RHAPSODY-2 PROTOCOL SYNOPSIS

Investigational Product	3K3A-APC, a recombinant variant of human activated protein C (APC) in which 3 lysine residues (191-193) are replaced by 3 alanine residues
Full Protocol Title	ZZ-3K3A-301: A multicenter, randomized, placebo-controlled, double-blinded, Phase 3 study to evaluate the efficacy and safety of 3K3A-APC, a recombinant variant of human activated protein C, in combination with tissue plasminogen activator, mechanical thrombectomy, or both, in subjects with moderate to severe acute ischemic stroke
Short Protocol Title	RHAPSODY-2: Recombinant variant of Human Activated Protein C (APC), in combination with tissue plaSminOgen activator (thrombolysis) in moDeratelY severe acute hemispheric ischemic stroke
Study Design	This multicenter, randomized, placebo-controlled, double-blind, Phase 3 study is being performed in coordination with StrokeNet to evaluate the efficacy and safety of 3K3A-APC following administration of thrombolysis, mechanical thrombectomy, or both, in subjects with moderate to severe acute ischemic stroke. Subjects will be considered for the study if they are eligible for thrombolysis administration, mechanical thrombectomy, or both, for acute ischemic stroke with National Institutes of Health Stroke Scale (NIHSS) score ≥5. The study will be conducted at approximately 60 United States (US) sites and approximately 40 non-US sites. The study will be conducted in 2 phases: the lead-in dose-finding phase and the definitive phase. During the lead-in dose-finding phase, a maximum of 360 subjects will be randomized to 3K3A-APC (10, 15, or 30 mg) or placebo using a Bayesian adaptive approach. Randomized subjects will receive 3K3A-APC or placebo (control) every 12 hours for 5 doses (approximately 3 days) or until discharge from the hospital (whichever occurs first). Subjects will be monitored through Day 7/Discharge and on Days 30, 60 (phone/virtual), and 90. The lead-in phase will transition to 1 selected 3K3A-APC dose and recruitment will continue when a single dose proves superior to control, as measured by the proportion of intracerebral bleeding or death. If more than 1 dose proves superior to control, the lower dose will advance to the next phase. The entire study will stop if no dose proves superior to control during the lead-in phase. The definitive phase will continue with the 1 selected dose of 3K3A-APC from the lead-in phase. Approximately 1040 subjects will be randomized during the definitive phase. Subjects from the lead-in phase who receive the dose selected for the definitive phase will be included in the final data analysis.
Objectives, Endpoints, and Analyses	Table S1 summarizes the primary and key secondary objectives, endpoints, and analyses. A full list of objectives, endpoints, and analyses is provided in Section 2 and Section 3.2 of the protocol.

Table S1. Primary and Key Secondary Objectives, Endpoints, and Analyses

	Objective	Endpoint	Analyses
Primary:			
- Lead-in phase:	To evaluate the effect of 3K3A-APC on bleed-free survival at Day 30	Intracerebral bleeding (any blood detected on SWI-MRI) or death at 30 days after ischemic stroke	Bayesian adaptive analysis of posterior probabilities that the proportion of bleeding or death for best dose is lower than control
- Definitive phase:	To evaluate the effect of 3K3A-APC on 90-day disability	Day 90 mRS	Day 90 mRS scores will be compared between groups using Bayesian ordinal (shift) analysis
Key secondary:			
- Definitive phase:	To evaluate the effect of 3K3A-APC on bleed-free survival at Day 30	Intracerebral bleeding (any blood detected on SWI-MRI) or death at 30 days after ischemic stroke	Comparison of the proportion of intracerebral bleeding or death at 30 days for the selected dose of 3K3A-APC versus control, using Fisher's exact test

MRI=magnetic resonance imaging; mRS=modified Rankin Scale; SWI=susceptibility-weighted imaging

Interventions and Duration	3K3A-APC will be provided as a lyophilized powder to be reconstituted in sterile water and diluted in 0.9% sodium chloride in water and administered as a 100 mL intravenous (IV) infusion over 15 minutes. Three dose-tiers of 3K3A-APC will be used in the lead-in phase of this study: 10, 15, and 30 mg; transitioning into one dose in the definitive phase. Placebo will be 0.9% sodium chloride in water, visually indistinguishable from the test product, and will be administered in the same manner as the active product. Adult subjects will receive 3K3A-APC or matching placebo given as a 15-minute infusion no later than 120 minutes following completion of thrombolysis infusion or initiation of mechanical thrombectomy (arterial puncture), whichever is sooner. Subjects will receive another 15-minute infusion of 3K3A-APC or placebo every 12 hours (±1 hour) for 5 total infusions (or until discharge, whichever occurs first).
Study and Treatment Duration	Subjects will be considered for the study if they are eligible for thrombolysis administration, mechanical thrombectomy, or both, for acute ischemic stroke with NIHSS score ≥5. Randomized subjects will receive 3K3A-APC or placebo every 12 hours for 5 doses (approximately 3 days) or until discharge from the hospital (whichever occurs first). Subjects will be monitored through Day 7/Discharge and on Days 30, 60 (phone/virtual), and 90. The use of anticoagulants such as vitamin K antagonists, factor Xa inhibitors, and thrombin inhibitors are prohibited from the time of study drug initiation to 4 hours following the last dose of 3K3A-APC. Antiplatelet medication must be started in all subjects prior to discharge unless clinically contraindicated (eg, hemorrhagic transformation). Note: the use of unfractionated or low molecular-weight heparin in accordance with standard of care (SOC) for the prevention of deep vein thrombosis, 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 subject safety. 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 subject safety.
Criteria for Evaluation	**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. Subjects will become eligible for this clinical study if they receive IV thrombolysis per local SOC or begin mechanical thrombectomy, subject to the following: Subjects who present within 24 hours of last known well time will be eligible for enrollment if they fulfill local suitability criteria that 1) must be approved in advance of study enrollment by the Lead Investigator, and 2) must follow nationally recognized guidelines. Note: Subjects must receive IV thrombolysis OR begin mechanical thrombectomy before they can receive 3K3A-APC or placebo. 4. NIHSS score ≥5 at time of randomization 5. Signed informed consent form (ICF) by the subject or authorized representative 6. Agreement to use effective birth control throughout the study (ie, Day 90): a. Males - barrier method of contraception plus a spermicide b. Females of childbearing potential (ie, not surgically sterile or post-menopausal, defined as >51 years of age 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. Able to receive the first dose of study drug within 120 minutes following the time thrombolysis infusion is completed or mechanical thrombectomy (arterial puncture) begins, whichever is sooner. **Exclusion Criteria** <u>Neurological</u> 1. Presenting neurologic deficit is non-disabling. A disabling deficit is one that, if unchanged, would prevent the subject from performing basic activities of daily living (bathing, ambulating, toileting, hygiene, and eating) or returning to work. 2. History of stroke or penetrating head injury within 90 days prior to enrollment 3. History of previous or current diagnosis of intracranial hemorrhage (ie, intracerebral, epidural, subdural, or subarachnoid) 4. Known Moyamoya disease, cerebral arteriovenous malformation, or unsecured aneurysm requiring intervention during the acute study period (Days 1 to 30 after enrollment) 5. Presence of tandem lesions, suggesting a likely need for proximal artery stenting during the thrombectomy procedure that would mandate post-operative dual antiplatelet therapy

	6. Presence of other known neurological or non-neurological co-morbidities (eg, intracerebral neoplasm, metabolic encephalopathies, hemiplegic migraine, multiple sclerosis, convulsive disorder, monocular blindness) that, in the PI's opinion, may lead, independently of the current stroke, to further deterioration in the subject's neurological status during the study period, or may render the study's neurological assessments inconclusive for the purpose of evaluating the effect of the investigational product on the stroke 7. Presence of premorbid neurological deficits and functional limitations, assessed by a pre-stroke modified Rankin scale (mRS) of ≥2 <u>Non-Neurological</u> 8. Prolonged prothrombin time (PT) and international normalized ratio (INR) >1.7, at the time of study drug initiation. Prior use of novel oral anticoagulants is allowed if PT and INR are within this limit AND allowed by local guidelines. 9. Prolonged activated partial thromboplastin time (aPTT) that exceeds the upper limit of normal (ULN), at the time of study drug initiation. 10. Use of heparin within the 48 hours prior to enrollment, except to maintain catheter patency. Note: Heparin use after enrollment is allowed during thrombectomy. Prior to or following enrollment, heparin or low-molecular weight heparin used subcutaneously to prevent deep venous thrombosis in hospitalized subjects is allowed. 11. Severe hypertension (systolic blood pressure >185 mmHg or diastolic blood pressure >110 mmHg) or hypotension (systolic blood pressure <90 mmHg), as measured by ≥2 consecutive supine measurements 10 minutes apart, that does not respond to simple treatment (eg, 1 dose of labetalol or nicardipine infusion) 12. Blood glucose concentration <50 mg/dL 13. Prior exposure to any exogenous form of a recombinant variant of human activated protein C (APC) (eg, plasma-derived APC, 3K3A-APC, XIGRIS®, drotrecogin alfa [activated]) <u>General</u> 14. Weight >130 kg 15. Unable to undergo a magnetic resonance imaging (MRI) procedure per local guidelines 16. Pregnancy or breastfeeding 17. Known current abuse of alcohol or illicit drugs. Any recreational drug or alcohol use that, in the opinion of the PI, may interfere with study participation or assessments. 18. Received treatment with an investigational drug or device within 30 days prior to enrollment 19. Any other condition that, in the opinion of the PI, may adversely affect the safety of the subject, the subject's ability to complete the study, or the outcome of the study
Statistical Methods	

Analysis Populations	There are 2 analysis populations in the study: 1. Modified Intent-to-Treat (mITT) population, defined as all subjects for whom study drug was initiated 2. All-Treated population, defined as all subjects who receive ≥1 dose of study drug and who also have ≥1 evaluable response assessment The primary analysis will be conducted using the mITT population. Subjects enrolled in either the lead-in or definitive phase will be included in the primary estimand analysis. Missing data will be imputed. All additional secondary and exploratory efficacy analyses will be performed on the All-Treated population using all available follow-up assessments. All safety analyses will be performed on the mITT population.
Sample Size	The entire study will have a maximum of 1400 subjects in the mITT sample (maximum of 360 subjects in the lead-in phase). Accounting for 2% dropout rate, the maximum sample size was inflated from 1372 subjects to 1400 subjects (354 to 360 in the lead-in phase). This sample size was selected to achieve a probability of type I error less than 0.025 and power of at least 80% when the effect size for bleed/death endpoint is 16% and the effect size for the difference in mean mRS is $\mu p - \mu T = 0.4$, determined by simulation. This difference corresponds to a 7% absolute increase in the proportion of subjects with a 90-day mRS of 0 or 1.
Randomization Scheme	The objective of the lead-in phase is to select the best treatment arm relative to control using a Bayesian outcome adaptive design. Initially, 60 subjects will be randomized equally among each of the 4 arms (3K3A-APC 10, 15, or 30 mg or placebo) with equal probability. After that, adaptive randomization will be used in such a way that subjects are more likely to be allocated to the best treatment arm (ie, the arm with the smallest rate of death or bleeding relative to the other treatment arms). Advantages of this approach relative to a completely randomized design include the ability to allocate more subjects to the apparently superior arm, stop the lead-in phase early for efficacy, drop a poorly performing arm, and thus, the design may result in more subjects being treated successfully. At the end of the lead-in phase, the dose that proves superior to control (placebo), as measured by the proportion of intracerebral bleeding or death, will be selected, and the trial will transition to a definitive comparison of that dose against control. If more than 1 dose proves superior to control, the lower dose will advance to the next phase. If no arm appears superior to control at the end of the lead-in phase, the trial will stop. In the definitive phase, subjects will be randomly assigned to the control or treatment arm, balanced by site, age (<70 vs ≥70 years), NIHSS (<11 vs ≥11), time (<6 hours vs 6-24 hours from last known well time to thrombolysis or arterial puncture, whichever is sooner), and study site. At the end of the lead-in phase, the placebo and selected treatment arm will likely be imbalanced with respect to these stratification factors, so the randomization scheme going forward in this definitive phase will use a “biased coin” algorithm to ensure eventual ‘catch-up’ and balance of the placebo and treatment group subjects on these stratification variables.

Analyses	Primary and key secondary analyses are summarized in Table S1. Subgroup analyses will be performed by age, sex, thrombolysis use, time of therapy, and region (US vs outside US). Additional assessments during the study include the NIHSS, mRS, BI, and EQ-5D-5L.