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:

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 100 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) >2xULN, 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 tPA[8]. 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 100 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 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