Final Results of the RHAPSODY Trial: A Multi-Center, Phase 2 Trial Using a Continual Reassessment Method to Determine the Safety and Tolerability of 3K3A-APC, A Recombinant Variant of Human Activated Protein C, in Combination with Tissue Plasminogen Activator, Mechanical Thrombectomy or both in Moderate to Severe Acute Ischemic Stroke

Patrick Lyden, MD,[1] Kent E. Pryor, PhD,[2] Christopher S. Coffey, PhD,[3] Merit Cudkowicz, MD, MSc,[4] Robin Conwit, MD,[5] Ashutosh Jadhav, MD, PhD,[6] Robert N. Sawyer, Jr MD,[7] Jan Claassen, MD,[8] Opeolu Adeoye, MD,[9] Shlee Song, MD,[1] Peter Hannon, MD,[10] Natalia S. Rost, MD, MPH,[4] Archana Hinduja, MD,[11] Michel Torbey, MD, MPH,[11] Jin-Moo Lee, MD, PhD,[12] Curtis Benesch, MD,[13] Michael Rippee, MD,[14] Marilyn Rymer, MD,[14] Michael T. Froehler, MD, PhD,[15] E. Clarke Haley, MD,[16] Mark Johnson, MD,[17] Jon Yankey, MS,[3] Kim Magee, MS,[3]

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Address correspondence to Dr Patrick Lyden, Department of Neurology, 127 South San Vicente Boulevard, AHSP A8318, Los Angeles, CA 90048. Email: lydenp@cshs.org

[†]NN104 Investigators are listed in the Appendix.

From the [1]Cedars-Sinai Medical Center, Los Angeles, CA; [2]ZZ Biotech, LLC, Houston, TX; [3]The University of Iowa, Iowa City, IA; [4]Massachusetts General Hospital, Neurological Clinical Research Institute, Boston, MA; [5]National Institutes of Health, National Institute of Neurological Disorders and Stroke, Bethesda, MD; [6]University of Pittsburgh Medical School, Pittsburgh, PA; [7]State University of New York-University at Buffalo, Buffalo, NY; [8]Neurological Institute, Columbia University, New York, NY; [9]Department of Emergency Medicine, University of Cincinnati, Cincinnati, OH; [10]University of Utah, Salt Lake City, UT; [11]Ohio State University Medical Center, Columbus, OH; [12]Barnes-Jewish Hospital, St. Louis, MO; [13]University of Rochester Medical Center, Rochester, NY; [14]The University of Kansas Hospital, Kansas City, KS; [15]Cerebrovascular Program, Vanderbilt University Medical Center, Nashville, TN; [16]University of Virginia, Charlottesville, VA; [17]Southwestern Medical Center, University of Texas, Dallas, TX; [18]Consultant, ZZ Biotech, LLC; [19]The MRI Institute for Biomedical Research, Bingham Farms, MI; [20]Department of Medical Pharmacology, College of Medicine, University of Arizona, Tucson, AZ; [21]Laboratory of Neuro Imaging, Institute of Neuroimaging and Informatics, Keck School of Medicine, University of Southern California Los Angeles, Los Angeles, CA; [22]The Scripps Research Institute, La Jolla, CA; and [23]Zilkha Neurogenetic Institute and Department of Physiology and Neuroscience, Keck School of Medicine, University of Southern California Los Angeles, Los Angeles, CA

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Julie Qidwai, MS,[3] Howard Levy, MD, PhD,[18] E. Mark Haacke, PhD,[19] Miller Fawaz, MS,[19] Thomas P. Davis, PhD,[20] Arthur W. Toga, PhD,[21] John H. Griffin, PhD,[22] and Berislav V. Zlokovic, MD, PhD[23]; and the NeuroNEXT Clinical Trials Network NN104 Investigators[†]

Objective: Agonism of protease-activated receptor (PAR) 1 by activated protein C (APC) provides neuro- and vasculoprotection in experimental neuroinjury models. The pleiotropic PAR1 agonist, 3K3A-APC, reduces neurological injury and promotes vascular integrity; 3K3A-APC proved safe in human volunteers. We performed a randomized, controlled, blinded trial to determine the maximally tolerated dose (MTD) of 3K3A-APC in ischemic stroke patients.
Methods: The NeuroNEXT trial, RHAPSODY, used a novel continual reassessment method to determine the MTD using tiers of 120, 240, 360, and 540 μg/kg of 3K3A-APC. After intravenous tissue plasminogen activator, intra-arterial mechanical thrombectomy, or both, patients were randomized to 1 of the 4 doses or placebo. Vasculoprotection was assessed as microbleed and intracranial hemorrhage (ICH) rates.
Results: Between January 2015 and July 2017, we treated 110 patients. Demographics resembled a typical stroke population. The MTD was the highest-dose 3K3A-APC tested, 540 μg/kg, with an estimated toxicity rate of 7%. There was no difference in prespecified ICH rates. In exploratory analyses, 3K3A-APC reduced ICH rates compared to placebo from 86.5% to 67.4% in the combined treatment arms ($p = 0.046$) and total hemorrhage volume from an average of 2.1 ± 5.8 ml in placebo to 0.8 ± 2.1 ml in the combined treatment arms ($p = 0.066$).
Interpretation: RHAPSODY is the first trial of a neuroprotectant for acute ischemic stroke in a trial design allowing thrombectomy, thrombolysis, or both. The MTD was 540 μg/kg for the PAR1 active cytoprotectant, 3K3A-APC. A trend toward lower hemorrhage rate in an exploratory analysis requires confirmation.
Clinical Trial Registration: Clinical Trial Registration-URL: http://www.clinicaltrials.gov. Unique identifier: NCT02222714.

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Activated protein C (APC) is a blood protease with anticoagulant and cell-signaling activities mediated by protease-activated receptor 1 (PAR1).[1] APC and analogs with cell-signaling cytoprotective activities provided beneficial effects in preclinical models of central nervous system disorders, including stroke, brain trauma, multiple sclerosis (MS), and amyotrophic lateral sclerosis (ALS); systemic disorders such as sepsis, ischemic, and reperfusion injury of heart and kidney; liver, pulmonary, kidney, and gastrointestinal inflammation; and diabetes and lethal whole-body radiation.[1,2] To decrease bleeding risks, the native APC structure was mutated: 3 lysines were replaced by alanines (K191A-K192A-K193A), removing >90% of the anticoagulant activity while preserving multiple cell-signaling activities.[3–5] The resultant molecule, 3K3A-APC, exhibits powerful cytoprotection in rodent stroke models,[6–8] brain trauma,[9] ALS,[10] and MS.[11] 3K3A-APC acts by transmembrane G-protein-coupled PAR1 to protect neurons, brain endothelial cells, and blood–brain barrier (BBB) directly in vivo and in vitro,[8,12–14] and it also reduces "reperfusion injury" by multiple vasculoprotective effects.[21] 3K3A-APC has satisfied 10 in 10 of the Stroke Treatment Academic Industry Roundtable (STAIR) suggestions for preclinical drug safety and efficacy assessment[2] and has an established safety and pharmacokinetic profile in human volunteers.[15,16]

The combination of 3K3A-APC with recanalization reduced postreperfusion hemorrhages in a preclinical model using recombinant tissue plasminogen activator (rt-PA) after embolic stroke.[12] Additional studies in spontaneously hypertensive rats and aged female mice showed that 3K3A-APC and rt-PA treatment reduced infarction, and that 3K3A-APC extended the therapeutic window of t-PA.[8] When rt-PA was withheld for 4 hours after ischemia onset, it provided no benefit, but introduced bleeding; 3K3A-APC provided benefit in the absence of rt-PA, showed the same benefit when given after the ineffective rt-PA, and eliminated rt-PA–associated bleeding.

The NeuroNEXT trial NN104 (RHAPSODY) was a randomized, controlled, blinded dose-escalation safety trial for 3K3A-APC. The primary objective of this phase 2A trial was to evaluate the safety of ascending intravenous (IV) doses of 3K3A-APC in adult patients presenting with acute ischemic stroke who were eligible for thrombolysis, thrombectomy, or both. There were 2 secondary objectives: to determine the pharmacokinetic parameters of 3K3A-APC in stroke patients (to be reported elsewhere) and to seek evidence of vasculoprotection, measured as reduced hemorrhage frequency, or severity attributable to 3K3A-APC treatment during recanalization therapy.

Participants and Methods

The RHAPSODY trial (a multicenter, phase 2 trial using a continual reassessment method to determine the safety and tolerability of 3K3A-APC, a recombinant variant of human APC, in combination with t-PA, mechanical thrombectomy, or both in moderate-to-severe acute

ischemic stroke; NCT02222714) protocol has been published.[17] The final version of the RHAPSODY protocol is available from the authors. We conducted a multicenter, prospective, randomized, controlled, double-blinded, phase 2a trial to evaluate the safety, pharmacokinetics, and preliminary efficacy of 3K3A-APC following t-PA or mechanical thrombectomy or both in participants with moderate-to-severe acute ischemic stroke. Subjects who signed consent (or whose representatives provided surrogate consent) and met all inclusion and exclusion criteria were randomized into the trial using the NeuroNEXT Interactive Web Response System. Twenty-two cohorts of 4 trial participants each were randomized, and each cohort included 3 participants randomized to the study drug dose chosen for that cohort and 1 participant randomized to placebo. Trial inclusion/exclusion criteria are shown in Table 1. Randomized subjects who experienced significant neurological improvement (defined as an National Institutes of Health Stroke Scale [NIHSS] score < 5) before receipt of study drug—"Early Responders"—and other randomized subjects who did not receive study drug for any reason were not included in the study and were replaced in the randomization sequence and Continual Reassessment Method (CRM) cohort. Any subject who received any amount of study drug or placebo was considered "enrolled" and followed to the end of the trial. In

TABLE 1. Excerpted Inclusion/Exclusion Criteria used in RHAPSODY

Inclusion	Exclusion
1. Age 18 to 90 years, inclusive	1. Rapid spontaneous improvement
2. Acute ischemic stroke defined as focal, neurological deficit(s), secondary to a presumed vascular occlusive event	2. History of stroke within 90 days
3. Able to receive IV t-PA per local standard of care *or* begin mechanical thrombectomy per local standard of care	3. History of previous or current diagnosis of intracranial hemorrhage
4. NIHSS score ≥ 5 at time of randomization	4. Moyamoya disease, cerebral AVM, or known unsecured aneurysm requiring intervention
5. Signed informed consent by subject or authorized representative	5. Presence of other neurological or non-neurological comorbidities
6. Agreement to use effective birth control throughout the study (ie, day 90)	6. Premorbid mRS score of ≥2
7. Willing (subject and/or caretaker) to commit to follow-up assessments	7. Mechanical thrombectomy subjects only: baseline noncontrast 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
8. Mechanical thrombectomy subjects only: onset (last-seen-well) time to arterial puncture time < 6 hours	Non-neurological
	8. Prolonged prothrombin time (INR >1.7)
	9. Prolonged PTT
	10. Use of heparin within 48 hours
	11. Severe hypertension (systolic BP >185 mmHg or diastolic BP >110 mmHg) or hypotension (systolic BP <90 mmHg) that does not respond to simple treatment (eg, 1 dose of labetalol or nicardipine infusion)
	12. Estimated GFR <35 ml/min
	13. Blood glucose concentration < 50 mg/dL
	14. Past exposure to any exogenous form of APC
	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 preceding 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

This table shows an abbreviated version of the inclusion and exclusion criteria. A full version is available from the authors.
AVM = arteriovenous malformation; APC = activated protein C; CT = computed tomography; GFR = glomerular filtration rate; INR = international normalized ratio; IV = intravenous; MRI = magnetic resonance imaging; mRS = modified Rankin Score; NIHSS = National Institutes of Health Stroke Scale; PTT = partial thromboplastin time; t-PA = tissue plasminogen activator.

response to reported efficacy of intra-arterial thrombectomy (IAT), the protocol was amended on May 1, 2015 to include patients undergoing IAT within 6 hours of symptom onset.[17] Additional changes extended the upper time window for administration of investigational drug from 90 to 120 minutes; extended the upper age range from 80 to 90 years; and broadened the NIHSS range to >5 (from 7 to 20 previously).[18] Criteria specific to IAT were also added: onset time to arterial puncture time < 6 hours and a baseline computed tomography (CT) excluding large core infarctions.

The primary outcome of this trial was to establish a maximum tolerated dose (MTD)[19] of the study drug using a modified version of the CRM. We defined the MTD as the highest study dose with an estimated dose limiting toxicity (DLT) rate of ≤10%. We assessed for DLTs until 48 hours following the last study drug dose. Predefined DLTs included: symptomatic intracerebral hemorrhage (ICH), defined as blood present on CT or magnetic resonance imaging (MRI) brain image associated with neuroworsening (≥4-point increase on the NIHSS not attributed to iatrogenic causes such as sedation) that, in the opinion of the investigator, represented a clinically significant change attributable to the hemorrhage (assumed background rate of 3–6%[20–24]); an activated partial thromboplastin time (aPTT) at 1 hour after treatment that reached 2× the local upper limit of normal defined; findings that meet all 3 components of Hy's law[25,26]; any other bleeding event classified as serious by the site investigator, or any bleeding that required more than 2 units of packed red blood cells over any 2 consecutive days; any CTCAE v4.03 Grade 3 laboratory value[27] that, in the opinion of the investigator, was related to treatment; or any adverse event that, in the opinion of the investigator, was related to treatment and led to cessation of further dosing. Subarachnoid hemorrhage in subjects who received IAT was *not* considered a DLT.

In a CRM approach, dose cohorts are filled sequentially, with a dose-blinded safety review after each cohort. Based on preclinical work and a pilot phase 1 trial, we selected 4 dose levels of 3K3A-APC to test: 120, 240, 360, and 540 μg/kg. To initiate the CRM process, the following DLT frequencies were predefined in each dose tier: 120, 5%; 240, 7%; 360, 9%; and 540, 11%. The initial patient cohort was started at the lowest dose level (120 μg/kg). From there, dose escalations were driven by the CRM. After each cohort of 4 patients (1 placebo and 3 treated) was filled, an Internal Medical Monitor (IMM) blinded to dose level reviewed the cohort for adverse events that could be considered a DLT. The IMM or site investigator (also blinded to dose) referred such events to a Safety Review Committee (SRC) who reviewed each proposed event against the protocol definitions of DLT. During the SRC safety review period, the interactive randomization system assigned patients to placebo only, to maintain trial momentum and avoid frequent trial halts and restarts. Once the blinded safety review was completed, confirmed DLTs were reported to the statistical coordinating center and the CRM re-estimated the DLT rate so that a dose adjustment could be made: increase, decrease, or hold. Sites were not informed when the trial was undergoing safety review, so as to maintain trial enthusiasm and momentum. The new dose level was added to the randomization system and the next cohort was opened, again without the sites knowing of the change. After the final group of subjects was enrolled, the final MTD would be the highest dose with estimated toxicity probability ≤10%. The study was to stop once the maximum number of cohorts (22) was observed or 1 of the prespecified safety or efficacy stopping guidelines was triggered.

3K3A-APC was administered as a 100-ml intravenous infusion over 15 minutes every 12 hours (±1 hour) for 5 doses. Drug or visually indistinguishable placebo was prepared by an unblinded pharmacist; all treatment personnel were unaware of the dose and group assignment. Study drug (3K3A-APC or placebo) began no sooner than 30 minutes after the end of the rt-PA infusion, for safety reasons, and no later than 120 minutes following completion of rt-PA infusion or initiation of mechanical thrombectomy (skin puncture), whichever occurred sooner. Within these time limits, the investigators were encouraged to begin infusion as soon as possible after consent. Participants were considered confirmed randomizations, that is, part of the intent-to-treat cohort, only after they received any amount of study drug. Thus, subjects who became ineligible (eg, rapid responders whose NIHSS dropped to <5) between randomization and initiation of study drug were to be removed from the trial and replaced. Detailed brain imaging was required before enrollment, but perfusion imaging was not used to select patients for trial enrollment.

Safety evaluations were collected through day 7 following stroke onset. Subjects were evaluated for DLTs from administration of the first dose to 48 hours following the last dose of treatment. Subjects were seen for assessments on days 7, 14, 30 and 90. MRI scans, using a common protocol that included gradient echo (GRE) or susceptibility-weighted image (SWI) sequences, were obtained at 7, 30, and 90 days after stroke. As with all trials in the NeuroNEXT network, a central institutional review board (cIRB) approved this trial and the consent form. Each trial-site IRB reviewed and accepted the cIRB approval and consent form. Informed consent was obtained from every patient, or an authorized caregiver.

Intracerebral hemorrhage volume—including hematomas, petechiae, and cerebral microbleeds (CMBs)—was quantified from susceptibility-sensitive sequences directly by a central analyst blind to treatment assignment. We used the accepted definition of CMB.[28] The method used to quantify brain hemorrhage is illustrated in Figure 1. Using day 30 magnetic resonance imaging (MRI) as the outcome, we considered a scan "positive for hemorrhage" (including hematoma, petechiae, and CMB) if the corresponding total hematoma volume was larger than 0.06 ml (5 mm in diameter). This value was chosen because it corresponds to the definition of 1 microbleed, and we reasoned that our threshold for detection should be set to miss no more than 1 CMB. Because of the potential for artifact in the semiautomated image analysis (eg, calcium, vein), we also performed a sensitivity analysis for the presence of hemorrhage using several volume thresholds for the positive identification of hemorrhage, using the equivalent of 0, 1, 2, or 3 microbleeds (0, 0.06, 0.5, and 1.8 ml, respectively).

Ischemic lesion and infarction territories were identified on MRI as increased signal intensity in various regions of white matter and gray matter. We labeled lesion and calculated volume using ITK-SNAP software (version 3.6) based on signal intensity as well as considering neuroanatomical features observed on T2-weighted MRI. We first labeled the lesions in the axial plane as our MRI data were acquired axially, and the best resolution was appreciated on this plane. We refined the label shape through the other views (coronal, sagittal) and finally performed volumetry on the primary lesion.

Statistical Analysis

Primary Endpoint. For this implementation of a CRM, we sought 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.[29] For this study, a hyperbolic tangent dose-response model was used, in which

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

After each group of subjects was treated, and their status observed as to whether or not each had a DLT, the posterior mean of a was calculated. Based upon that estimate, $\hat{a}$, the highest dose with estimated toxicity probability, $\psi(x_i^*, a) \leq 10\%$ was allocated to the next group of subjects. If this dose was more than 1 level higher than the previous dose allocated, the dose was to be increased 1 level only. After the final group of subjects was enrolled, the final MTD was to be defined as the highest dose with estimated toxicity probability less than or equal to the target toxicity level of 10%. The study was to stop once either the maximum number of cohorts (22) was observed or prespecified safety or efficacy stopping guidelines had been achieved per the study protocol.

Adverse Events. Adverse event data were summarized based on the MedDRA coding system. Data were summarized within MedDRA preferred terms and overall as percent of subjects within treatment groups (both combined treatment group and individual dose levels) that experienced an event. Adverse events were also summarized according to whether the independent medical monitor deemed the event related to study drug and whether the event was expected. Fisher's exact test was used to compare the percent of subjects with a given event between treatment groups.

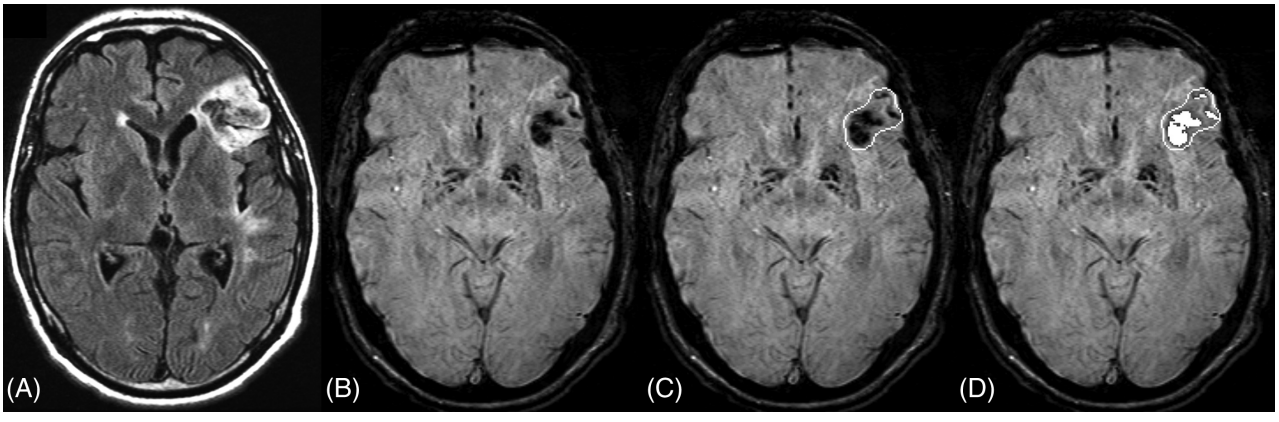


FIGURE 1: Image analysis method for hemorrhage volume quantification. The analyst (unaware of treatment assignment) identified the infarct region using FLAIR (A), then reviewed the susceptibility sequence (B). An object was drawn around abnormal findings (C), and a threshold was applied within the object to outline any hemorrhage (D). The number of pixels lower than the threshold was produced through SPIN software and converted to areas (Spintech Inc., Bingham Farms, MI). FLAIR = fluid-attenuated inversion injury.

Hemorrhage Incidence at Day 30. Analysis of hemorrhage incidence was based on the results of the day 30 MRI scans. Subjects were considered to have had a hemorrhage if the bleeding volume was >0.06 ml. The percent of subjects that experienced a hemorrhage was tabulated by combined treatment group and also by dose groups. The Pearson chi-square test was used to compare rates of hemorrhage between the combined treatment group and the placebo group. If any of the expected cell counts were too small to justify the use of the Pearson chi-square test, Fisher's exact test was used. Additional sensitivity analyses using alternative volume thresholds of >0, >0.5, and > 1.8 ml to determine hemorrhage rates were also performed.

Microbleed Incidence at Day 30. The rates of microbleeding within the infarct zone and outside the infarct zone at day 30 were analyzed separately to compare hemorrhage rates between the 2 groups. Subjects were considered positive if they experienced 1 or more microbleeds.

Modified Rankin Score at Day 90. The modified Rankin score was analyzed by comparing the percent of subjects with a score of 0 or 1 between treatment groups. Comparisons were performed using the same approach to compare hemorrhage rates between the 2 groups.

Barthel Index at Day 90. The Barthel Index was analyzed by comparing the percent of subjects with a score of 90 or larger between treatment groups. Comparisons were performed using the same approach to compare hemorrhage rates between the 2 groups.

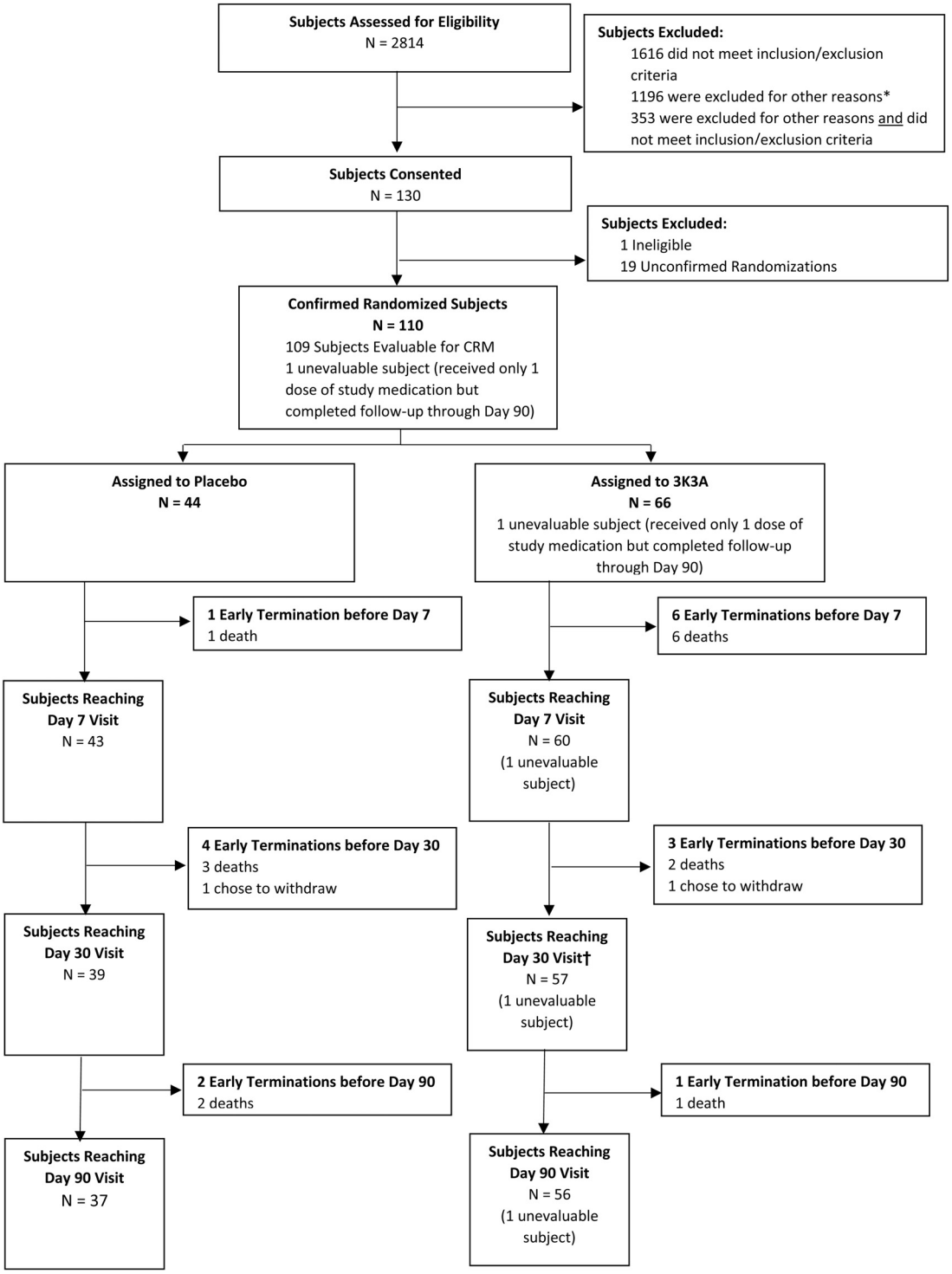
Results

Patients were enrolled between January 2015 and April 2017. Because of the time-compressed nature of acute stroke clinical trials, we screened 2,814 patients, obtained consent from 130 patients, and we enrolled 110 patients into the protocol (Fig 2). Of the 20 consented/not-enrolled patients, 1 patient was found to be ineligible before first dose, 7 became clinically unstable, 7 did not receive study drug before expiration of the enrollment window, and 5 cleared symptoms to NIHSS <5. We filled all planned 22 cohorts of 4 patients and enrolled 22 additional placebo patients during SRC review periods. Of the 110 enrolled subjects, 44 were randomized to placebo and 66 to 3K3A-APC. To maintain trial enthusiasm and enrollment momentum, the trial remained open during safety reviews—although sites were unaware—thus, more placebo patients were enrolled than would have happened by a strict enrollment ratio. The primary analysis—a modified ITT—included all subjects who were confirmed randomizations (received at least 1 dose of study drug) and either: received 2 or more doses of study drug or suffered a confirmed DLT. For the secondary analyses, we included all patients with an evaluable day 30 MRI scan. Demographics of the trial groups are shown in Table 2. Distribution of risk factors traditionally associated with adverse events after stroke were relatively well balanced between placebo and 3K3A-APC–treated patients.

The primary outcome—the highest dose associated with an estimated toxicity <10%—was the 540-μg/kg tier, with an estimated toxicity (DLT rate) of 7%. The final actual DLT rates are shown in Table 3 for all dose tiers compared to placebo. Specifically, DLTs occurred in 4 (9%) placebo-treated patients and in 3 (5%) 3K3A-APC–treated patients. The CRM model estimated the DLT frequencies in each group: 3% for 120 μg/kg, 4% for 240 μg/kg, 5% for 360 μg/kg, and 7% for the 540 μg/kg. Thus, the final MTD estimated in the trial is 540 μg/kg.

Adverse events by dose level are shown in Table 3. Adverse events, serious adverse events (SAEs), and hemorrhages all occurred with similar frequency in drug- and placebo-treated groups. Neuroworsening—defined as an increase in the NIHSS by more than 4 points—occurred equally in both groups, 8 (12.1%) 3K3A-APC treated and 8 (15.9%) placebo treated. Mean aPTT after each dose of study drug was not statistically significantly elevated by treatment. There was no evidence of increased hemorrhage rate or volume related to 3K3A-APC, despite its potential anticoagulant property (Table 4). Using the prespecified volume threshold (0.06 ml) for defining "hemorrhage-positive" scans, we found hemorrhage in 56% of 3K3A-APC–treated versus 68% of placebo-treated patients (p = 0.28). In a sensitivity analysis using several volume thresholds for positive identification of hemorrhage, we found less-frequent hemorrhage in the 3K3A-APC–treated group, although this numerical difference reached statistical significance only using the 0-ml threshold (p = 0.046, uncorrected for multiple comparisons). We pursued further evidence of an effect on bleeding with post-hoc, exploratory analyses (Table 4): Using quantitative volumetry, there were smaller hemorrhages in the 3K3A-APC–treated group, but this difference was not statistically significant (p = 0.07). Between the placebo and 3K3A-APC treatment groups, there was no difference in the numbers of microbleeds observed within or outside the infarct zone.

Efficacy outcomes were collected to demonstrate feasibility of collection in future efficacy studies. Incidence of favorable outcome (90-day mRS 0 or 1) was not statistically significantly different from placebo, including all dose tiers together (28 [45.2%] treatment versus



*Other reasons include: window for study drug administration expired (468); subject unable/unwilling to participate (110).
†Six subjects assigned to 3K3A did not have Day 30 visits but completed follow-up through Day 90.

FIGURE 2: CONSORT diagram showing patients screened and then ultimately enrolled. Because of the time-compressed nature of acute stroke clinical trials, we screened 2,814 patients, obtained consent from 130 patients, and enrolled 110 patients into the protocol. Of the 20 consented/not-enrolled patients, 1 patient was found to be ineligible before first dose, 7 became clinically unstable, 7 did not receive drug before expiration of the enrollment window, and 5 cleared symptoms to NIHSS <5. These patients were replaced in the randomization scheme. The primary analysis—a modified intent-to-treat—included all subjects who were confirmed randomizations (n = 110). CRM = Continual Reassessment Method; NIHSS = National Institutes of Health Stroke Scale.

TABLE 2. Demographic Results in Patients Treated With Placebo or Any Dose of 3K3A-APC

	Placebo (n = 44)	3K3A-APC (n = 66)
Males	24 (55%)	29 (44%)
White	36 (82%)	52 (79%)
Hispanic/Latino	7 (16%)	4 (6%)
Age, yr	64 (12.0)	64 (15.2)
Weight (kg)	84 (18.0)	84 (19.1)
Platelet count ≥100 K	42 (96%)	65 (99%)
History: diabetes	18 (41%)	18 (27%)
History: hypertension	33 (75%)	52 (79%)
NIHSS before recanalization[a]	13.5 (5 – 30)	13 (5 – 30)
NIHSS eligibility[a]	11.5 (5 – 28)	12 (5 – 40)
Modified Rankin		
0	41 (93%)	58 (88%)
1	3 (7%)	8 (12%)
≥ 2	0 (0%)	0 (0%)
Recanalization therapy		
- IV t-PA only	24 (55%)	35 (53%)
- IAT only	2 (5%)	3 (5%)
- IV t-PA and IAT	18 (41%)	28 (42%)
Stroke onset to first therapy		
- Time to t-PA initiation	2:07 (1:01)	2:13 (0:58)
- Time to first skin puncture	1:59 (0:44)	2:16 (0:49)
- Time to t-PA initiation or first	5:39 (0:21)	3:31 (1:37)
Skin puncture	1:53 (0:35)	2:02 (1:00)

Patients treated with placebo are compared against patients treated with any dose of 3K3A-APC. Likely attributed to small sample sizes, there were no differences in distribution of any variable among the 5 dose tiers of 3K3A-APC, so these were combined. Continuous variables are shown with standard deviation.

[a]Median (IQR) NIHSS are shown because of the skewed nature of the observed distributions for these variables. Significance testing was not done because of the small sample sizes and exploratory intent of the trial.

APC = activated protein C; IAT = intra-arterial thrombectomy; IV = intravenous; NIHSS = National Institutes of Health Stroke Scale; t-PA = tissue plasminogen activator.

27 [62.8% placebo]); because of small numbers, this result was expected. Similarly, incidence of a favorable 90-day Barthel Index (≥90) was not significantly different among groups, 40 (76.9%) treated versus 34 (91.9%) placebo. Median interquartile range 90-day NIHSS was 1.5 (0.0–4.0; n = 56) in the 3K3A-APC group and 1 (0.0–3.0; n = 37) in the placebo group (difference not significant). Infarct volume (mean ± standard deviation [SD]) at 90 days was similar among all dose tiers and the placebo group; 26.2 ± 32.6 ml in 3K3A-APC–treated patients (n = 56) versus 26.0 ± 42.1 ml in placebo-treated patients (n = 37).

Discussion

In the RHAPSODY trial, we found that all 3K3A-APC dose tiers were well-tolerated. The highest tolerated dose, 540 μg/kg, was associated with an acceptable DLT rate not statistically different from placebo. Rates of adverse events (serious and nonserious) were acceptable and comparable to placebo at all dose tiers. Bleeding (incidence and volume of intracerebral hemorrhage) was not elevated in 3K3A-APC–treated patients. In exploratory analysis, hemorrhage rate and size trended lower in the 3K3A-APC group, compared to placebo, but confirmation of this trend will require a larger clinical trial. Although the trial was designed to find the MTD—and the trial succeeded in this—the optimal dose for further study will likely be lower, given the equivalence among doses.

Pharmacological 3K3A-APC for human use retains the full cytoprotective and regenerative activities of wt-APC with <10% of its anticoagulant activity.[3–5] This decrease in anticoagulant activity of 3K3A-APC relative to wt-APC should reduce the risk of bleeding in patients, which was a serious side effect of wt-APC or DrotAA.[30] In animal models of stroke,[12,13,31] traumatic brain injury,[9] and ALS,[10] 3K3A-APC exerted beneficial effects that were equivalent to, and sometimes greater than, those of wt-APC.[2,6,7,32]

The mechanisms for 3K3A-APC neuroprotection are partly elucidated.[13,14] In extensive murine studies, PAR1 activation by APC exerts protective effects throughout the neurovascular unit because the PAR1 receptor functions on neurons, microglia, and endothelial cells.[2] Although PAR1 was initially discovered and then studied for many years as a thrombin receptor,[33,34] APC signaling by PAR1—which is key for neuroprotection—differs remarkably from PAR1 signaling caused by thrombin. This striking phenomenon whereby 2 agonists, thrombin and APC, cause very different cell signaling effects by the same G-protein–coupled receptor, PAR1, is termed "biased agonism."[1,32,35] Thus, we hypothesize that 3K3A-APC–mediated biased agonism of PAR1 represents a novel strategy that beneficially targets the entire neurovascular unit comprising distinct subsets of cells at the BBB.

TABLE 3. Frequency of Dose-Limiting Toxicity and AEs by Dose Tier

	N (%)				Total Treated vs Total Placebo		
Dose (μg/kg) **N = number treated**	**120** **N = 12**	**240** **N = 16**	**360** **N = 6**	**540** **N = 9**	**3K3A-APC** **N = 43**	**Placebo** **N = 37**	***p***
Dose-limiting toxicities (primary outcome)	0 (0)	2 (8)	1 (8)	0 (0)	3 (5)	4 (9)	0.43
Any AE	12 (80)	20 (83)	9 (75)	15 (100)	56 (85)	41 (93)	0.24
Asymptomatic ICH	2 (13)	4 (17)	4 (33)	5 (33)	15 (23)	17 (39)	0.09
Symptomatic ICH	0 (0)	3 (13)	0 (0)	2 (13)	5 (8)	2 (5)	0.70
Headache	1 (7)	6 (25)	2 (7)	3 (20)	12 (18)	10 (23)	0.63
Any SAE	8 (53)	9 (38)	4 (33)	9 (60)	30 (46)	18 (41)	0.70
Any related SAE	1 (7)	4 (17)	1 (8)	0 (0)	6 (9)	4 (9)	1.00
Any unanticipated SAE	1 (7)	1 (4)	1 (8)	2 (13)	5 (8)	7 (16)	0.22
Any related and unanticipated SAE	0 (0)	0 (0)	1 (8)	0 (0)	1 (2)	1 (2)	1.00
Neuroworsening	2 (13)	3 (13)	0 (0)	3 (20)	8 (12)	7 (16)	0.58

Patients were enrolled nonsequentially into 1 of 4 doses of 3K3A-APC or placebo using an implementation of a Continual Reassessment Method (CRM). Every cohort included 1 placebo and 3 treated patients who all received the dose assigned to that cohort. Extra placebo patients were enrolled during trial pauses for safety review. After filling all 22 planned cohorts, there were 44 placebo-treated and 66 3K3A-APC–treated patients assigned to the dose levels shown. SAEs are summarized by dose level as number (percentage) of subjects with any SAE. SAEs were considered "related" if the Internal Medical Monitor (IMM) categorized the event as probably or possibly related to treatment. An event was "unanticipated" if the event was not previously reported or associated with stroke or 3K3A-APC treatment. Neuroworsening was defined as a ≥ 4-point worsening in the National Institutes of Health Stroke Scale on 2 occasions not attributable to iatrogenic events such as administration of sedation. Dose-limiting toxicities were defined in the protocol (see Participants and Methods) and used to drive the CRM. Distribution of events was balanced across all dose tiers.

AEs = adverse events; APC = activated protein C; ICH = intracranial hemorrhage; SAEs = serious adverse events.

Extensive preclinical testing was done to assure that 3K3A-APC satisfied the consensus statements regarding putative neuroprotective preclinical stroke therapies (STAIR).[36] No therapy, however, has yet proven successful in humans, even some that ostensibly satisfied STAIR guidelines. Stroke models in animals replicate some, but not all, aspects of human strokes: Experimental models typically involve young animals free of comorbid conditions (eg, diabetes, hypertension) that impact outcome after stroke, although notably here, the putative compound in the RHAPSODY trial, 3K3A-APC, was effective in aged and in hypertensive animals.[8] 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 required study drug infusion as soon as possible after consent, but no later than 120 minutes after skin puncture (or rt-PA completion). In this way, the protocol was optimized to show benefit because the drug was combined with attempted recanalization, which included thrombectomy in approximately half the subjects; unfortunately, the protocol did not include post-thrombectomy documentation of recanalization.

Our findings should be interpreted with some limitations in mind. Most important, the trial sites in RHAPSODY currently participate in the National Institute of Neurological Disorders and Stroke–sponsored NeuroNEXT clinical trial network and in general were also certified Comprehensive Stroke Centers. The trial was small from the perspective of detecting an effect on hemorrhage or 90-day outcomes. Although the reduced frequency and volume of hemorrhages seems to favor 3K3A-APC for vasculoprotection, larger samples will be needed to conclude this with confidence. Finally, although treatment with thrombolysis or thrombectomy or both was required in this trial, successful recanalization was not documented in all cases.

In conclusion, we found the MTD for 3K3A-APC to be 540 μg/kg. The drug was well tolerated over several doses, so further dose finding will be necessary based on

TABLE 4. Hemorrhage Incidence, Type, and Volumes 30 Days After Stroke and Treatment

		Dose (μg/kg)						
Hemorrhage Type	**Total**	**120 N = 12**	**240 N = 16**	**360 N = 6**	**540 N = 9**	**3K3A-APC N = 43**	**Placebo N = 37**	***p***
Prespecified in statistical analysis plan								
Patients with ≥1 microbleeds in the infarct zone: N (%)	15 (18.5)	1 (8.3)	2 (12.5)	3 (50.0)	1 (11.1)	7 (16.3)	8 (21.6)	0.54
Hemorrhage (>0.06 ml) Incidence by day 30: N (%)	49 (61.3)	7 (58.3)	11 (68.8)	2 (33.3)	4 (44.4)	24 (55.8)	25 (67.6)	0.28
Sensitivity analysis using alternative volume thresholds								
(>0 ml): N (%)	61 (76.3)	8 (66.7)	13 (81.3)	3 (50.0)	5 (55.6)	29 (67.4)	32 (86.5)	0.046
(>0.5 ml): N (%)	24 (30.0)	4 (33.3)	5 (31.3)	0 (0.0)	1 (11.1)	10 (23.3)	14 (37.8)	0.16
(>1.8 mL): N (%)	13 (16.3)	1 (8.3)	2 (12.5)	0 (0.0)	1 (11.1)	4 (9.3)	9 (24.3)	0.07
Exploratory analyses								
Total hemorrhage volume day 30: ml ± SD	1.4 ± 4.3	0.9 ± 1.9	1.2 ± 2.9	0.04 ± 0.10	0.5 ± 1.2	0.8 ± 2.1	2.1 ± 5.8	0.07
Microbleeds outside the infarct zone (N ± SD)	0.8 ± 2.7	1.8 ± 5.7	1.0 ± 2.6	1.8 ± 3.3	0.2 ± 0.4	1.2 ± 3.6	0.4 ± 0.8	0.75
Patients with ≥1 microbleeds outside the infarct zone: N (%)	20 (25.0)	3 (25.0)	4 (25.0)	2 (33.3)	2 (22.2)	11 (25.6)	9 (24.3)	0.90

Intracerebral hemorrhage was identified from 30-day magnetic resonance imaging. In 63 cases, the imaging was available from susceptibility weighted imaging (SWI) and in 17 cases the gradient echo (GRE) sequence was used. There was no difference among the groups in the frequency of SWI vs GRE. A central reader unaware of any group assignments first assessed total hemorrhage volume using the method shown in Figure 1. Then, the number of microbleeds found in the infarct area or remote from the infarct area were counted. The secondary endpoints that were prespecified in the Statistical Analysis Plan are listed first. Then, we conducted a sensitivity analysis using several volume thresholds for declaring the image "positive" for hemorrhage. Finally, 2 analyses were performed post hoc for exploratory purposes only. Because all these analyses are considered hypothesis generating, there was no correction for multiple comparisons.

SD = standard deviation.

activity and efficacy measures. A suggestion of possible vasculoprotection (fewer and smaller hemorrhages) requires confirmation.

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Author Contributions

P.L., K.P., C.C., M.C., R.C., H.L., E.M.H., M.F., T.D., A.T., J.G., and B.Z. contributed to the conception and design of the study. P.L., C.C., M.C., A.J., R.X., J.C., O.A., S.S., P.H., N.R., A.H., M.T., J.-M.L., C.B., M.R., M.R., M.F., E.C.H., M.J., J.Y., M.K., J.Q., M.H., M.F., and A.T. contributed to the data acquisition and analysis. P.L., K.P., C.C., A.J., R.X., J.C., O.A., S.S., P.H., N.R., A.H., M.T., J.-M.L., C.B., M.R., M.R., M.F., E.C.H., M.J., J.Y., K.M., J.G., and B.Z. drafted and revised the manuscript and figures. All data were collected independently of the corporate sponsor and stored at the University of Iowa, per Network of Excellence in Neuroscience Trials (NeuroNEXT) Standard Operating Procedures. All analyses were approved by the PI and the senior biostatistician. Although company representatives were present on team phone calls, the investigators retained full control over data, event adjudication, analyses, interpretation, and drafting of the manuscript.

Potential Conflicts of Interest

K.P. is an employee of ZZ Biotech LLC. H.L., T.P.D., and J.H.G. are consultants to ZZ Biotech, LLC. B.V.Z. is scientific founder of ZZ Biotech LLC and chairs its Scientific Advisory Board.

References

1. Griffin JH, Zlokovic BV, Mosnier LO. Activated protein C: biased for translation. Blood 2015;125:2898–2907.
2. Zlokovic BV, Griffin JH. Cytoprotective protein C pathways and implications for stroke and neurological disorders. Trends Neurosci 2011; 34:198–209.
3. Guo H, Zhao Z, Yang Q, et al. An activated protein C analog stimulates neuronal production by human neural progenitor cells via a PAR1-PAR3-S1PR1-Akt pathway. J Neurosci 2013;33:6181–6190.
4. Wang Y, Zhao Z, Rege SV, et al. 3K3A-activated protein C stimulates postischemic neuronal repair by human neural stem cells in mice. Nat Med 2016;22:1050–1055.
5. Mosnier LO, Gale AJ, Yegneswaran S, Griffin JH. Activated protein C variants with normal cytoprotective but reduced anticoagulant activity. Blood 2004;104:1740–1744.
6. Griffin JH, Fernandez JA, Lyden PD, Zlokovic BV. Activated protein C promotes neuroprotection: mechanisms and translation to the clinic. Thromb Res 2016;141(suppl 2):S62–S64.
7. Griffin JH, Mosnier LO, Fernandez JA, Zlokovic BV. 2016 Scientific Sessions Sol Sherry Distinguished Lecturer in Thrombosis: thrombotic stroke: neuroprotective therapy by recombinant-activated protein C. Arterioscler Thromb Vasc Biol 2016;36:2143–2151.
8. Wang Y, Zhao Z, Chow N, et al. Activated protein C analog protects from ischemic stroke and extends the therapeutic window of tissue-type plasminogen activator in aged female mice and hypertensive rats. Stroke 2013;44:3529–3536.
9. Walker CT, Marky AH, Petraglia AL, et al. 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.
10. Zhong Z, Ilieva H, Hallagan L, et al. 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: 3437–3449.
11. Han MH, Hwang SI, Roy DB, et al. Proteomic analysis of active multiple sclerosis lesions reveals therapeutic targets. Nature 2008;451: 1076–1081.
12. Wang Y, Zhang Z, Chow N, et al. An activated protein C analog with reduced anticoagulant activity extends the therapeutic window of tissue plasminogen activator for ischemic stroke in rodents. Stroke 2012;43:2444–2449.
13. Guo H, Singh I, Wang Y, et al. Neuroprotective activities of activated protein C mutant with reduced anticoagulant activity. Eur J Neurosci 2009;29:1119–1130.
14. Guo H, Wang Y, Singh I, et al. Species-dependent neuroprotection by activated protein C mutants with reduced anticoagulant activity. J Neurochem 2009;109:116–124.
15. Williams PD, Zlokovic BV, Griffin JH, Pryor KE, Davis TP. Preclinical safety and pharmacokinetic profile of 3K3A-APC, a novel, modified activated protein C for ischemic stroke. Curr Pharm Des 2012;18:4215–4222.
16. Lyden P, Levy H, Weymer S, et al. Phase 1 safety, tolerability and pharmacokinetics of 3K3A-APC in healthy adult volunteers. Curr Pharm Des 2013;19:7479–7485.
17. Lyden P, Weymer S, Coffey C, et al. Selecting patients for intra-arterial therapy in the context of a clinical trial for neuroprotection. Stroke 2016;47:2979–2985.
18. Lyden P. Using the National Institutes of Health Stroke Scale: a cautionary tale. Stroke 2017;48:513–519.
19. Goodman SN, Zahurak ML, Piantadosi S. Some practical improvements in the continual reassessment method for phase I studies. Stat Med 1995;14:1149–1161.
20. Hacke W, Kaste M, Bluhmki E, et al. Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. N Engl J Med 2008;359: 1317–1329.
21. Goyal M, Demchuk AM, Menon BK, et al. Randomized assessment of rapid endovascular treatment of ischemic stroke. N Engl J Med 2015;372:1019–1030.
22. Campbell BC, Mitchell PJ, Kleinig TJ, et al. Endovascular therapy for ischemic stroke with perfusion-imaging selection. N Engl J Med 2015;372:1009–1018.
23. Berkhemer OA, Fransen PS, Beumer D, et al. A randomized trial of intraarterial treatment for acute ischemic stroke. N Engl J Med 2015; 372:11–20.
24. Saver JL, Goyal M, Diener HC; SWIFT PRIME Investigators. Stent-retriever thrombectomy for stroke. N Engl J Med 2015;373:1077.
25. Temple R. Hy's law: predicting serious hepatotoxicity. Pharmacoepidemiol Drug Saf 2006;15:241–243.
26. Reuben A. Hy's law. Hepatology 2004;39:574–578.
27. U.S. Department of Health and Human Services, National Institutes of Health, National Cancer Institute. Common Terminology Criteria for Adverse Events (CTCAE). (V4. 03). NIH Publication. 2015(09-5410). https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40

28. Greenberg SM, Vernooij MW, Cordonnier C, et al. Cerebral microbleeds: a guide to detection and interpretation. Lancet Neurol 2009; 8:165–174.

29. 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.

30. 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;344:699–709.

31. Wang Y, Thiyagarajan M, Chow N, et al. Differential neuroprotection and risk for bleeding from activated protein C with varying degrees of anticoagulant activity. Stroke 2009;40: 1864–1869.

32. Sinha RK, Wang Y, Zhao Z, et al. PAR1 biased signaling is required for activated protein C in vivo benefits in sepsis and stroke. Blood 2018;131:1163–1171.

33. Coughlin SR. Thrombin signalling and protease-activated receptors. Nature 2000;407:258–264.

34. Coughlin SR. Protease-activated receptors in hemostasis, thrombosis and vascular biology. J Thromb Haemost 2005;3:1800–1814.

35. Mosnier LO, Sinha RK, Burnier L, Bouwens EA, Griffin JH. Biased agonism of protease-activated receptor 1 by activated protein C caused by noncanonical cleavage at Arg46. Blood 2012;120:5237–5246.

36. Fisher M, Feuerstein G, Howells DW, et al. Update of the stroke therapy academic industry roundtable preclinical recommendations. Stroke 2009;40:2244–2250.