19:1071-1075.
49. Hesse K, Fulton RL, Abdul-Rahim AH, Lees KR, VISTA Collaborators. Characteristic adverse events and their incidence among patients participating in acute ischemic stroke trials. *Stroke*. 2014;45(9):2677-2682.
50. Perry L., Love C. P. Screening for dysphagia and aspiration in acute stroke: a systematic review. *Dysphagia*. Winter 2001;16(1):7-18.
51. Hilker R., Poetter C., Findeisen N., et al. Nosocomial pneumonia after acute stroke: implications for neurological intensive care medicine. *Stroke*. Apr 2003;34(4):975-981.
52. Indredavik B., Rohweder G., Naalsund E., Lydersen S. Medical complications in a comprehensive stroke unit and an early supported discharge service. *Stroke*. Feb 2008;39(2):414-420.
53. Rocco A., Pasquini M., Cecconi E., et al. Monitoring after the acute stage of stroke: a prospective study. *Stroke*. Apr 2007;38(4):1225-1228.
54. van de Beek Diederik, Wijdicks Eelco F. M., Vermeij Frederique H., et al. Preventive Antibiotics for Infections in Acute Stroke: A Systematic Review and Meta-analysis. *Arch Neurol*. September 1, 2009 2009;66(9):1076-1081.

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Version date 7-Oct-2016

55. O'Quigley J, Pepe M, Fisher L. Continual Reassessment Method: A Practical Design for Phase 1 Clinical Trials in Cancer. *Biometrics*. 1990;46:33-48.
56. Kimura K, Aoki J, Shibasaki K, Saji N, Uemura J, Sakamoto Y. New Appearance of Extradisemic Microbleeds on T2*-Weighted Magnetic Resonance Imaging 24 Hours After Tissue-type Plasminogen Activator Administration. *Stroke*. 2013;44:epub.
57. Derex L, Nighoghossian N, Hermier M, Adeleine P, Philippeau F, Honnorat J, et al. Thrombolysis for ischemic stroke in patients with old microbleeds on pretreatment MRI. *Cerebrovasc Dis*. 2004;17:238-241.
58. Harada M, Morita N, Uno M, Satomi J, Izumi Y, Asanuma K, et al. Incidence and clinical correlation of intracranial hemorrhages observed by 3-tesla gradient echo T(2)*-weighted images following intravenous thrombolysis with recombinant tissue plasminogen activator. *Cerebrovasc Dis*. 2010;29:571-575.
59. Shoamanesh A, Kwok CS, Lim PA, Benavente OR. Post thrombolysis intracranial hemorrhage risk of cerebral microbleeds in acute stroke patients: a systematic review and meta-analysis. *Int J Stroke*. 2013;8:348-356.
60. Kidwell CS, Chalela JA, Saver JL, Starkman S, Hill MD, Demchuk AM, et al. Comparison of MRI and CT for detection of acute intracerebral hemorrhage. *Journal of the American Medical Association*. 2004;292:1823.
61. Fiehler J, Albers GW, Boulanger JM, Derex L, Gass A, Hjort N, et al. Bleeding risk analysis in stroke imaging before thrombolysis (BRASIL): pooled analysis of T2*-weighted magnetic resonance imaging data from 570 patients. *Stroke*. 2007;38:2738-2744.
62. Cheng AL, Batool S, McCreary CR, Lauzon ML, Frayne R, Goyal M, Smith EE. Susceptibility-weighted imaging is more reliable than T2*-weighted gradient-recalled echo MRI for detecting microbleeds. *Stroke*. 2013;44(10):2782-2786.
63. Wang Y, Zhang Z, Chow N, Davis TP, Griffin JH, Chopp M, Zlokovic BV. An Activated Protein C Analog With Reduced Anticoagulant Activity Extends the Therapeutic Window of Tissue Plasminogen Activator for Ischemic Stroke in Rodents. *Stroke*. 2012;43(9):2444-2449.