Selecting Patients for Intra-Arterial Therapy in the Context of a Clinical Trial for Neuroprotection

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Background and Purpose—The advent of intra-arterial neurothrombectomy (IAT) for acute ischemic stroke opens a potentially transformative opportunity to improve neuroprotection studies. Combining a putative neuroprotectant with recanalization could produce more powerful trials but could introduce heterogeneous and adverse event possibilities. We sought to demonstrate feasibility of IAT in neuroprotectant trials by defining IAT selection criteria for an ongoing neuroprotectant clinical trial.

Methods—The study drug, 3K3A-APC, is a pleiotropic cytoprotectant and may reduce thrombolysis-associated hemorrhage. The NeuroNEXT trial NN104 (RHAPSODY) is designed to establish a maximally tolerated dose of 3K3A-APC. Each trial site provided their IAT selection criteria. An expert panel reviewed site criteria and published evidence. Finally, the trial leadership designed IAT selection criteria.

Results—Derived selection criteria reflected consistency among the sites and comparability to published IAT trials. A protocol amendment allowing IAT (and relaxed age, National Institutes of Health Stroke Scale, and time limits) in the RHAPSODY trial was implemented on June 15, 2015. Recruitment before and after the amendment improved from 8 enrolled patients (601 screened, 1.3%) to 51 patients (821 screened, 6.2%; odds ratio [95% confidence limit] of 4.9 [2.3–10.4]; P<0.001). Gross recruitment was 0.11 patients per site month versus 0.43 patients per site per month, respectively, before and after the amendment.

Conclusions—It is feasible to include IAT in a neuroprotectant trial for acute ischemic stroke. Criteria are presented for including such patients in a manner that is consistent with published evidence for IAT while still preserving the ability to test the role of the putative neuroprotectant.


Key Words: activated protein C ■ clinical trial ■ neuroprotective agents ■ thrombectomy ■ thrombolysis

Clinical trials of neuroprotection for acute ischemic stroke, including rapid prehospital treatment, continue to fail. Possible contributors to this failure include the unsuitability of preclinical animal models,\textsuperscript{1} inadequate clinical trial design,\textsuperscript{2,3} and inappropriate patient selection.\textsuperscript{4} One of the most obvious and potentially powerful differences between animal and human neuroprotection studies is the influential role of recanalization/reperfusion. Recanalization refers to the reopening of the principal artery supplying a vascular territory; reperfusion implies successful restoration of flow in the microvasculature. Recanalization without reperfusion fails to salvage brain after larger artery occlusion.\textsuperscript{5} Preclinical studies...
repeatedly document that putative neuroprotectants provide greater benefit when combined with recanalization. Many neuroprotectants show benefit in animal stroke models, but they all work best if given in combination with recanalization. In contrast, documentation of arterial recanalization has not been required during human neuroprotection trials, and this could be one of the many reasons for clinical trial failures. Even after the advent of intravenous thrombolysis as an effective stroke therapy, the typical clinical neuroprotectant stroke trial did not require documentation of successful recanalization as an enrollment criterion. If recanalization enhances the chance of successful neuroprotection, then the failure of so many stroke neuroprotection trials seems less surprising.

The advent of intra-arterial neurothrombectomy (IAT) for acute ischemic stroke opens a potentially transformative opportunity for improved study of neuroprotection. Combining a putative neuroprotectant with IAT could result in more powerful clinical trials because all enrolled patients would have documented recanalization. On the contrary, allowing IAT in a clinical trial brings additional heterogeneity into the response outcome variable, introduces additional adverse event possibilities, and may limit generalizability of the trial results to centers that offer such therapy. As well, the published trials of IAT used a wide variety of inclusion/exclusion criteria, possibly making it difficult to design a clinical trial protocol that is consistent with published data. Health organizations and professional societies have published guidelines for clinical implementation of IAT that seek to allow a wide range of options for physicians. Wider latitude of treatment options allows individual physicians to personalize their approach to each patient with acute ischemic stroke, but practice variation among clinical trial study sites could introduce variation in the primary outcome sufficient to obscure treatment benefit or adverse effects of the study therapy. The different, competing priorities of a clinical trial compared with clinical practice are summarized in Table 1. Thus, the clinical trial design and protocol must specify a range of IAT treatment options that is sufficient to allow sites to use the therapy in a wide variety of patients without introducing excessive variability into the primary efficacy and safety outcomes.

We sought to define IAT selection criteria for an ongoing clinical trial of a putative neuroprotectant. The study drug takes advantage of the neuroprotective and vasculoprotective effects of activated protein C (APC), which is naturally active at the protease activated receptor-1. To avoid anticoagulation without changing the cytoprotective effects, the native APC structure was mutated in a targeted manner, such that 3 lysines were replaced by alanines removing >90% of the anticoagulant activity with preserved cytoprotection. The resultant molecule, 3K3A-APC, exhibits considerable cytoprotective effects in vitro and in animal stroke models. 3K3A-APC protects neurons, astrocytes, and endothelial cells directly, via action on the transmembrane G-coupled protein protease activated receptor-1, and also shows vasculoprotective effects that could be interpreted as protection from reperfusion injury. The drug shows greatest effect when combined with recanalization and is effective after treatment delay of ≤12 hours after stroke onset. 3K3A-APC has satisfied the Stroke Treatment Academic Industry Roundtable suggestions for preclinical drug assessment and has an established safety and pharmacokinetic profile in human volunteers. The NeuroNEXT trial NN104 (RHAPSODY) is a dose-escalation trial designed to establish a maximally tolerated dose of 3K3A-APC (protocol synopsis in the online-only Data Supplement). Although 3K3A-APC exhibits beneficial effect after permanent occlusion, the effect is greater in preclinical models that include recanalization. Thus, the RHAPSODY trial provides an opportunity to test whether allowing IAT (and thereby documenting recanalization) contributes favorably or negatively to clinical trial recruitment. We convened a panel of experts in both clinical trial design and IAT to review and critique proposed inclusion/exclusion criteria. Shortly after the publication of the key IAT studies, we surveyed our clinical trial sites to assess their local implementation of IAT selection criteria. On the basis of the summarized published primary results and published society guidelines, expert reviews, and local implementation at our sites, we defined IAT selection criteria for RHAPSODY. We adopted a clinical trial protocol amendment to allow IAT based on these selection criteria and carefully monitored the implementation of the amended protocol in the study sites. Here, we report on the results of the review process and the impact of the amendment on study recruitment.

### Methods

The RHAPSODY trial (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 APC, in combination with tissue-type plasminogen activator, mechanical thrombectomy, or both in moderate-to-severe acute ischemic stroke, NCT02222714)
is a multicenter, prospective, randomized, controlled, double-blinded phase 2 study intended to evaluate the safety, pharmacokinetics, and preliminary efficacy of 3K3A-APC after tissue-type plasminogen activator or mechanical thrombectomy or both in participants with moderate-to-severe acute ischemic stroke. Approximately 100 participants will be randomized, which includes 88 participants in groups of 4 randomized to either 3K3A-APC or placebo (3:1 ratio) and additional placebo participants enrolled during safety review pauses. This study uses a modified version of the continual reassessment method to establish a maximum tolerated dose.23 For the purposes of this study, we assumed an established background symptomatic intracerebral hemorrhage rate of 3% to 6%.24,24 Correspondingly, the maximum tolerated dose is defined as the highest dose with a dose-limiting toxicity rate of ≤10%. Participants are enrolled at the dose estimated from the assumed dose–response model and previous data to be closest to the maximum tolerated dose. Dose-limiting toxicities are assessed from the first dose to 48 hours after the last dose of study treatment. An elevation of partial thromboplastin time, symptomatic intracerebral hemorrhage, systemic bleeding requiring transfusion, or hepatic toxicity that seems related to study treatment is considered a dose-limiting toxicity. Abnormal laboratory values and any event that prompts cessation of study drug are evaluated as possible dose-limiting toxicity as well.

3K3A-APC is administered as a 100 mL IV infusion over 15 minutes every 12 hours (±1 hour) for 5 doses. Four dose levels of 3K3A-APC will be considered: 120, 240, 360, and 540 µg/kg. Matching placebo, visually indistinguishable from the drug, is administered in the same manner as the active product. The drug may not begin until 30 minutes after the end of the recombinant tissue-type plasminogen activator (r-tPA) infusion, for safety reasons, and no later than 120 minutes after completion of r-tPA infusion or initiation of mechanical thrombectomy (skin puncture), whichever is sooner, although investigators are encouraged to begin study drug infusion as soon as possible. Patients will be examined on days 7, 14, 30, and 90 for safety and outcome. Participants are not considered part of the intent-to-treat cohort until they receive any amount of 3K3A-APC or placebo. Thus, subjects who become ineligible (eg, rapid responder whose NIHSS [National Institutes of Health Stroke Scale] drops to <5) between randomization and initiation of study drug will be removed from the study and replaced. Detailed brain imaging is required before enrollment and at 24 hours, 7 days, 30 days, and 90 days after enrollment and will be analyzed for ischemic stroke size and hemorrhagic transformation.

After the publication of studies reporting efficacy of IAT, we sought to assess the readiness of our study sites to include IAT in the treatment of enrolled patients. Each site provided any written guidance they used for specifying the patients they would consider eligible for IAT therapy. The results were summarized and sites that provided insufficient responses were contacted and asked to consider resubmitting. Next, we assembled a panel of experts including principal investigators of some of the main IAT publications. The IAT selection document from each site was then reviewed and approved, rejected, or queried. Finally, using the input from the expert panel and the sites, the trial leadership designed study selection criteria for allowing IAT-treated patients. Clinical trial recruitment was tracked before and after adoption of the protocol amendment. Additional changes implemented as part of the same protocol amendment were as follows: extended the upper time window for administration of investigational drug from 90 to 120 minutes, extended the upper age range from 80 to 90, and broadened the NIHSS range to 25 (from 7–20 previously). Criteria specific to IAT were also added: onset time to arterial puncture time ≤6 hours and a baseline computed tomography revealing large core occlusions as defined by local protocol, ASPECTS score, or computed tomography perfusion.

### Results

The key variables that influence efficacy and safety outcomes included in the published IAT trials are summarized in Table 2. Despite very different trial designs, the 5 trials actually enrolled samples that showed relatively consistent age, severity, and times to important milestones. The unexpected demographic consistency among these trials allows some specificity in designing clinical trial selection criteria.

The most critical factor influencing outcome after IAT is the incidence of successful recanalization. The main trials’ recanalization rates are summarized in Table 3. Because of a wide variation in the method for determining recanalization, the rates summarized in Table 3 are not uniformly comparable among the trials. All trials except ESCAPE (Endovascular Treatment for Small Core and Anterior Circulation Proximal Occlusion With Emphasis on Minimizing CT to Recanalization Times) used the mTICI score (modified Thrombolysis in Cerebral Infarction) to assess recanalization in the intervention arm; ESCAPE used the TICI score. ESCAPE used the mAOI score (modified Arterial Occlusive Lesion) to assess recanalization on repeat computed tomographic angiogram (within 2–8 hours of tissue-type plasminogen activator) in the control arm; some other trials assessed recanalization/reperfusion in the control arm (not in all patients) after 24 hours.23,25 The critical safety outcomes include symptomatic intracerebral hemorrhage and mortality. As shown in Table 3, the rate of symptomatic intracerebral hemorrhage in the published IAT trials is comparable, or perhaps lower than that seen in previous trials of intravenous thrombolysis. Mortality is similarly comparable to published trials of intravenous thrombolytic therapy.

After reviewing the assembled protocols from our study sites, as well as the results from published IAT trials, we

### Table 2. Intra-Arterial Trials’ Study Factors Relevant to Neuroprotection

<table>
<thead>
<tr>
<th>Trial</th>
<th>Sample Size, n</th>
<th>Median Time to IAT, min</th>
<th>Maximum Time to IAT, h</th>
<th>Mean Age, IA vs Control, y</th>
<th>Median NIHSS, IA vs Control</th>
<th>Median Time to IVT, IA vs Control, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESCAPE</td>
<td>316</td>
<td>200</td>
<td>12</td>
<td>71/70</td>
<td>16/17</td>
<td>110/125</td>
</tr>
<tr>
<td>EXTEND-IA</td>
<td>70</td>
<td>210</td>
<td>6</td>
<td>69/70</td>
<td>17/13</td>
<td>127/145</td>
</tr>
<tr>
<td>MR CLEAN</td>
<td>500</td>
<td>260</td>
<td>6</td>
<td>66/66</td>
<td>17/18</td>
<td>85/87</td>
</tr>
<tr>
<td>REVASCAT</td>
<td>206</td>
<td>269</td>
<td>8</td>
<td>66/67</td>
<td>17/17</td>
<td>117/105</td>
</tr>
<tr>
<td>SWIFT PRIME</td>
<td>196</td>
<td>224</td>
<td>6</td>
<td>65/66</td>
<td>17/17</td>
<td>110/117</td>
</tr>
</tbody>
</table>

For the 5 major trials of IAT for acute stroke that showed benefit, the key factors are summarized. Median time to IAT is the median of the time from symptom onset (or last known well) to the initiation of the procedure, in min. Maximum time to IAT is the maximum protocol-allowed time to initiate recanalization therapy after stroke symptom onset. Mean age, median NIHSS, and mean (or median in ESCAPE and MR CLEAN) time to bridging therapy with IV r-tPA were comparable in the groups compared in all studies. IA indicates intra-arterial; IAT, intra-arterial neurothrombectomy; IVT, intravenous therapy; NIHSS, National Institutes of Health Stroke Scale; and r-tPA, recombinant tissue-type plasminogen activator.
Recanalization is predicted to improve the odds of success in a neuroprotectant clinical stroke trial. The outcomes of most relevance in designing selection criteria for a neuroprotection trial are rates of successful reperfusion, eventual disability (mRS), sICH, and mortality. Although trials reported successful recanalization at different times, we looked for late recanalization meaning sometime after the immediate treatment period, typically 24 h after IAT. IAT, intra-arterial neurothrombectomy; mAOL, modified Arterial Occlusive Lesion; mRS, modified Rankin Score; sICH, symptomatic intracerebral hemorrhage; and TICI, Thrombolysis in Cerebral Infarction.

*Defined as achieving TICI score of 2b/3.
†Defined as achieving mAOL score of 2 or 3.
‡Defined as reperfusion >90% without sICH.
§Defined as achieving the modified TICI 2b/3.
‖Defined as reperfusion ≥90%.

Table 3. Intra-Arterial Trial Results Relevant to Neuroprotection

<table>
<thead>
<tr>
<th>Trial</th>
<th>Late Recanalization or Reperfusion (%) IA vs Control</th>
<th>mRS 0–2 IA vs Control</th>
<th>sICH, % IA vs Control</th>
<th>Mortality IA vs Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESCAPE</td>
<td>72.4%/31.2†</td>
<td>53/29.3</td>
<td>3.6/2.7</td>
<td>10.4/19.0</td>
</tr>
<tr>
<td>EXTEND-IA</td>
<td>89%/34‡</td>
<td>71/40</td>
<td>0/6</td>
<td>9/20</td>
</tr>
<tr>
<td>MR CLEAN</td>
<td>84%/57.5‡</td>
<td>32.6/19.1</td>
<td>7.6/6.4</td>
<td>21/22</td>
</tr>
<tr>
<td>REVASCAT</td>
<td>66%/not reported</td>
<td>43.7/28.2</td>
<td>1.9/1.9</td>
<td>18.4/15.5</td>
</tr>
<tr>
<td>SWIFT PRIME</td>
<td>83%/40I</td>
<td>60/35</td>
<td>0/3</td>
<td>9/12</td>
</tr>
</tbody>
</table>

Derived a set of selection criteria that reflect consistency among the sites and comparability to the published IAT trials (Table 4). The protocol amendment allowing IAT (as well as relaxed age, NIHSS, and time limits) in the RHAPSODY trial was adopted on May 1, 2015, and fully implemented at all sites by June 18, 2015. For the purposes of this analysis, we examined recruitment as of February 22, 2016. Recruitment before and after the amendment is shown in the Figure and in Table 5, which document considerable improvement in trial recruitment. Before the amendment, 8 patients were enrolled from 601 screened patients (1.3%), whereas after the amendment, 51 patients (2 IAT only, 25 IV r-tPA with IAT, and 24 IV r-tPA only) were enrolled from 821 screened patients (6.2%)

Table 4. Selection Guidelines in a Neuroprotectant Trial

<table>
<thead>
<tr>
<th>Variable</th>
<th>Allowed Range</th>
<th>Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>&lt;80–90</td>
<td>&lt;90</td>
</tr>
<tr>
<td>Time: door to puncture, min</td>
<td>90–120</td>
<td>90</td>
</tr>
<tr>
<td>Time: LKNW to revascularization, h</td>
<td>5–8</td>
<td>6</td>
</tr>
<tr>
<td>Devices</td>
<td>Any second- or third-generation device</td>
<td>Stent retrievers</td>
</tr>
<tr>
<td>NIHSS</td>
<td>5 to 32</td>
<td>≥5</td>
</tr>
<tr>
<td>Imaging modality</td>
<td>CT or MRA</td>
<td>NCCT</td>
</tr>
<tr>
<td>Vascular imaging</td>
<td>ELVO on any modality</td>
<td>ELVO on CTA/MRA</td>
</tr>
<tr>
<td>Perfusion imaging</td>
<td>Mismatch</td>
<td>Salvageable penumbra</td>
</tr>
<tr>
<td>Core size</td>
<td>ASPECTS &gt;6, CT hypo &gt;1/3, DWI &gt;70 cc</td>
<td>ASPECTS &gt;7</td>
</tr>
</tbody>
</table>

Based on our review of the published trials, as well as the user survey from our 15 clinical trial sites, we were able to identify an allowed range of selection criteria. The RHAPSODY target criteria are selected from the allowed range for our use in the RHAPSODY trial, but future clinical trialists could consider any value within the allowed range, based on their own considerations of the stroke population they wish to target. For example, an ASPECTS score >7 was required by the RHAPSODY steering committee to elevate the presumed safety of this early development, phase 2 trial, even though some IAT trials admitted patients with more severe core size measurements. ASPECTS indicates Alberta Stroke Program Early CT Score; CT, computed tomography; CTA, computed tomographic angiogram; CT hypo, early hypodensity visible on NCCT; DWI, diffusion-weighted imaging hyperintensity; ELVO, eligible large vessel occlusion; IAT, intra-arterial neurothrombectomy; LKNW, last known normal or well; MRA, magnetic resonance angiography; MRI, magnetic resonance imaging; NCCT, noncontrast cranial computed tomography; and NIHSS, National Institutes of Health Stroke Scale.

With no change in the number of sites that were recruiting, clinical trial recruitment depends on the number of active enrolling sites and the inclusion/exclusion criteria: the gross recruitment rate before the amendment was 0.11 patients per site per month and after the amendment was 0.43 patients per
site per month. The final version of the RHAPSODY patient selection criteria is listed in the online-only Data Supplement.

Discussion

We sought to incorporate IAT into a clinical trial of neuroprotection for acute ischemic stroke. To our knowledge, there is no previous demonstration of the feasibility of this approach. On the basis of a summary of the published IAT trials and the selection criteria in use at 15 clinical trial study sites, we identified a range of acceptable selection criteria that could be used in similar trials (Table 4). After implementing these criteria, as well as relaxed age, NIHSS, and timing limits, trial recruitment accelerated dramatically (Figure). The data suggest that it is feasible and likely desirable to allow IAT in the context of a neuroprotectant stroke trial.

A plethora of putative neuroprotectants has emerged from preclinical testing, and many have shown early promise in small phase 1 or phase 2 trials. After testing in large, adequately powered, properly blinded, and randomized pivotal trials, however, no neuroprotectant strategy has proven successful. Preclinical studies typically include recanalization (although permanent vessel occlusion can be modeled), and combining a putative neuroprotectant with recanalization may provide the greatest chance for success. Some putative neuroprotectants successfully reduced infarct volumes in animals only if administered before recanalization/reperfusion. Such drugs might not be practical for clinical use in which recanalization with IV r-tPA or IAT must occur as quickly as possible after hospital arrival. Clinical trialists designing protocols for such agents may, therefore, wish to modify the selection criteria offered here.

Other differences between human and preclinical studies also matter: such experimental models typically involve young animals free of comorbid conditions (eg, diabetes mellitus and hypertension) that impact outcome after stroke, although the putative compound in the RHAPSODY trial, 3K3A-APC, has shown effectiveness in aged and in hypertensive animals. Another critical factor in demonstrating neuroprotection is timing: drugs that show benefit in experimental models when given within minutes or an hour of recanalization have rarely been so tested in clinical trials. In RHAPSODY, we require study drug infusion as soon as possible after consent, but no later than 120 minutes after skin puncture (or r-tPA completion). In this way, we suggest that the protocol is optimized to show benefit because the drug will be combined with proven recanalization as verified during IAT. An alternative approach would be to require recanalization before study drug treatment, thus limiting generalizability to patients who receive IAT alone or r-tPA plus IAT. This approach could be the ideal one, especially for a treatment in which preclinical testing suggests that recanalization is absolutely necessary for drug efficacy. Although we chose in RHAPSODY to allow patients treated with only IV r-tPA to allow broader generalization of the results, we suggest that other trialists may choose differently under different circumstances.

The selection criteria listed in Table 4 represent a compromise between the need for loosened criteria to allow more rapid enrollment versus tighter criteria to focus the study on patients most likely to respond. An upper limit on age is arbitrary but needed for regulatory approval of the clinical trial protocol. The limit on times (door to puncture and last known normal or well to revascularization) are the most important because no variable impacts the likelihood of favorable outcome more than time. The lower limit on NIHSS serves to select a population more likely to show large vessel occlusion, a requirement for IAT. The requirement for demonstration of salvageable penumbra or limited volume of irretrievable core, or both, is controversial: although there is no sense—and some potential risk—in reperfusing dead tissue, it is unclear whether current imaging methods unequivocally identify brain beyond salvage. Nevertheless, for acceptance at both regulatory and site-investigator levels, some limit on infarct volume seemed important.

Our findings should be interpreted with some limitations in mind. Most importantly, the 15 study sites in RHAPSODY are carefully selected and currently participate in the National Institute of Neurological Disorders and Stroke-sponsored NeuroNEXT clinical trial network. The sites were selected through a competitive process and have demonstrated commitment to clinical trial quality, regulatory compliance, and recruitment/retention. They all joined the study simultaneously, and participation in competing clinical trials was discouraged. On the contrary, when NeuroNEXT was being organized, there was no premium allotted to selecting study sites with demonstrated capability as comprehensive stroke centers. Thus, the trial includes some centers with world-class recognition for their neurointerventional programs and other sites with more typical capabilities in delivering IAT. Therefore, our results would likely predict the experience to be seen in other clinical


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Disclosures

P. Lyden is the recipient of U01 NS088312 and K. Pryor the recipient of X01 NS088312, both titled “A multicenter, phase 2 study using a CRM to determine the safety and tolerability of 3K3A-APC in combination with tPA in moderately severe acute hemispheric ischemic stroke.” K. Pryor is an employee of, and H. Levy is consultant to, ZZ Biotech, LLC. The other authors report no conflicts.


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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):
  - $\geq 3 \times \text{ULN}$ of alanine aminotransferase (ALT) or aspartate aminotransferase (AST),
  - Serum total bilirubin (TBL) $>2\times\text{ULN}$, without initial findings of cholestasis (serum alkaline phosphatase (ALP) activity $>2 \times \text{ULN}$,
  - 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\cite{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

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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