STRATEGY FOR IMPROVING STROKE TREATMENT RESPONSE (SISTER) TRIAL

Protocol Number: TS23/DS9231-U202

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CONFIDENTIALITY STATEMENT

SUMMARY OF CHANGES FROM PREVIOUS VERSION:

Version	Summary of Revisions Made	Rationale
2.0	Tenecteplase removed as study drug	NIH reviewer and FDA suggestions
2.0	Four doses of TS23 now studied	NIH reviewer and FDA suggestions
2.0	Statistical plan modified	NIH reviewer and FDA suggestions
2.0	Minor clarifications and refinements throughout	Improve operations and communication
2.1	Minor clarifications; typo & grammar errors corrected. Addition of statement that subjects should not receive antithrombotics for at least 24 hours after study drug administration	Improve communication; decrease risk of bleeding
2.2	Page 26: 30-day lab draw language removed	Correction of an error as the 30-day lab draw had been deleted from schedule
3.0	Table of contents – 30±6 hrs. visits changed to ±4 hrs. and consistently changed throughout protocol	Ease of data collection
	1.1 – Endpoints – CT scan changed to 30±4 hrs. and throughout protocol; NIHSS changed to 72 hrs.; SITS-MOST definition added to symptomatic ICH endpoint	Clarification of timing; addition of language
	1.2 Schema amended to reflect the 30 ±4 hr. change	Clarification of timing
	1.3 SoA amended to reflect 30 ±4 hr., PT/INR & aPTT add, 72 hr. NIHSS, and discharge visit changes	Clarification of timing; Addition of research labs
	4.1 Dose escalation adjusted	DSMB request
	6.2.2 Appearance of study drug language	Changed to match IB
	8.1 Laboratory evaluation language change – findings on first imaging added, language to location of vessel occlusion changed' lab processing	Improve communication
	8.2 Relabeling of procedures; CBC language removed as it is SOC	Clarification of timing and processing
	8.3 Relabeling of procedures; 3 ±1 hr. fibrinogen, α2AP, MMP-9 and PKs moved to 8.3, separation of labs for processing	Clarification of timing and processing
	8.4 Renumbered, CT & perfusion language clarified, separation of labs for processing and addition of PT/INR & aPTT, addition of SITS-MOST definition of symptomatic ICH	Improve communication and clarification of timing and processing; additional labs added; language addition
	8.5 72 hr. visit added to include NIHSS and antithrombotic statement	Clarification of timing and addition of language
	8.6 Discharge Visit – NIHSS removed; TOAST specific criteria added	Clarification of timing and language

	8.7 & 8.8 renumbered	Addition of visits prior
	8.8 90 Day visit	Added clarification
	9.3.7 Adverse Events of Special Interest	Addition of symptomatic ICH
	10.2.2 Primary efficacy outcome amended	Improve communication

STATEMENT OF COMPLIANCE

The trial will be conducted in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP), applicable United States (U.S.) Code of Federal Regulations (CFR), and the NINDS Terms and Conditions of Award. The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the Investigational New Drug (IND) sponsor, funding agency and documented approval from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Participants Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

TABLE OF CONTENTS

TABLE OF TABLES

TABLE OF FIGURES

1 PROTOCOL SUMMARY

1.1 Synopsis

Title: Strategy for Improving Stroke Treatment Response (SISTER)

Study Description: *SISTER is a Phase-2, prospective, randomized, placebo-controlled, blinded, dose finding trial that aims to determine the safety and preliminary efficacy of TS23, a monoclonal antibody against the alpha-2 antiplasmin (a2-AP), in acute ischemic stroke.*

Objectives: Primary Objective: To identify a dose of TS23 that is safe and more efficacious than placebo for the treatment of patients from 4.5 to 24 hours of ischemic stroke onset (or last known well), who have evidence of core-penumbra mismatch on perfusion imaging and are not a candidate for standard of care reperfusion therapies.

Endpoints:

Primary Endpoints:

1. Safety: ANY intracranial hemorrhage (ICH) visualized on the CT scan 30 (±4) h after study drug administration.
2. Efficacy: NIH Stroke Scale Score at 30 (±4) h after study drug administration (adjusted for the baseline in analysis)

Secondary Endpoints:

Biomarker Efficacy:

3. a2AP activity at 3 (±1) h after completion of therapy
4. Matrix metalloproteinase-9 (MMP-9) plasma level 3 (±1) h after completion of therapy.
5. % tissue reperfusion on 30 (±4) h perfusion scan compared to the baseline

Clinical Efficacy**:**

1. Improvement in level of global disability (Modified Rankin Scale (mRS) distribution) at 90 (±7) days.
2. Frequency of excellent functional outcome (mRS 0-1) at 90 (±7) days.
3. National Institutes of Health Stroke Scale (NIHSS) at 72 (±12) h (or discharge if sooner; adjusted for the baseline in analysis)

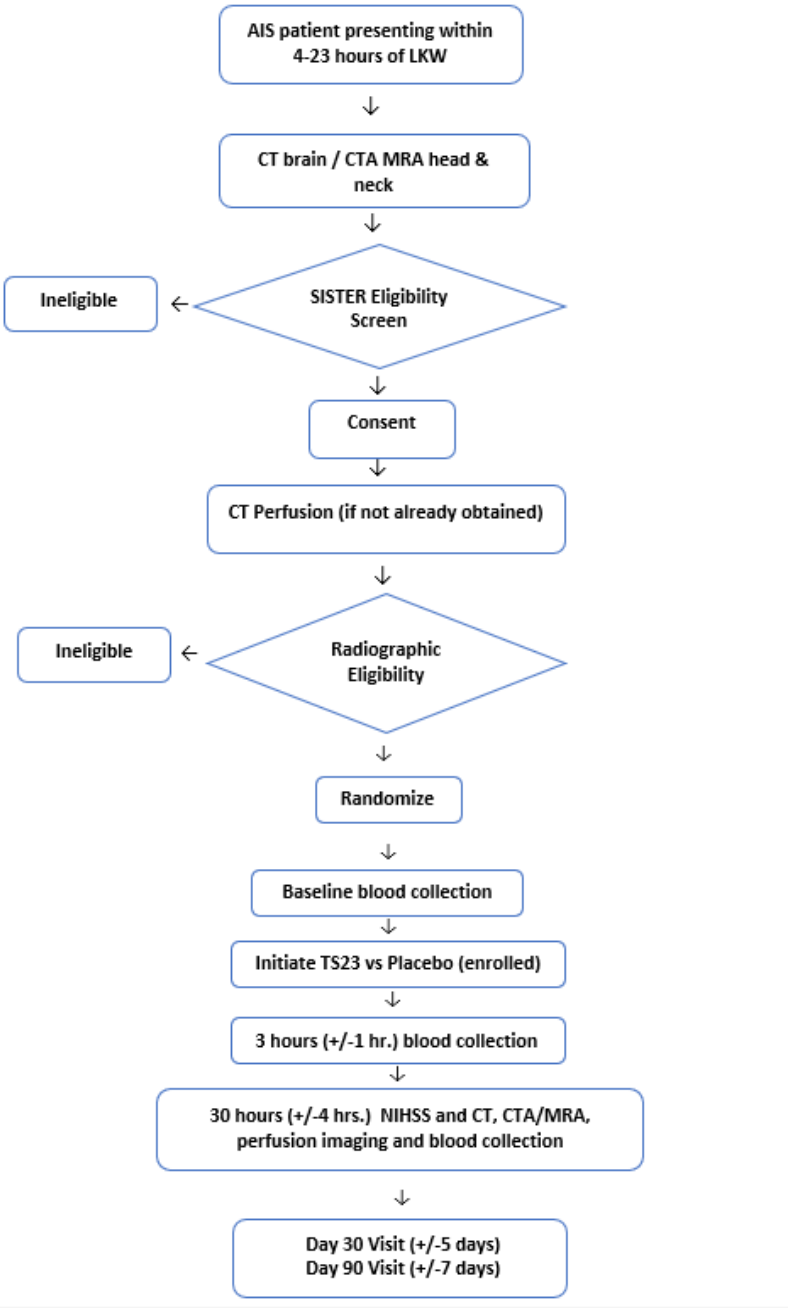
	Pharmacokinetics and anti-drug antibodies: 1. Pharmacokinetic (PK) profile of TS23 at 3 (+/-1) h and 30 (±4) h after completion of therapy and 90 (±7) days. 2. Evaluation of anti-drug antibodies to TS23 (at baseline and 90 (±7) d follow-up visit). Safety: 1. Incidence of symptomatic ICH within 30 (±6) h of study drug administration [SITS-MOST definition (a local or remote type II parenchymal hemorrhage within 30 (±4) h after treatment associated with a ≥ 4-point deterioration on the NIHSS score from baseline or from the lowest score from baseline to 24 hours, or leading to death.)] 2. Incidence of non-ICH major or clinically relevant non-major bleeding within 30 days of study drug administration. 3. Non-bleeding, serious adverse events (SAEs) within 90 (±7) days 4. Incidence of stroke-related and all-cause deaths within 90 (±7) days 5. Plasma fibrinogen levels at 3 (+/-1) h after completion of study drug administration
Study Population:	We will enroll a total of 300 adults (≥18 years) with acute ischemic stroke who have a baseline NIHSS ≥6 and are able to receive study drug within 4.5-24 hours after stroke onset (or last known well) who have evidence of core-penumbra mismatch on baseline perfusion imaging and are not planned for endovascular intervention or standard of care intravenous (IV) thrombolysis.
Phase:	2
Description of Sites/Facilities Enrolling Participants:	Consecutive eligible patients will be screened and enrolled in up to 50 comprehensive stroke centers in the U.S. Currently, no sites outside of the U.S. are planned.
Description of Study Intervention:	The study intervention arms are TS23 (dosed at 3, 5, 7, or 10 mg/kg) or matching placebo. Study drug is administered intravenously as

short IV infusion after reconstitution over ~15 minutes (max dose of 1000 mg).

Study Duration: 54 months

Participant Duration: 3 months

1.2 Schema



1.3 Schedule of Activities (SoA)

Schedule of Activities								
			Time after initiation of study drug administration					
	Baseline	**Study drug administration**	**3 (±1) hours**	**30 (±4) hours****	**72 (±12)/ Discharge, whichever is 1st**	**Discharge Visit**	**Day 30±5**	**Day 90±7**
Screening & Eligibility	X^R							
Medical History	X^S							
Laboratory Studies	X^S & X^R	X^{R*}	X^{R*}	X^S & X^{R**}				X^R
Pre-stroke mRS	X^R							
NIH stroke scale	X^R			X^R	X^R		X^R	X^R
Vital Signs	X^S			X^S				
CT brain	X^S			X^S or X^R				
CT/MR Perfusion	X^S or X^R			X^R				
CTA/MRA Head & Neck	X^S			$X^{R\#}$				
Informed Consent	X^R							
Randomization	X^R							
Study drug administration		X^R						
Discharge Summary						X^S		
Concomitant Medications	X^S	X^R	X^R	X^R	X^R	X^R	X^R	X^R
Adverse Events		X^R	X^R	X^R	X^R	X^R	X^R	X^R
Serious Adverse Events		X^R	X^R	X^R	X^R	X^R	X^R	X^R
mRS						X^R	X^R	X^R
End of Study								X^R

X^S: Standard of Care; X^R: Research Procedure; D/C = Discharge; CTA/MRA = CT pr MR Angiogram; mRS: Modified Rankin Scale score
*PK/PD & Fibrinogen lab draw BEFORE and 3(+/-1) hour after study drug administration
**PK/PD, Fibrinogen, PT/INR, aPTT and CBC (SOC) lab draw 30(+/-4) hour after study drug administration
#Required only if visible vessel occlusion present on the baseline CTA or MRA per site read

2 INTRODUCTION

2.1 Study Rationale

Stroke is a leading, (Murray, Vos et al. 2012) and growing, (Feigin, Krishnamurthi et al. 2015) cause of disability in the U.S. and worldwide. Between 2012 and 2030, U.S. annual stroke-related medical costs are projected to triple, from $72 billion to $184 billion, with much of the cost related to disability due to significant brain injury. (Ovbiagele, Goldstein et al. 2013) **Acute stroke reperfusion therapies—IV thrombolysis using alteplase (r-tPA) and endovascular therapy (EVT)—are some of the most impactful treatments in medicine, but only a minority (~20%) of patients are eligible for them and the majority of those treated suffer enduring disability (70%).** (Campbell, Donnan et al. 2015) Discovering more effective treatment strategies is a public health priority as it would alleviate suffering and disability, and save billions of dollars annually.

Presently, recombinant tissue plasminogen activator (r-tPA, alteplase) is the only effective drug treatment for acute ischemic stroke. The U.S. Food and Drug Administration (FDA) approved r-tPA for administration within 3 hours, though clinical guidelines support treatment up to 4.5 hours from stroke onset or last known well. (Powers, Rabinstein et al. 2019) Although approved ~25 years ago, only about 5-10% of all ischemic stroke patients in the U.S. are eligible for r-tPA, and only ~5% receive it. (Adeoye, Hornung et al. 2011) The benefit of r-tPA, reperfusion, is highly time-dependent, offset by risk of hemorrhage. Overall, r-tPA reduces the risk of 90-day disability by 7.5% if given within 4.5-hour window, but beyond that there is no definitive evidence of benefit. Within the 4.5h window, r-tPA has limited efficacy for large vessel occlusions (LVOs) and recanalizes only 25%. (Menon, Al-Ajlan et al. 2018)

EVT has revolutionized treatment for a limited subset of acute stroke patients with LVOs but remains inaccessible for many. For instance, EVT remains unproven for a substantial proportion of LVO patient population (~ 50%) who have a mild deficit, relatively large infarct core, or M2 or other vessel occlusions. Access to EVT is limited in low- and middle-income countries, and even in parts of the U.S., where only 50% of the population lives within an hour distance from a thrombectomy-capable hospital. (Adeoye, Albright et al. 2014)

No drug treatment is established for most patients beyond 4.5 h from symptom onset. The DAWN and DEFUSE-3 trials provided recent, incontrovertible proof for perfusion imaging-based selection of patients to benefit from EVT in this “extended time-window” up to 24h. Limited evidence exists for thrombolysis within 4.5 to 9h of stroke onset, (Ma, Campbell et al. 2019, Ringleb, Bendszus et al. 2019) and no randomized evidence is available beyond 9h to support thrombolytic treatment.

Tenecteplase (TNKase) may emerge as an alternative to r-tPA in clinical practice but is unlikely to fully address the limitations of r-tPA. TNKase is a derivative of r-tPA with mutations at 3 sites that activates plasminogen by the same mechanism. (Meretoja and Tatlisumak 2008) The primary advantage of TNKase is an 8.4-fold longer half-life that permits bolus dosing, which is a logistical advantage for transferring stroke patients between facilities. (Meretoja and Tatlisumak 2008) Although pivotal trials demonstrating noninferiority of TNKase to r-tPA are awaited, clinical studies performed in other countries suggest that TNKase has similar efficacy to r-tPA within 4.5h, while more limited studies suggest a higher rate for reperfusion and improved outcomes in patients with LVOs. (Haley,

Thompson et al. 2010, Parsons, Spratt et al. 2012, Huang, Cheripelli et al. 2015, Logallo, Novotny et al. 2017, Campbell, Mitchell et al. 2018)

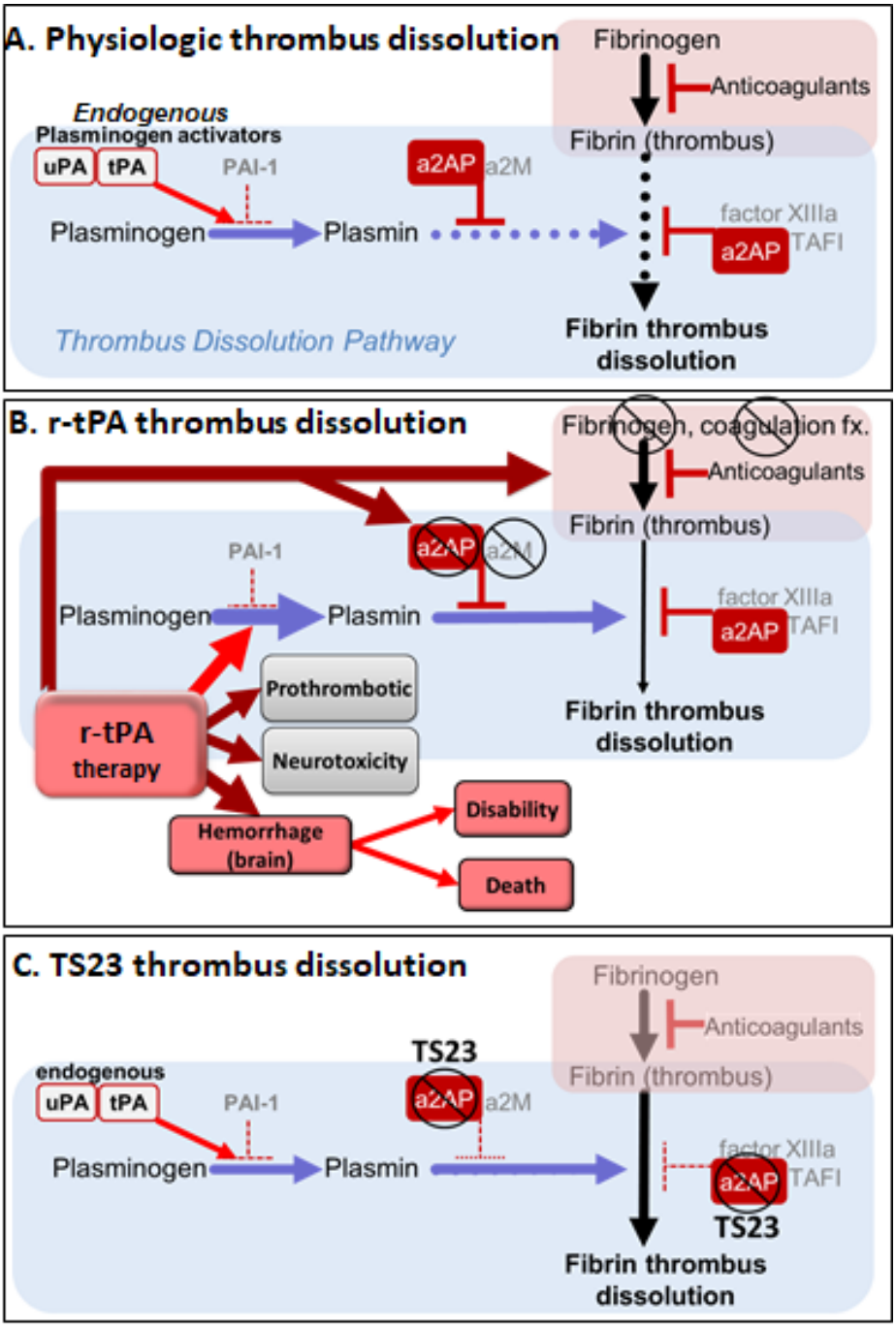
Exogenous plasminogen activators (e.g., r-tPA, TNKase and others) dissolve thrombi, but have inherent safety concerns, including hemorrhage and neurotoxicity. Ischemic strokes are caused predominantly by an occlusive fibrin thrombus that reduces blood flow below levels necessary for brain viability. (Kim, Lee et al. 2005, Donnan, Davis et al. 2011, Liebeskind, Sanossian et al. 2011) Ischemia triggers complex pathophysiologic mechanisms that involve metabolic depletion, membrane failure, excitotoxicity, dysfunction of the neurovascular unit, oxidative-nitrative stress and inflammation (reviewed in (Dirnagl, Iadecola et al. 1999, Hallenbeck 2010)). By catalyzing the dissolution of fibrin thrombi, plasminogen activators can restore blood flow. At the same time, they can induce brain hemorrhage by increasing blood brain barrier disruption and matrix metalloproteinase activation. (Lapchak, Chapman et al. 2000, Wang, Lee et al. 2003, Wang, Tsuji et al. 2004, Jickling, Liu et al. 2014) Symptomatic or radiologically large hemorrhages (i.e., parenchymal hematomas), which cause major morbidity or mortality in the majority, are seen in ~6% of r-tPA treated patients, whereas control rates are 0-1%. (NINDS t-PA Stroke Study Group.1997) Additionally, asymptomatic, or radiologically more minor hemorrhage (i.e., hemorrhagic infarction), is increasingly shown to be associated with worse outcomes as well, occurring in 30-40% of r-tPA treated patients by 24h, and only 10-20% of controls. (Dzialowski, Pexman et al. 2007, Lees, Bluhmki et al. 2010, Jiang, Zhao et al. 2019, van Kranendonk, Treurniet et al. 2019) Paradoxically, r-tPA therapy also increases fibrin clot formation. (Owen, Friedman et al. 1988, Tripodi, Bottasso et al. 1990, Mak, Lee et al. 2002)

Plasminogen activators have well-known neurotoxic effects in experimental models that may reduce optimal outcomes in humans. (Tsirka, Rogove et al. 1996, Wang, Tsirka et al. 1998) Through the n-methyl-d-aspartate receptor (NMDA) receptor complex, they may increase calcium currents to worsen damage during excitotoxic neurotoxicity; (Nicole, Docagne et al. 2001) reduce brain cell viability by degrading laminin between neurons and interfering with cell matrix signaling needed for survival; (Indyk, Chen et al. 2003) and increase neuronal apoptosis. (Liu, Cheng et al. 2004) Additional potential mechanisms of r-tPA mediated direct and indirect neurotoxicity have been proposed. (Wang, Tsirka et al. 1998, Nagai, Vanlinthout et al. 1999, Wang, Lee et al. 2003, Kaur, Zhao et al. 2004, Liu, Cheng et al. 2004, Abu Fanne, Nassar et al. 2010)

The proposed trial, "Strategy for Improving Stroke Treatment Response" or SISTER, is based on a paradigm shift in our understanding of how thrombus dissolution (fibrinolysis) is regulated, which allows clinical practice to advance beyond plasminogen activators alone. Once fibrin-containing thrombi form, they can only be dissolved by the enzyme plasmin that cuts fibrin into small, soluble pieces in a process called endogenous or physiologic fibrinolysis (Figure 1A). When thrombi form, endogenous tissue plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA) are produced to convert plasminogen to plasmin. In ischemic stroke, physiologic dissolution of the culprit thrombus is minimal, which has been attributed, in our current paradigm to low endogenous levels of r-tPA/uPA. (Walsh, Stengle et al. 1969, Sasahara, Sharma et al. 1972, 1988, Topol, George et al. 1988, Tanswell, Tebbe et al. 1992, Goldhaber, Agnelli et al. 1994, Cannon, McCabe et al. 1997, Vanderschueren, Van Vlaenderen et al. 1997, Wang-Clow, Fox et al. 2001, Hacke, Albers et al. 2005, Haley, Lyden et al. 2005, Kearon, Kahn et al. 2008, Tebbe, Bramlage et al. 2009) To address this paradigmatic problem, numerous pharmacologic plasminogen activators were developed (e.g., r-tPA

(alteplase), r-tPA mutants such as TNKase, or desmoteplase, etc.) and different dosing was tested. Treatment with r-tPA or TNKase, increases blood levels by ~1000-fold over endogenous tPA levels. (Garabedian, Gold et al. 1988, Ganti, Potti et al. 2002) At these levels the specificity of r-tPA as a thrombus-targeting agent is lost and the toxic effects of r-tPA (mentioned above) are magnified (Figure 1B). (Garabedian, Gold et al. 1988, Ganti, Potti et al. 2002) Pharmacologic r-tPA generates large amounts of plasmin (Figure 1B) to overcome the inhibitory effects of a2AP, an endogenous protein that inactivates plasmin. While other molecules affect thrombus dissolution (Figure 1, e.g., activated Factor XIII, a2-macroglobulin, plasminogen activator inhibitor-1 and thrombin-activated fibrinolysis inhibitor), studies have shown that a2AP is the dominant inhibitor. (Bajzar 2000, Lee, Lee et al. 2000, Tsikouris, Suarez et al. 2002, Mutch, Thomas et al. 2007) We propose *a new monoclonal antibody therapy that specifically targets and inactivates a2AP (a2AP-I) called TS23* (Figure 1C). (Mutch, Thomas et al. 2007) By itself, TS23 or an a2AP-I triggers safe thrombus dissolution by physiologic plasminogen activators on the fibrin surface (Figure 1C) without overwhelming other regulatory molecules that prevent bleeding.

Figure 1. Regulation of Thrombus Dissolution (Fibrinolysis)



An a2AP-I alone or in combination with lower doses of plasminogen activators, is safer and more effective than a plasminogen activator alone in experimental ischemic stroke. Section 2.2 reviews the extensive experimental data showing that in comparison to r-tPA, an a2AP-I significantly reduces brain injury, hemorrhage, disability and mortality in ischemic stroke. Additional studies show that an a2AP-I safely dissolves thrombi and prevents microvascular thrombosis without causing bleeding.

(Reed, Houng et al. 2014) Lifelong deficiency of a2AP is tolerated in mice and humans, suggesting that transient a2AP-I is unlikely to cause a serious risk of bleeding. In contrast, r-tPA and TNKase cause a predictable, dose-related risk of brain. (Turi, Goldberg et al. 1993, Haley, Lyden et al. 2005) r-tPA causes a 'systemic lytic state' with diffuse plasmin generation in the circulation leading to degradation of fibrinogen and a2AP.(Cannon, McCabe et al. 1997, Vanderschueren, Dens et al. 1997) Bleeding is not increased in a2AP-deficient mice vs. normal mice treated with anticoagulants or with antiplatelet agents. (Matsuno, Kozawa et al. 2002, Investigator's Brochure, Section 4.1) Finally, serious hemorrhage after r-tPA or TNKase therapy are often linked to adverse effects of these therapies on coagulation, (Yaghi, Eisenberger et al. 2014) however even the highest doses of TS23 don't affect clotting.

In summary, there is an urgent need for safe and effective medical therapies for ischemic stroke, particularly in those who present beyond the 4.5 h-window. These therapies should reduce disability, carry a low risk of hemorrhage, be broadly available and easy to administer. TS23, is a novel, paradigm-changing, a2AP-I therapy that can address these critical needs, as shown in Preliminary Studies, below.

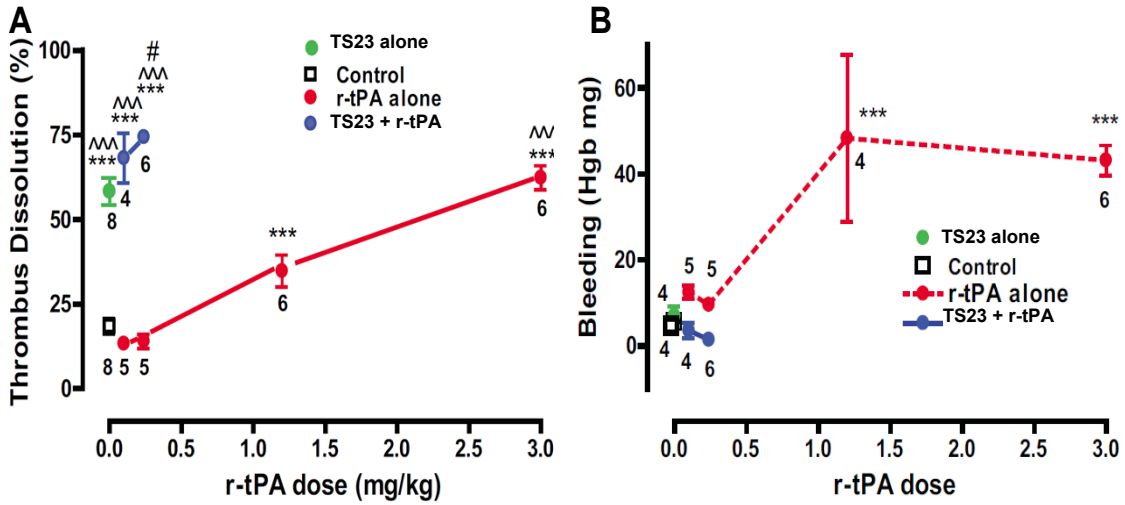
2.2 Background

Extensive data different labs show the enormous therapeutic potential of a2AP-I alone, or in synergistic combination with r-tPA. These experimental data, studies of a2AP-deficient humans and animals, and clinical studies of reperfusion therapies in the extended time window, informed our trial design.

TS23 (a2AP-I) alone dissolves thrombi with the potency of higher dose r-tPA without causing bleeding.

Mechanistic studies show that a2AP-I synergistically dissolves human clots with all types of plasminogen activators (Reed, Matsueda et al. 1990) because it prolongs the half-life of the plasmin they generate (Figure 1). To examine this *in vivo*, we assessed the potency and safety of TS23 alone for dissolving thrombi and in combination with r-tPA in a *humanized* model of pulmonary embolism (PE) in mice. (Singh, Houng et al. 2019) (Note: r-tPA was used as TNKase is not approved for PE or ischemic stroke). Mice were treated 30 min after embolization with r-tPA (0, 1.2 or 3 mg/kg) over 1 h (similar to the FDA-approved dose), TS23 (a2AP-I) alone (10 mg/kg, stoichiometric with a2AP), TS23 with a 12.4 to 25-fold lower doses of r-tPA or no agent (controls). Treatment with r-tPA caused more thrombus dissolution than controls ($p<0.05$, Figure 2A). TS23 alone or in combination with very low dose r-tPA ($p<0.001$, Figure 2A) caused significantly more thrombus dissolution vs control ($p<0.001$) or vs. higher dose r-tPA-treated (1.2 mg/kg) mice ($p<0.001$, Figure 2A). TS23 treatment alone, or in combination with very low dose r-tPA, did not cause more surgical bleeding vs. control, untreated mice (Figure 2B) In contrast, r-tPA increased bleeding vs. TS23-treated mice (Fig. 2B, $p<0.01$) or controls ($p<0.001$). Thus, TS23 alone or in combination with very low dose r-tPA significantly increased the dissolution of pulmonary emboli vs. r-tPA alone and controls and, importantly, without increasing bleeding.

Figure 2. Effects of a2AP Inactivation (a2AP-I) and Pharmacologic r-tPA on Thrombus Dissolution and Bleeding in Mice with Experimental Pulmonary Emboli

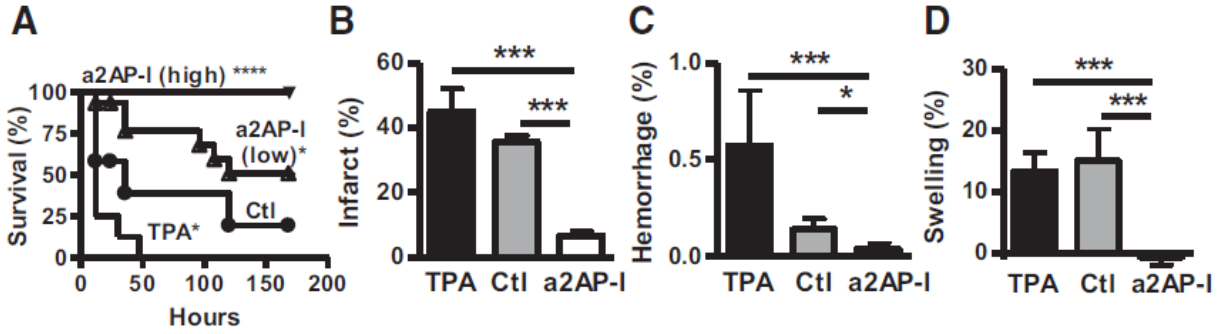


Anesthetized a2AP$^{-/-}$ mice were replaced with physiologic amounts of human a2AP by infusion. (Singh, Houng et al. 2019) Human PE were made from pooled fresh-frozen human plasma labeled with trace amounts of ^{125}I-human fibrin and embolized into the lungs. Mice were treated thirty minutes after thromboembolism with r-tPA, an a2-antiplasmin-inactivating antibody (a2AP-I, TS23) alone, the combination of very low dose r-tPA and a2AP-I or nothing (Control) as shown, N=48. A) Four hours after PE, the experiments were terminated and thrombus dissolution was measured. The number of mice per dose is shown. Differences assessed by one-way ANOVA. *** $p<0.001$ vs. control; ^^^ $p<0.001$ vs. r-tPA 0.1, 0.24 or 1.2 mg/kg; $^{\#}$ $p<0.05$ vs. r-tPA 3 mg/kg. B) Tail tip bleeding was measured as hemoglobin (Hgb) loss by Drabkin's reagent in the same experimental groups. N per group is shown. Differences assessed by one-way ANOVA. ***$p<0.001$ vs. controls.

a2AP-I alone reduces brain injury, bleeding, and mortality in experimental ischemic stroke

Ischemic stroke is a rigorous test of drug safety because the ischemic brain is exquisitely sensitive to potential toxicities, including hemorrhage. We examined the effects of a2AP inactivation vs. r-tPA in a thromboembolic model of proximal middle cerebral artery (MCA) stroke. This model incorporates Stroke Academic Industry Roundtable (STAIR) guidance for the pre-clinical development of neuroprotective agents (dose response, therapeutic time windows, experimental models, physiologic monitoring and outcome measures) (Stroke Therapy Academic Industry Roundtable, 1999, 2001,Gladstone, Black et al. 2002, Fisher 2003, Wahlgren and Ahmed 2004, Albers, Goldstein et al. 2011) Mice were treated with IV r-tPA, a2AP-I or nothing, 30 min. after a severe, proximal MCA thromboembolic stroke. (Reed, Houng et al. 2014) Figure 3 shows that the a2AP-I saved lives, in a dose-related fashion by comparison to r-tPA-treated ($p<0.0005$) or control mice ($p<0.004$). Both Fab (partial protein segment) and whole immunoglobulin G (IgG) forms of a2AP-I conferred full protection from mortality. (Reed, Houng et al. 2014) There was no neurobehavioral disability in a2AP-I treated mice at 7 days vs. sham mice (without stroke) in a standardized test (Rotarod) of motor coordination and balance after stroke (not shown). (Hunter, Hatcher et al. 2000)

Figure 3. Inactivation of a2AP (a2AP-I) after MCA Thromboembolism Saves Lives and Reduces Ischemic Brain Injury in Survivors

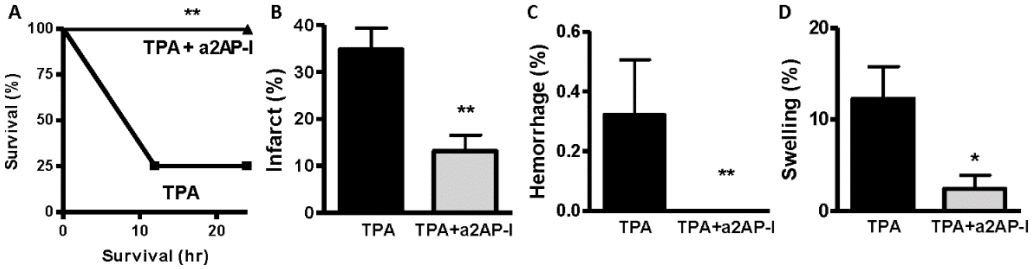


A) Survival in treated or control mice during a 7-day observation period. Mice were treated with low dose a2AP-I (9 mg/kg), or high dose a2AP-I (as whole IgG (21.3 mg/kg) or Fab (9.3 mg/kg)), r-tPA (standard rodent dose,10 mg/kg, which reflects 10-fold lower catalytic activity of r-tPA with mouse plasminogen) (Korninger and Collen 1981) or no agent (Ctl), 30 minutes after stroke onset. Effects on B) brain infarction (volume %), C) brain hemorrhage (volume %) and D) brain swelling (volume %) after thromboembolism in surviving mice. Survival groups N= 12 in each cohort, data represent means ± SE. *p≤0.05, ***p≤0.001, ****p≤0.0001 vs. control.

a2AP-I combined with low-dose r-tPA reduces brain injury, bleeding, and mortality in experimental ischemic stroke.

The effects of combination treatment with low dose r-tPA and a2AP-I were examined in acute ischemic stroke caused by MCA thromboembolism. (Houng, Wang et al. 2014) By comparison to standard dose r-tPA, the combination of a 5-fold lower dose of r-tPA + a2AP-I significantly increase reduced brain infarction, brain hemorrhage and brain edema (Figure 4B-D). Low dose r-tPA + a2AP-I also significantly reduced acute mortality (Figure 4A). In other experiments (see ref. (Houng, Wang et al. 2014)) the combination of low dose r-tPA + a2AP-I significantly enhanced thrombus dissolution vs. low dose r-tPA and also significantly reduced brain infarction and brain hemorrhage.

Figure 4. Treatment with Low-dose r-tPA (TPA) with a2AP-I Reduces Mortality, brain Infarction, Hemorrhage and Swelling vs. Standard Dose r-tPA Alone



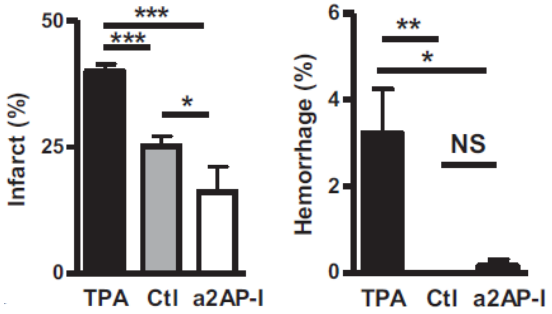
Mice were treated 30 min after MCA thromboembolism with standard rodent dose r-tPA alone (10 mg/kg) (Korninger and Collen 1981) or a 5-fold lower dose or r-tPA with a2AP-I (21.3 mg/kg). Survival was monitored and brains of mice surviving at least 12 h were analyzed. A) Effect on 24 h survival. B) Effect on brain infarction in surviving mice. Percent infarction was determined by TTC staining. C) Effect on brain swelling. D) Effect on hemorrhage as described above. Data represent mean ± standard errors. Survival analyses N= 17, otherwise N = 5 per group. *p<0.05, **p<0.01, vs. r-tPA alone.

a2AP-I has a longer therapeutic window than r-tPA in thromboembolic ischemic stroke.

r-tPA has a limited therapeutic time window in humans and mice. Thirty min after thromboembolic stroke in mice, r-tPA treatment no longer reduced brain infarction by comparison to controls (Figure 3). In contrast, treatment with a2AP-I 30 min after thromboembolism significantly reduced brain infarction, hemorrhage and mortality vs. control and r-tPA treated mice (Figure 3). Even when administered 2.5 hours after thromboembolism, a2AP-I treatment significantly reduced brain infarction vs. control and r-tPA (Figure 5A). (Reed, Houng et al. 2014) In contrast to r-tPA, a2AP-I did not significantly increase brain bleeding vs. controls (Figure 5B). (Reed, Houng et al. 2014)

The combination of a2AP-I with r-tPA (10 mg/kg or 2 mg/kg) also significantly reduced brain infarction, hemorrhage and thrombus dissolution vs. the same dose of r-tPA alone, even when given 2.5 hour after thromboembolic ischemic stroke. (Houng, Wang et al. 2014) Even though the mouse penumbra is considered to be much shorter in duration than the human one, these studies suggest that an a2AP-I alone or in combination with r-tPA significantly reduced ischemic brain injury over a several fold-longer time window than r-tPA alone.

Figure 5. Treatment with a2AP-I has a Longer Therapeutic Time Window than r-tPA in Thromboembolic Ischemic Stroke



Effects of an a2AP-I (9 mg/kg) or TPA (10 mg/kg) treatment given 2.5 hour after thromboembolism on the percent (**A**) brain infarction and (**F**) brain hemorrhage. Brains were examined 6 hour after cerebral thromboembolism. Infarct volume (%) was measured by TTC staining of serial brain slices followed by digital imaging by a blinded observer. (Swanson, Morton et al. 1990) Hemorrhage volume % was determined by digital imaging of serial brain slices by a blinded observer. Data represent the means±SE, n=7 per group. *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; NS, not significant; one-way analysis of variance.

Preclinical studies of a2AP and a2AP-I show consistent findings across four different laboratories.

Study quality and STAIR criteria: All reported studies used appropriate controls; some studies were blinded. Different doses and types of a2AP-I were used, but all had consistent effects. All doses of a2AP-I were given intravenously, as a2AP circulates in the blood and there is only trace a2AP in the brain. There is no indication of differing outcome effects in these studies. Studies have been performed in mice, rabbits, ferrets. Dose-response data effects were demonstrated in a pre-clinical study of thromboembolic stroke on survival. Dose-response effects on a2AP-I-induced fibrinolysis were seen in Good Laboratory Practice (GLP) studies in non-human primates and in a Phase I trial in humans (Table 1). The time window for stroke treatment was examined in an experimental model of

thromboembolic stroke. Studies were performed using permanent, transient, and thromboembolic models of ischemic stroke. There was experimental evidence for histological, enzymatic, functional and survival outcomes. The key findings were: 1) genetic deficiency (gene knock-out, KO) of a2AP or a2AP-I increases dissolution of pulmonary emboli, cerebral emboli, venous and arterial thrombi in mice, rabbits and ferrets; 2) an a2AP-I was synergistic with all types of plasminogen activators in thrombus dissolution; 3) In different ischemic stroke models from different laboratories, genetic deficiency of a2AP and a2AP-I significantly decreased brain infarction, brain bleeding, disability and mortality.

Table 1. Pre-clinical Studies of the Effects of a 2AP and a2AP-I

Experimental Model	Species Ref.	Model	Treatment v. Control	Thrombus Dissolution vs. Control	Outcomes vs Controls	Bleeding vs. Control
Ischemic Stroke	Mice (Houng, Wang et al. 2014)	MCA thromboembolism, blinded	KO vs. WT a2AP-I v. saline	⇧ ⇧	⇧survival (dose-response), ⇩infarction, ⇩bleeding, ⇩disability	⇩
Ischemic Stroke	Mice (Reed, Houng et al. 2014)	MCA thromboembolism, blinded	a2AP-I + tPA v. Tpa	⇧	⇧survival, ⇩infarction, ⇩bleeding	⇩
Ischemic Stroke	Mice (Nagai, De Mol et al. 2001)	MCA ligation	KO v. WT a2AP-I vs. control	NA	⇩ infarct	NA
Venous & arterial thrombosis	Mice (Matsuno, Kozawa et al. 2002)	Arterial & venous endothelial injury	KO v. WT	⇧	Spontaneous reperfusion	No change
Venous & arterial thrombosis	Rabbits (Reed, Matsueda et al. 1990)	Jugular vein thrombosis	a2AP-I v. tPA	⇧	NA	Not determined
VTE prevention	Mice (Singh, Houng et al. 2019)	IVC stasis thrombosis	a2AP-I v. control, KO v. WT	⇧	⇩venous thrombosis	⇔
Pulmonary embolism	Mice (Singh, Houng et al. 2017)	Autologous PE	a2AP-I v tPA, control; a2AP-I +tPA v. r-tPA	⇧	Synergism of a2AP-I with r-tPA	⇩
Pulmonary embolism	Mice (Matsuno, Okada et al. 2003)	Photochemical thrombosis	KO v. WT vs r-tPA	⇧	a2AP KO and tPA synergistic,⇧survival	No change
Pulmonary embolism	Mice (Dewerchin, Collen et al. 2001)	Autologous PE	a2AP KO v. WT	⇧	Spontaneous dissolution of PE in a2AP KO mice	No change
Pulmonary embolism	Ferret (Butte, Houng et al. 1997)	Human PE	a2AP-I, a2AP-I + r-tPA, r-tPA, Control	⇧	Synergism of a2AP-I + lower dose r-tPA > than higher dose r-tPA-	No change

Insights from humans with complete genetic deficiency of a2AP support a2AP-I safety and efficacy

The hallmark of humans with a2AP deficiency is that their blood clots spontaneously dissolve in test tubes, while normal blood clots do not. (Lijnen, Okada et al. 1999, Favier, Aoki et al. 2001, Harish, Zhang et al. 2006) This occurs without adding pharmacologic r-tPA or other plasminogen activators. Similarly, a2AP-deficient ($a2AP^{-/-}$) mice also show spontaneous lysis of blood clots injected *in vivo*

(Lijnen, Okada et al. 1999) and a2AP deficiency reduces the mortality from PE. (Matsuno, Okada et al. 2003)

Individuals with complete genetic a2AP deficiency do not show fibrinogen or clotting factor depletion, indicating that the absence of a2AP does not cause a systemic fibrinolytic state or other abnormalities. (Lijnen, Okada et al. 1999, Favier, Aoki et al. 2001, Harish, Zhang et al. 2006) None of the 14 humans with a2AP-deficiency described in a compendium of the world literature had central nervous system (CNS) hemorrhage. (Favier, Aoki et al. 2001) These individuals may have enhanced bleeding after trauma or laceration. This bleeding is usually controlled by standard measures or with antidotes -- chemical inhibitors (tranexamic acid, epsilon amino caproic acid) that prevent plasmin from binding to fibrin. (Harish, Zhang et al. 2006) Indeed, a2AP-deficient patients have successfully undergone heart surgery with these approaches. Importantly, bleeding was not observed with a2AP-I or TS23, which only transiently inactivate a2AP. *One of the most compelling arguments for the safety of an a2AP-I, which transiently induces a2AP deficiency, is that complete, life-long deficiency of a2AP is tolerated in humans and in mice.* (Lijnen, Okada et al. 1999, Harish, Zhang et al. 2006) Data from mice with genetic a2AP-deficiency also support the therapeutic potential and safety of using an a2AP-I. Complete a2AP deficiency is associated with normal fertility, viability, and development. (Lijnen, Okada et al. 1999) Bleeding times are also normal. (Lijnen, Okada et al. 1999) In mice, a2AP-deficiency accelerates wound healing, perhaps through an increase in the release of vascular endothelial growth factor. (Kanno, Hirade et al. 2006) Inhibitors of mouse a2AP increase liver repair after injury when compared to controls. (Okada, Ueshima et al. 2004) Deficiency of a2AP also decreased arteriosclerosis after vascular injury. (Matsuno, Ishisaki et al. 2003) Importantly, deficiency of a2AP does not increase bleeding when animals are treated with r-tPA. (Matsuno, Okada et al. 2003) These findings parallel our a2AP-I testing in non-human primates at TS23 doses sufficient to inhibit a2AP for one week, and in both in experimental thrombosis *in vivo* and in Phase I trials of humans.

2.3 Safety-Toxicology and Toxicokinetics of TS23

The FDA agreed during our Pre-IND meeting that non-human primates were the only pharmacologically relevant species for testing the safety and toxicology of TS23. We completed pivotal safety-toxicology studies investigating the toxicokinetics of TS23 in 32 cynomolgus monkeys following a single IV injection. This study was conducted according to GLP as set forth in Title 21 of the CFR, Part 58. Please see the current version of the Investigator's Brochure for a summary of the safety toxicology studies and results for TS23 (a2AP-I).

2.4 Phase 1 Study of TS23 (a2AP-I)

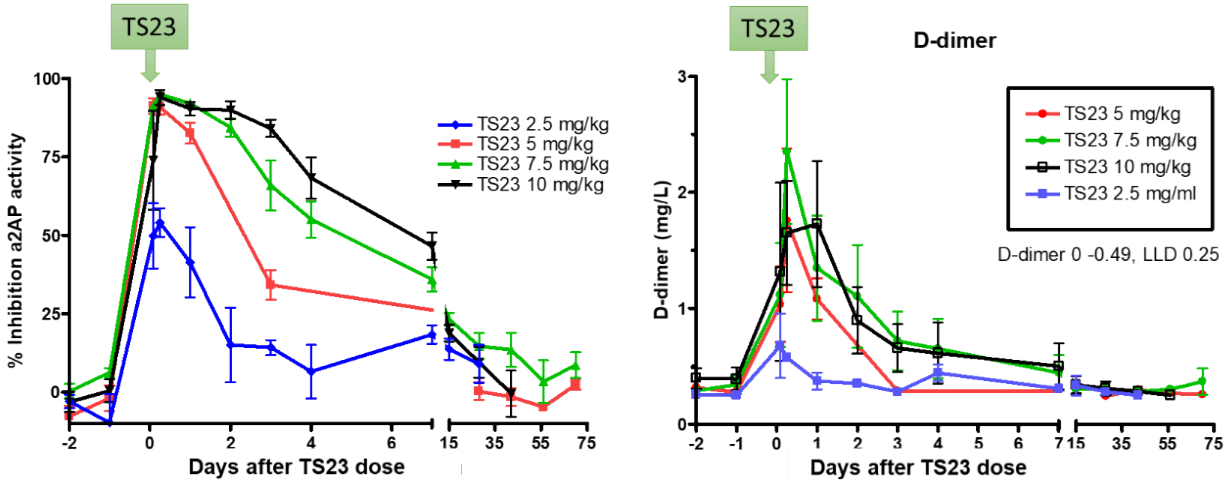
A first-in-human Phase 1, single-ascending dose-escalation study was done to investigate the safety, tolerability, PK and pharmacodynamics (PD) of TS23 in healthy normal human volunteers. This open-label trial monitored participants before and after a single IV dose of TS23. Four dose cohorts of six participants received 2.5 to 10 mg/kg of TS23. After receiving IV TS23 over ~ 20 min, participants were monitored for 10 weeks (cohort 1) and 16 weeks (cohort 2, 3, 4) to assess the half-life of TS23, perform safety assessments, toxicity monitoring, PK and PD measurements.

Safety evaluations included electrocardiograms (ECGs), weights, physical examinations, neurologic examinations, vital signs, laboratories (hematology, chemistry, PT, aPTT, fibrinogens, urinalyses), and

PD data (α2AP activity and D-dimer levels). Vital signs (including temperature, pulse rate, respiratory rate, and blood pressure) were recorded immediately before administration of TS23, 10 minutes into the infusion, at the end of the infusion, and every 15 minutes for the first hour following the infusion. PK, PD, and other safety assessments, including toxicity assessments and clinical laboratory tests, were performed for 4 weeks. All doses of TS23 were safe and well tolerated. There were no clinically relevant trends in the clinical lab results, vital signs, ECGs, or physical examinations, and no significant adverse events (AEs) attributable to TS23.

TS23 inhibited a2AP activity in all participants, in a dose-related fashion, with maximum effects in the initial blood samples after IV dosing (Figure 6A). The duration of inhibition of a2AP was also dose related. a2AP activity was ~>50% inhibited for hours to 4 days at dose 2.5 to 7.5 mg/kg. TS23 immediately activated fibrinolysis after dosing and increased D-dimer levels relative to baseline levels; D-dimer levels were higher in cohorts receiving higher doses of TS23 (Figure 6B). (D-dimer is a specific biomarker that indicates endogenous thrombus dissolution, even in normal, asymptomatic individuals). In addition, clots formed *ex vivo* from subject plasma obtained 24h after TS23 administration rapidly dissolved in a dose-dependent fashion, while control clots from normal blood donors did not dissolve within 120 min, the maximum time of monitoring. Thus, TS23 safely engaged and inactivated a2AP, in a dose-related fashion, triggering endogenous fibrinolysis *in vivo* and clot dissolution *ex vivo*.

Figure 6. Dose-related Effects of TS23 on a2AP Activity and D-dimer Levels in Humans



A) a2AP inhibition was measured in a clinically validated plasma assay at baseline and at the indicated time points following a dose of TS23. B) D-dimer levels were measured in participants at baseline and at the indicated time points following a dose of TS23. Means ± SE are shown for each dose cohort.

Please see the current version of the Investigator's Brochure for further information on the Phase 1 Clinical study results for TS23 (a2AP-I).

2.5 Risk/Benefit Assessment

2.5.1 Known Potential Risks

Risks associated with TS23: Our study drug, TS23, is a chimeric monoclonal antibody that inactivates a2-antiplasmin (a2AP). TS23 is produced under current Good Manufacturing Practice (cGMP) conditions and must pass stringent testing prior to human use. In pre-clinical testing and in the Phase I study of TS23 in normal volunteers, TS23 was well tolerated and did not cause any serious adverse effects. However, potential risks associated with TS23 in acute stroke patients may include:

Risk of intracranial hemorrhage. The currently approved thrombolytic, r-tPA, causes symptomatic intracranial hemorrhage in 3-6% of acute stroke patients who receive the medication, and any intracranial hemorrhage in 30-40% by 36 hours from treatment. (Dzialowski, Pexman et al. 2007, Lees, Bluhmki et al. 2010, Jiang, Zhao et al. 2019, van Kranendonk, Treurniet et al. 2019) Available data suggest comparable risk from tenecteplase. In preclinical studies of mouse models of acute stroke, a TS23 surrogate antibody was associated with significant reductions in intracranial hemorrhage compared to rtPA alone or controls (Reed, Houng et al. 2014). Pre-clinical data, studies of a2AP-deficient animals and humans and the Phase I data suggest that the risk of intracranial hemorrhage will be low. However, we will monitor for ICH in every patient and through our independent medical monitor and Data and Safety Monitoring Board (DSMB) (please see Data and Safety Monitoring Plan for further details).

Risk of systemic hemorrhage. Because TS23 is a thrombus-dissolving agent, it may increase the risk of systemic bleeding. We have carefully defined study exclusion criteria to minimize enrolment of patients who would otherwise be at risk of systemic hemorrhage. We will closely monitor this event stringently as outlined in the DSMB plan.

There is a potential risk of a mild infusion reaction (redness, itching, swelling, rash or pain) associated with administration of TS23. There is a low risk of a severe infusion reaction (fever, chills, dizziness, headache, muscle ache, low blood pressure, or trouble breathing). For this, patients will be monitored in high acuity hospital settings for prompt identification and treatment.

Because this drug is investigational, all of its side effects may not be known. There may be rare and unknown side effects. This will also be monitored closely.

There is a small chance that participants may develop antibodies to the study drug, which will be monitored as outlined in the protocol.

Risks associated with radiation: The study requires a repeat computed tomography (CT) of the head. It will also require a -angiogram (CTA) if there is a visible vessel occlusion on the baseline image. An additional perfusion and angiogram scan that uses computed tomography (CT) is required for centers that do not perform magnetic resonance imaging (MRI)-based perfusion or angiograms. CT perfusion exposes patients to radiation that may have long term risks. The study team will share best practice CT perfusion protocols to minimize radiation exposure. The radiation exposure a participant will get from this study is approximately 0.19 rem (a rem is a unit of absorbed radiation). This is less than 0.3 rem that the average person in United States gets each year from natural sources such as sun, outer space,

air, food, and soil. The risk of radiation exposure from this research study is considered very small and is not likely to adversely affect the participants.

Risks associated with collection of protected health information (PHI): Collection of PHI for research involves a small risk for violation of patient confidentiality. To minimize this risk, only the minimum amount of PHI needed to conduct the study will be collected. All data collected will be generated during clinical care, and no additional data will be collected for research. At no time will we reveal subject identities in any manner, including research presentation, descriptions, or publications. All patients will be assigned a unique patient identifier upon enrollment in the study. Patient identifiers will only be accessible to the study PIs and a select few research staff. Once the study results have been published, all study records will be stripped of any PHI in order to maximize patient and surrogate confidentiality.

2.5.2 Known Potential Benefits

The proposed study is urgent. Currently there are no approved treatments for the patients intended for enrollment in SISTER who present within the 9-24 hour window. The alternative to participation in SISTER trial would be "no treatment" for these patients. Thus, SISTER participants stand to directly benefit from the potential of receiving a thrombus-dissolving medication that may be safe and may improve their chances of achieving a good outcome. Even though the EXTEND trial has shown potential benefit of rtPA treatment within the 4.5-9 h of stroke onset, in a patient population similar to the intended SISTER population, this practice is not yet widely adopted in the U.S. as there are several concerns regarding generalizability of EXTEND results. (Ma, Campbell et al. 2019, Ringleb, Bendszus et al. 2019) This practice is also not adopted in the US acute stroke clinical guidelines.(Powers, Rabinstein et al. 2019) Thus, patients who present within 4.5-9 who are not deemed eligible for rtPA by local investigators stand to benefit from participation in the study. For society, this study offers benefits including availability of a safe and effective acute treatment for patients who otherwise are untreated. In the long-term, this study has the potential for providing a safer and more effective alternative to plasminogen activator thrombolytics for all acute stroke patients, possibly obviating the need for procedural intervention in the large number of patients who don't have ready access to these procedures.

2.5.3 Assessment of Potential Risks and Benefits

The proposed strategy for SISTER has gone through stages of review, critique, and revision from experts in the fields of stroke neurology, cardiology, and biostatistics. The inclusion and exclusion criteria are specifically chosen to only exclude conditions that may require special safety considerations for thrombolytics. The imaging selection component adds another layer of protection by ensuring that patients will only be enrolled that have significant salvageable brain tissue, and minimal amount of irreversible injured tissue (which is most likely to cause intracranial bleeding). The Multiple Principal Investigators team (MPIs), Independent Medical Monitor, and DSMB will closely and comprehensively monitor pre-specified safety events, AEs, SAEs and unanticipated problems (UPs), according to plans outlined in the Data and Safety Monitoring Plan attachment. They will be reported to the IRB and the sponsor as outlined in the protocol, who may suggest study modifications as appropriate to protect human participants. After the study drug infusion, patients will be monitored for immediate infusion reactions in a high acuity hospital setting for prompt identification and treatment.

Further, the study drug will be shipped and maintained under strict conditions according to our FDA-approved IND. We will strictly adhere to the study protocol, collect high-quality data, and thoroughly train and, periodically retrain, all research and clinical personnel involved with the study regarding the details of study protocol and procedures. During the study period, all patients will receive care according to the local and most recent acute stroke guidelines. Study personnel will be readily available to answer questions at any time during the study course. The small risk for violation of confidentiality will be minimized by training all study personnel with the web-based Responsible Conduct of Research Training Program.

Inventing a safer and more effective alternative to the current paradigm of thrombolytic, i.e., plasminogen activators, addresses an urgent gap in the acute stroke treatment that needs to be filled. SISTER trial will test a new paradigm in acute stroke thrombolytic therapy using a monoclonal antibody against a2-antiplasmin. If successful, this trial will provide important insights into safety and preliminary efficacy of this new molecule for the treatment of acute ischemic stroke patients, which will be immediately translated into a phase 2/3 pivotal trial. The findings of SISTER trial will also be relevant to the broader acute ischemic stroke population including those presenting within the earlier time window and harboring a large cerebral blood vessel occlusion.

3 OBJECTIVES AND ENDPOINTS

3.1 Primary Objective

To identify a dose of TS23 that is safe and potentially more efficacious than placebo for the treatment of patients from 4.5 to 24 hours of ischemic stroke onset, who have evidence of core-penumbra mismatch on perfusion imaging.

3.2 Endpoints

Primary Endpoints:

1. Safety: ANY ICH visualized on the CT scan obtained 30 (±44) hours after study drug administration.

2. Efficacy: National Institutes of Health (NIH) Stroke Scale Score at 30 (±4) h after study drug administration (adjusted for the baseline NIHSS in analysis)

Secondary Endpoints:

Clinical Efficacy (In hierarchical order):

1. Improvement in level of global disability (mRS distribution) at 90 (±7) days.

2. Frequency of excellent functional outcome (mRS 0-1) at 90 (±7) days.

3. NIHSS at 72 (±12) h (or discharge if sooner; adjusted for the baseline in analysis)

Biomarker Efficacy:

1. a2AP activity at 3 (±1) h after completion of study drug administration

2. Matrix metalloproteinase-9 plasma level 3 (+/-1) h after completion of study drug administration.

3. % tissue reperfusion on perfusion scan obtained at 30 (±4) h after study drug administration compared to the baseline

Pharmacokinetics and anti-drug antibodies:

1. PK profile of TS23 at 3 (±1) h, and 30 (±4) h, and 90 (±7) days, after completion of study drug administration.

2. Evaluation of anti-drug antibodies to TS23 (at baseline and 90 (±7) days follow-up visit).

Safety:

1. Incidence of symptomatic ICH within 30 (±4) h of study drug administration (SITS-MOST definition) (Wahlgren, Ahmed et al. 2007)

2. Incidence of non-ICH major or clinically relevant non-major bleeding within 30 days of study drug administration.

3. Non-bleeding, SAEs within 90 (±7) days

4. Incidence of stroke-related and all-cause deaths within 90 (±7) days

5. Plasma fibrinogen levels at 3 (±1) h after completion of TS23 therapy

3.3 Rationale and assessment of endpoints

NIHSS: We choose 30 (±4) h NIHSS (adjusted by baseline NIHSS) as our primary efficacy outcome measure as an ideal combination of good surrogate properties, feasibility, and strong biological rationale. NIHSS is a common surrogate marker for early-phase stroke trials (Lyden, Lu et al. 2001, Saver, Goyal et al. 2015, Lapergue, Blanc et al. 2017, Goldstein, Lennihan et al. 2018) and, when analyzed appropriately using regression methods, (Young, Weir et al. 2005, Meyer, Broocks et al. 2020, Mistry, Yeatts et al. 2020, Mistry, Yeatts et al. 2021) is strongly associated with patient-centered outcomes, including the 90d mRS global disability scale. A 72-hour NIHSS will be measured as a key secondary outcome to capture the prolonged neurological effects of TS23 in the subacute period. To minimize bias, the NIHSS will be assessed by a certified rater who will be blinded to the patients' study group assignment.

Any ICH: We choose ANY ICH to evaluate the safety of TS23 more robustly, as opposed to the commonly used symptomatic ICH outcome. In recent studies, ANY ICH is shown to be associated with worse 90-day global disability outcomes. (Gay, Collie et al.) In fact, we account for this safety outcome in our sample size and power calculations and are well-positioned to readily detect safety

issues with TS23. EXTEND trial data in the non-LVO subset (personal communication, Dr. Bruce Campbell) and data pooled from other major thrombolytic trials (Lees, Bluhmki et al. 2010) informed our assumptions.

mRS analyzed as ordinal global disability scale and dichotomous excellent outcome (mRS 0-1): mRS is the most commonly used, patient-reported, functional disability outcome in acute stroke trials. (Lees, Bath et al. 2012, Lees, Khatri et al. 2016) To increase outcome comparability and minimize bias, the modified Rankin Scale assessments of global disability will be obtained at all sites using the formal, algorithmic Rankin Focused Assessment-Ambulatory (RFA-A) method by trained raters. (Saver, Filip et al. 2010)

a2AP and MMP-9 level at 3(+/-1)-hour after completion of therapy: TS23 mechanistically lowers a2AP activity by binding to the a2AP molecule to prevent it from inhibiting plasmin. It also lowers the MMP-9 activity, which is a biomarker linked to stroke severity, as noted above in pre-clinical and phase-1 human studies. We will measure the levels of a2AP and MMP-9 in the blood of patients who receive study drug and placebo to establish mechanistic efficacy in AIS patients via a study-specific lab draw.

Tissue reperfusion: An attractive property of TS23, that stands in favorable distinction from plasminogen activators, is that is reduces micro thrombosis and thus improves tissue reperfusion, which we will measure on the 30 (±4) h follow-up CT or MR perfusion scan and calculate the percentage of the baseline hypo perfused tissue (volume of T_{max}>6s) that remains hypo-perfused at 30 (±4) h. A study-specific perfusion scan, using the same perfusion modality that was used at baseline, will be performed at 30 (±4) h after study drug infusion.

Symptomatic ICH and non-ICH major or clinically relevant non-major bleeding events are the most feared complications of thrombolytic medications. For SISTER, the symptomatic ICH will be defined using the SITS-MOST definition (Wahlgren, Ahmed et al. 2007) and other major or clinically relevant non-major bleeding events will be determined by the local study investigators as detailed in the protocol Section 9.

4 STUDY DESIGN

4.1 Overall Design

SISTER is a Phase IIa, Bayesian, adaptive, randomized dose-finding trial of TS23 in in patients with acute ischemic stroke. The study will randomize to 4 doses of TS23 (3, 5, 7, 10 mg/kg) and placebo. The trial will randomize and initiate study medication for 300 subjects. The first 50 subjects will be randomized in a dose escalation burn-in period, allocating 10 subjects to each group starting with the lowest dose. Response Adaptive Randomization updates will occur every 50 subjects, thereafter, (favoring doses with maximum utility). For each block of 50 subjects, 13 are allocated to control. The trial may stop early for safety.

Up to 50 sites are planned for the trial . We hypothesize that TS23 will be safe and potentially more efficacious than placebo for the enrolled group of patients.

4.2 Scientific Rationale for Study Design

TS23 is a novel molecule and the SISTER trial is testing this novel thrombolytic in patients with acute ischemic stroke for the first time. The best dose of TS23 for these patients is unknown. Pre-clinical studies have shown that TS23 works without increasing safety concerns. We will test TS23 alone against placebo and determine the dose of TS23 that has the best combination of safety (measured as any ICH on CT brain) and efficacy (measured on the NIHSS). By enrolling concurrently placebo-treated patients, we will be well-positioned to compare all TS23 arms against placebo in the final analysis.

4.3 Justification for Dose

The Phase II SISTER Trial seeks to identify the optimal dose of TS23 that is safest and most effective at reducing neurological deficits in ischemic stroke vs. control. The doses chosen in this study (3, 5, 7 and 10 mg/kg up to a max of 1000 mg) are within the range of TS23 dosing that was shown to be safe in phase-I human studies (2.5-10 mg/kg; as noted above). Since thrombus dissolution increases significantly when a2AP levels are less than ~50%, we selected a dose range of TS23 (3-10 mg/kg) that inhibits a2AP activity by ~50% to >95%.

4.4 End of Study Definition

A participant is considered to have completed the study if he or she has completed the 90-day visit shown in the SoA, see Section 1.3.

5 STUDY POPULATION

5.1 Inclusion Criteria

1. Age 18 years and older

2. Suspected anterior circulation acute ischemic stroke

3. Presenting NIH Stroke Scale score ≥ 6

4. Favorable baseline neuroimaging consisting of all of the following:

    a. ASPECTS of 6 or more on CT (or ASPECTS of ≥ 7 on MRI

    b. Favorable perfusion imaging on CT perfusion (CTP)/MR-perfusion weighted imaging (PWI) consisting of all of the following:

        i. Mismatch ratio of penumbra:core >1.2

        ii. Mismatch volume >10 cc

        iii. Core <70 cc

5. Able to receive assigned study drug within 4.5 to 24 hours of stroke onset or last known well.

6. Able to receive assigned study drug within 90 minutes of qualifying perfusion imaging.*

7. Informed consent for study participation obtained from participant or their legally authorized representative.

*Study drug administration is encouraged within 60 minutes after qualifying perfusion image but is allowed up to 90 minutes. After 90 minutes, another perfusion image to ensure that inclusion criteria are met is required.

5.2 Exclusion Criteria

1. Plan to receive endovascular treatment.

2. Received or plan to receive IV thrombolysis.

3. Pre-stroke modified Rankin score >2.

4. Previous treatment with TS23 or known previous allergy to antibody therapy.

5. Known pregnancy, women who are breastfeeding or plan to breastfeed within 3 months of receiving TS23 or have a positive urine or serum pregnancy test for women of childbearing potential.

6. Known previous stroke in the past 90 days.

7. Known previous intracranial hemorrhage, intracranial neoplasm, subarachnoid hemorrhage, or arterial venous malformation.

8. Known active diagnosis of intracranial neoplasm.

9. Clinical presentation suggestive of a subarachnoid hemorrhage, even if initial CT scan was normal.

10. Surgery or biopsy of parenchymal organ in the past 30 days.

11. Known trauma with internal injuries or persistent ulcerative wounds in the past 30 days.

12. Severe head trauma in the past 90 days.

13. Persistent systolic blood pressure >180mmHg or diastolic blood pressure >105mmHg despite best medical management.

14. Serious systemic hemorrhage in the past 30 days.

15. Known hereditary or acquired hemorrhagic diathesis, coagulation factor deficiency, or oral anticoagulant therapy with International Normalized Ratio (INR) >1.7.

16. Platelets <100,000/mm3.

17. Hematocrit <25 %.

18. Elevated aPTT above laboratory upper limit of normal.

19. Creatinine > 4 mg/dl, or patients receiving renal dialysis, regardless of creatinine.

20. Received heparin or low molecular weight heparins (such as Dalteparin, Enoxaparin, Tinzaparin) in full therapeutic dose within the previous 24 hours.

21. Received Factor Xa inhibitors (such as Fondaparinaux, apixaban or rivaroxaban) within the past 48 hours.

22. Received direct thrombin inhibitors (e.g., argatroban, dabigatran, bivalirudin, desirudin, lepirudin) within 48 hours.

23. Received glycoprotein IIb/IIIa inhibitors within the past 14 days.

24. Known pre-existing neurological or psychiatric disease, which would confound the neurological/functional evaluations.

25. Current participation in another research drug treatment protocol (i.e., participants could not start another experimental agent until after 90 days).

26. Concurrent acute myocardial infarction, pulmonary embolism, deep venous thrombosis, or other thrombotic event that requires anticoagulation or anti-platelet treatment.

5.3 Screen Failures

The population to be screened for the trial is defined as AIS patients presenting within 4-23 hours of last known well and who did not (or are not planned to) receive IV thrombolysis or endovascular treatment as standard clinical care. Among these, patients who are not consented are considered "screened, not consented." Patients who are consented but are not randomized due to study imaging ineligibility or other reasons, are considered "consented, not randomized." A participant, who is both randomized **and** received study drug initiation, is in the modified Intent-to-Treat (mITT) population. To ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements, and to respond to queries from regulatory authorities, we will collect demography, screen failure details, and eligibility criteria.

5.4 Strategies for Recruitment and Retention

The target sample size for SISTER trial is 300 participants in the mITT population enrolled over 36 months at 50 active sites at a given time (average accrual rate of 2.6 patient per site per year). All 50 active sites participating in the trial will screen and enroll consecutive patients who meet the trial criteria. The study personnel will not discriminate based on age, gender, race/ethnicity. Participants will be screened and enrolled in the emergency room of all participating comprehensive stroke centers.

The study personnel will work with local treatment teams to identify potential participants. The strategies of such identification will be outlined in the Manual of Procedures document as well as local site-level Standard Operating Procedures (SOPs) document.

By the nature of the condition, a considerable portion of patients with acute ischemic stroke may experience acute cognitive dysfunction. They are a vulnerable population. Inclusion of these patients is required to optimally inform safety and efficacy profile of the study drug for all acute stroke patients that meet the study criteria and to generate results that are generalizable. Exclusion of all patients with cognitive impairment at the time of enrollment will result in a study population that is not representative of AIS patients presenting within the 4.5-24-hour time window. Our team has extensive experience in undertaking investigations that involve vulnerable patients, and we will apply our expertise in minimizing risks for these study participants. Our study team has extensive experience with surrogate consent process as well. Because SISTER is a phase 2 trial testing safety of a novel drug, we will exclude pregnant women due to potentially unknown risks of the medication. Other special populations, such as fetuses, neonates, prisoners, and children, will not be eligible for inclusion.

6 STUDY INTERVENTION

6.1 Study Intervention(s) Administration

6.1.1 Study Intervention Description

TS23 is an IgG2 chimeric antibody that inactivates human a2AP. TS23 is expected to enhance the dissolution of thrombi by removing the inhibitory effects of a2AP on the fibrinolytic process.

Participants enrolled in SISTER should not receive antithrombotics for at least 24 hours after study drug administration.

The study order sets will specifically include an order not to administer antithrombotics within the first 24 hours after study drug administration. This will also be closely monitored as participants will be admitted to the intensive care unit setting for at least first 24 hours after drug admission.

Pharmaceutical Form

TS23 drug product is a liquid for IV administration. Each vial contains 10 mL of a 10.0 mg/mL solution of TS23 in a phosphate buffer-based formulation.

Dosage and Administration

TS23 will be administered as a ~15-minute IV infusion at the dose stipulated in study protocols. In the phase 1 study (TS CP01-2015) in healthy participants, TS23 was administered as single IV infusion dose ranging from 2.5 mg/kg to 10.0 mg/kg.

Special Populations

TS23 has not yet been tested in children, adults over 60 years of age, or participants with renal or hepatic impairment.

Contraindications

TS23 is contraindicated for participants with clinically significant active bleeding or for participants with known hypersensitivities to the drug substance (monoclonal antibodies) or to inactive ingredients in the formulation (such as sodium phosphate or sorbitol).

Warnings and Precautions

Bleeding: Due to the mechanism of action of TS23 on fibrinolysis, bleeding is a potential risk. Humans with α2AP deficiency have normal coagulation and platelet function. However, after coagulation, they may experience blood oozing from sites of external or internal injury. Because of the limited clinical data available with TS23, the potential for clinically significant or major bleeding in participants receiving DS- 9231 cannot be excluded. Participants should be carefully monitored for any signs of bleeding and treated appropriately. Antifibrinolytic agents such as epsilon amino caproic acid (Amicar) or tranexamic acid (Lysteda) should be considered if clinically indicated and used according to the package inserts.

No infusion-related or allergic reactions with TS23 have been observed in the only study in humans to date and the risk to participants remains unknown. Due to limited clinical experience, TS23 should be administered in clinical settings with access to resuscitation facilities and under the supervision of appropriately trained personnel. If severe allergic reactions occur, administration of TS23 should be immediately discontinued and the subject appropriately treated as per local guidelines.

Interactions

No data in humans available to date.

Pregnancy and Lactation

TS23 has not been studied in women and its potential effects on the fetus are unknown. Until conclusive studies have been conducted, TS23 should not be administered to pregnant women or women attempting to conceive, and adults of reproductive potential must adhere to contraception guidelines specified in the study protocols. No studies have been conducted to evaluate the potential of TS23 to be excreted in human breast milk. TS23 should not be administered to women who are breastfeeding or plan to breastfeed within 3 months.

Adverse Reactions or Undesirable Effects

TS23 will augment fibrinolysis and, similar to other thrombolytic agents, bleeding will be the most commonly anticipated AE. The bleeding events observed in the phase 1 human volunteer study were minor in nature, were transient and clinically inconsequential, did not require medical intervention, and were categorized as grade 1 events. Currently, there are no data available in such patients with active

illnesses who also may receive concomitant antithrombotic therapy. In pre-clinical studies, a surrogate a2AP-I for TS23 did not increase surgical bleeding in mice when used in combination with rivaroxaban, low molecular weight heparin or aspirin, by comparison to these agents alone (see Investigator's Brochure). However, in patients receiving concomitant antithrombotic therapy, the risk of bleeding may be higher. In case of severe bleeding requiring therapy, appropriate medical and surgical treatment should be instituted and antifibrinolytic agents such as epsilon amino caproic acid (Amicar) or tranexamic acid (Lysteda) may be considered, if clinically indicated, and used according to the package inserts (see Overdose, below).

TS23 is a chimeric monoclonal antibody and consequently a rare possibility of immunogenic response or infusion reaction exists. No infusion reactions were observed in the phase 1 human volunteer study. Furthermore, such reactions usually are expected when a subject receives a second or repeat dose of such therapy.

Clinical data from the only study with TS23 to date has not indicated any significant safety concerns, with a low number of treatment emergent adverse events (TEAEs) and no trends in the nature and/or severity of TEAEs detected. No deaths or treatment-related SAE have occurred in the development program. Observed bleeding TEAEs have been associated with venipuncture and have been of mild severity.

Reference Safety Information/Adverse Reactions

Based on the review of the available clinical data, currently there are no adverse reactions considered expected for TS23. All AEs are considered unexpected for regulatory reporting purposes.

Previous Clinical Experience with Products of the Same Class

No information about previous experience with products of the same class is available at this time.

Overdose

All attempts will be made to minimize overdosing by providing a simple dosing table to all sites (Table 2). There is currently no information available regarding potential overdose of TS23. In case of suspected overdose, participants should be closely monitored in an inpatient, supervised setting. Supportive care measures should be initiated as determined by the investigator's judgment. Due to the known mechanism of action of TS23, AEs events associated with TS23 overdose are likely to be related to increased fibrinolysis. In such cases, antifibrinolytic agents such as epsilon amino caproic acid (Amicar) or tranexamic acid (Lysteda) should be considered if clinically indicated and used according to the package inserts.

Drug Abuse and Dependence

There are currently no data on drug abuse or dependence with TS23; however, it is considered that no potential exists.

6.1.2 Dosing and Administration

In the SISTER trial, five different dose cohorts will be studied:

Cohort 1: placebo (for TS23)

Cohort 2: 3.0 mg/kg TS23

Cohort 3: 5.0 mg/kg TS23

Cohort 4: 7.0 mg/kg TS23

Cohort 5: 10.0 mg/kg TS23

The doses will be administered according to the following dosing provided in Table 2.

Table 2. Dosing Based on Patient Weight

TS23 dose mgs				
Weight (kg)	**3 mg/kg**	**5 mg/kg**	**7 mg/kg**	**10 mg/kg**
<60	180	300	420	600
60 to <70	210	350	490	700
70 to <80	240	400	560	800
80 to <90	270	450	630	900
≥90	300	500	700	1000

TS23 study drug is provided in 10 ml vials at a concentration of 10 mg/ml. The study drug will be diluted by the study pharmacist with sterile 0.9% saline to a total volume of 200 ml for infusion as described in the Pharmacy Manual. A placebo solution consisting of 200 ml of the diluent (0.9% saline for infusion) will be dosed in a similar manner to maintain the double-blind study design. TS23 diluted for infusion with 0.9% saline and 0.9% saline have a similar appearance. The diluted study drug or placebo will be given to the study team by an unblinded site pharmacist without indication of the identity of the drug, so as to maintain blinding. Participants will either receive the study drug (200 ml) or 0.9% saline placebo (200 ml), which will be given intravenously as a single dose, over an infusion time of about ~15 minutes. At the highest dose of TS23, this corresponds to a maximum rate of ≤0.67 mg/kg/min.

6.2 Preparation/Handling/Storage/Accountability

6.2.1 Acquisition and accountability

The StrokeNet Central Pharmacy will distribute study drug to participating sites using the WebDCU™ system. Initial investigational product supply will automatically be supplied to each site following collection of regulatory documents, site training, and completion of the site readiness call. Automatic resupply will occur when the WebDCU™ system determines additional investigational product is required at the site.

When a drug shipment is received, the site or site pharmacists will check the amount and condition of the drug, check for appropriate local language in the label, drug expiration date, and acknowledge receipt in WebDCU, and contact StrokeNet pharmacy as soon as possible if there is a problem with the shipment. A Drug Accountability Record will be provided for the investigational study drug. The record must be kept current and should contain the dates and quantities of study drug received, the subject to whom the study drug was dispensed (via identification number and/or initials or supply number as applicable), the date and quantity of study drug dispensed and remaining, as well as the initials of the dispenser.

At the end of the study, or as directed, all investigational product supplied by the Sponsor, with all discrepancy resolved, including unused, partially used, or empty containers, will be destroyed at the site according to the site's drug handling and disposition SOPs or returned to Strokenet Central Pharmacy if required by site institutional policy. A copy of these SOPs must be available onsite. The certificate of destruction must be provided to Translational Sciences that documents the drug, the quantity (vials), method of destruction, and date of destruction.

The Investigational Product will be destroyed or returned only after the study monitor or alternatively the site, according to their instructional SOPs, has completed a final inventory to verify the quantity to be destroyed or returned. The destruction of study drug must be documented, and the documentation filed and in case of returns included in the shipment. At the end of the study, a final study drug reconciliation statement must be completed by the Investigator or designee and provided to the Sponsor. All Investigational Product inventory forms must be made available for inspection by a Sponsor authorized representative or designee and regulatory agency inspectors. The Investigator is responsible for the accountability of all used and unused study supplies at the site.

6.2.2 Formulation, Appearance, Packaging, and Labeling

The investigational product TS23 is manufactured under the direction of Translational Sciences, Inc. by Cytovance Biologics, Inc. TS23 is supplied in 10 ml stoppered vials that will be labeled as an investigational drug with appropriate study identifiers to permit tracking of vials. Each vial contains 10 mL of a 10 mg/mL solution of TS23 in a phosphate buffer-based formulation (10 mM sodium phosphate, 50 mM sodium chloride, 5% Sorbitol, pH 6.0). It appears as a clear liquid, virtually free of particles.

Details of the packaging and labeling of TS23 10 mL of 10 mg/mL solution for infusion and matching placebo will be provided in the pharmacy instructions.

Packaging and labeling will be performed according to GMP.

6.2.3 Product Storage and Stability

TS23 drug supplies must be stored in a secure, limited access storage area at 2-8°C (36-46°F) and protected from light. In the event of an excursion from storage requirements, the site will quarantine the investigational product and consult the Sponsor to determine whether the investigational product can be used.

6.2.4 Preparation

The doses for administration of TS23 are weight-based. To preserve blinding, TS23 and its placebo will be administered as the same volumes and rates of IV infusion.

TS23 is available in single-use vials containing 10 mL of 10 mg/mL of product for IV infusion. This will be diluted in 0.9% saline approved for IV injection to a total volume 200 ml per subject. It will be infused over 15 min. as described in the Pharmacy Manual

The placebo will be sterile, 0.9% saline alone in a volume of 200 ml. It will also be infused over 15 min. as described in the Pharmacy Manual

6.3 Measures to Minimize Bias: Randomization and Blinding

6.3.1 Randomization

A web-based central randomization system will be developed by the National Data Management Center (NDMC) and available for use via WebDCU™. The objective of randomization is to prevent possible selection bias by providing random treatment assignment to each subject, and to prevent accidental treatment imbalances for the known prognostic variables. Balancing of prognostic variables will be conducted using the Minimal Sufficient Balance randomization algorithm which aims to maximize the treatment allocation randomness while containing the baseline covariate imbalances. The randomization will not be stratified by study site. With a total of 50 active sites enrolling at a given time during the study, who are enrolling at an average rate of 2.5 patients/year, it will be nearly impossible for local investigators to be able to inadvertently guess the randomization order.

Randomization will occur via the study-specific password-protected website accessed by an authorized research coordinator or investigator at the clinical site. If, in rare circumstances, the web system is not available, the coordinator or investigator will have access to a 24/7 emergency randomization hotline that will allow the site to randomize the subject. Upon randomization by the authorized person at each center, an e-mail notification will be sent to the Site Principal Investigator (PI), Site Primary Study Coordinator and relevant StrokeNet National Clinical Coordinating Center (NCC) and NDMC personnel.

6.3.2 Blinding

SISTER will employ a prospective, randomized, double-blinded, placebo-control trial design. Participants and local investigators will be blinded to the study group assignment. Only the local pharmacist will be aware of this group assignment; the local pharmacist will not have direct subject contact and will have no participation in outcome adjudication.

All clinical (including laboratory) and imaging outcome assessments will be performed by trained personnel who will be blinded to participants' study group assignment at 3(+/-1) hour, 30 (+/-4) hour, 30 (±5) days, and 90 (±7) days. NIHSS will be assessed by certified raters. The mRS will be rated by raters certified in the Rankin Focused Assessment (RFA) method. (Saver, Filip et al. 2010, Banzon,

Patel et al. 2013, Patel, Starkman et al. 2016) All imaging outcomes will be centrally adjudicated by trained readers who are blinded to the participant's study group assignment.

6.4 Study Intervention Compliance

The TS23 is given by short-lived administrations (15 mins). Thus, study intervention compliance will be monitored closely by direct observation of study drug administration by the local study staff. If the study drug is discontinued after initiation, the reason for discontinuation, total duration of administration and total dose administered will be noted in the Case Report Form (CRF) by the local study personnel.

6.5 Concomitant Therapy

For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in the CRF are concomitant prescription medications, over-the-counter medications, and supplements.

6.5.1 Rescue Medicine

In case of symptomatic ICH or major systemic bleeding event, antifibrinolytic agents such as epsilon amino caproic acid (Amicar) or tranexamic acid (Lysteda) should be considered and used according to the package insert.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 Discontinuation of Study Intervention

All patients enrolled in the SISTER trial will be monitored during the study drug administration and for at least 4 hours following the completing of drug administration in high acuity setting (such as the emergency department, intermediate care unit or ICU) for development of adverse reactions and without disruption of the standard clinical care. No dose reductions are allowed in this study. Temporary interruptions in the administration of study drug infusions, other than brief ones to address a blocked IV line, are also not allowed in this study. It is expected that all randomized participants will receive the complete treatment (~15 minutes infusion), unless there is a safety issue that prevents completion of the infusion. If the patient develops hemorrhagic complications or anaphylaxis to the drug administration, the study medication may be halted. In such an event, the patient will be treated for the complications per the local investigators' best judgement.

If there is a need to interrupt administration of the study drug infusion because of a safety concern, then study drug administration must be permanently discontinued. The reason for discontinuation and the amount of dose administered will be documented.

Information regarding the type of reaction leading to discontinuation of study intervention, total duration of administration, total dose administered, and treatment needed for the reaction will be noted

on the CRF. The event responsible for study drug discontinuation will be noted as an AE or SAE and/or UP (Please see Section 9 below).

Discontinuation from study intervention does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by the study protocol. If a clinically significant finding is identified (including, but not limited to changes from baseline) after enrollment, the investigator or qualified designee will determine if any change in participant management is needed. Any new clinically relevant finding will be reported as an AE.

7.2 Participant Discontinuation/Withdrawal from the Study

Participants are free to withdraw from participation in the study at any time upon request.

The reason for participant withdrawal from the study will be recorded on the CRF. Participants who sign the informed consent form and are randomized but do not receive the study intervention may be replaced. Participants who sign the informed consent form, and are randomized and receive the study intervention, and subsequently withdraw, or are withdrawn from the study, will not be replaced.

7.3 Lost to Follow-Up

When scheduling follow-up visits, multiple attempts should be made in order to reach the participant. If the participant cannot be reached, multiple attempts should be made to the person(s) listed as secondary contact. If no contact is made after multiple attempts, alternative methods should be considered in order to obtain contact with the participant and/or verify their living status. Examples of alternative methods of contact are fax, other scheduled medical appointments, and certified letter.

Efforts should be made for up to 150 days from randomization to determine the participants' 90-day status. A participant may be considered lost to follow-up if they cannot be reached for up to 150 days from randomization, despite following the procedures outlined above. The following are considered to be reasons a subject is lost to follow-up: unable to contact for follow-up, moved away from and unable to return for follow-up visits. Appropriate documentation of lost to follow-up will be maintained in the subject's research record at the site.

All effort is put forth to ensure complete follow-up for all participants that had study drug initiated, in particular with the assessment of the primary outcome at 24-30 hr. This is expected to be extremely rare for patients enrolled in SISTER given that majority of the patients will be admitted to the hospital at least for the duration of 24-30 hours following their acute stroke. The study personnel will note the patient's name, date-of birth, and medical record numbers upon enrollment. They will also clearly communicate and remind the clinical care teams about the study assessment timeline to from time-to-time. Other measures taken to minimize loss to follow-up will be outlined in site-specific SOPs. It is possible that a small fraction of patients may die before completing the 30 (±4) hour assessments. Our plan to account for such missing data is outlined in the statistical analysis plan (SAP).

8 STUDY VISITS

The following core data will be abstracted for all patients enrolled in the SISTER trial.

8.1 Baseline

Patients presenting with acute ischemic stroke will be evaluated by a stroke team physician, in accordance with institutional practice, to establish study eligibility. All consecutive patients meeting study entry criteria will be enrolled in the trial.

Participants enrolled in SISTER should not receive antithrombotics for at least 24 hours after study drug administration.

The study order sets will specifically include an order not to administer antithrombotics within the first 24 hours after study drug administration. This will also be closely monitored as participants will be admitted to the intensive care unit setting for at least first 24 hours after drug admission.

The following data will be documented at the baseline evaluation for randomized patients:

- Demographics: Age, sex, race/ethnicity
- Medical history regarding presence of concomitant medical conditions (e.g. hypertension, diabetes, hyperlipidemia, atrial fibrillation, prior ischemic stroke, prior hemorrhagic stroke, current or past smoking)
- Vital signs: Systolic and diastolic arterial blood pressure; heart rate, temperature
- Concomitant medications at time of stroke onset (anticoagulation, antiplatelets, antihypertensives, statins, antidiabetics, cholesterol reducers)
- Pre-stroke functional state: pre-stroke modified Rankin Scale, pre-stroke ambulatory status (independent, with assistance, unable)
- Laboratory Evaluation
    - Measurement within 12 hours prior to study drug administration (if there are multiple measurements, then consider the latest measurement)
        - SOC: aPTT, PT with INR, CBC, platelet count.
    - First measurements upon presentation to study hospital, within 2-3 hours of study drug administration):
        - SOC: Glucose, creatinine
    - First value collected during hospitalization:

      SOC: Hemoglobin A1C, Lipid Panel containing total cholesterol, HDL, LDL & triglyceride.
- Neurologic Deficit: NIHSS upon arrival to the study hospital

- Brain and Vascular imaging at presentation:
    - CT or MRI
    - CTA or MR angiography (MRA)
    - CTP or MRI-PWI, if applicable per institutional practices
  - Initial Workflow Time Metrics: Date and time of:
    - Last known well (LKW)
    - Symptoms first observed (SFO)
    - Outside hospital (OSH) date/times (if patient first presented to an OSH):
      - Arrival at OSH ("Door-In")
      - Departure from OSH ("Door-Out")
    - Study Hospital date/times:
      - Arrival at Study Hospital
      - Initial brain parenchymal imaging (NCCT or DWI MRI)
      - Initial vascular or perfusion imaging (CTA or MRA; CTP or PWI-MR)
  - Findings on First Imaging at Presentation (per site)
    - NCCT or MRI
      - ASPECTS
      - Any Radiologic Hemorrhage
    - CTA or MRA
      - Presence of a visible vessel occlusion
      - Location of vessel occlusion if present (per the central reader): ICA, M1 MCA, M2 MCA, M3/M4 MCA, A1 ACA, A2-5 ACA, VA, BA, P1 PCA, P2 PCA, P3/4 PCA, PICA, AICA, SCA, Other
    - CTP or Perfusion/Diffusion MR (PWI MR)

- Perfusion lesion volume (volumes of regions with T_{max} > 6 sec and >10 sec on CTP or PWI MR)
- Ischemic core lesion volume (on CTP -volume of region with rCBF < 30%; on DWI-volume of region with ADC < 620×10^{-3} mm/s)

8.2 Pre-Randomization/Randomization visit

- Time of randomization
- Time of study drug administration
- Time of study drug completion
- Baseline research related laboratory studies (prior to study drug administration):
  - Fibrinogen – processed and resulted at site.
  - a2AP, MMP-9 levels and PK studies – processed and frozen. This will be shipped to the central laboratory

8.3 3 (±1) Hours Post Study Drug Administration

- Research labs:
  - Fibrinogen - processed and resulted at site.
  - a2AP, and MMP-9 levels and PK studies - processed and frozen. This will be shipped to the central laboratory

8.4 30 (± 4) Hours Post Study Drug Administration

- Research related labs:
  - Fibrinogen, PT/INR, aPTT to be processed and resulted at site
  - a2AP, and MMP-9 levels and PK studies to be processed and frozen. This will be shipped to the central laboratory
- SOC Labs:
  - CBC

- NIHSS assessed by blinded, certified rater
- Basic metabolic panel (glucose, calcium, sodium, potassium, carbon dioxide, and chloride, BUN creatinine) at 30 (±4) hours post study drug administration if done as standard of care
- Vital Signs
- Non contrast CT Brain (reading will be performed centrally) **(Please note that an MRI cannot substitute for the CT)**
    - ASPECTS score
    - Any radiologic hemorrhage (further classified as SAH, HI1, HI2, PH1, PH2, RIH, IVH, SDH, EDH) on CT
- CTA or MRA head (required only if visible vessel occlusion present on the baseline CTA or MRA per site read).
    - Presence of a visible vessel occlusion
    - Location of vessel occlusion (per the central reader): ICA, M1 MCA, M2 MCA, M3/M4 MCA, A1 ACA, A2-5 ACA, VA, BA, P1 PCA, P2 PCA, P3/4 PCA, PICA, AICA, SCA, Other
- CT/MR perfusion
    - Perfusion lesion volume (volumes of regions with T_{max} > 6 sec and >10 sec on CTP or PWI MR)
    - Ischemic core lesion volume (on CTP -volume of region with rCBF < 30%; on DWI-volume of region with ADC < 620×10^{-3} mm/s)
    - If the participant underwent CT perfusion at baseline, then CT perfusion should be obtained at 30 (±4) hours.
    - If the participant underwent MR perfusion at baseline, then MR perfusion should be obtained at 30 (±4) hours.
- Symptomatic intracranial hemorrhage [symptomatic ICH, as per SITS-MOST definition (a local or remote type II parenchymal hemorrhage within 30 (±4) h after treatment associated with a ≥ 4-point deterioration on the NIHSS score from baseline or from the lowest score from baseline to 24 hours, or leading to death.)] (Wahlgren, Ahmed et al. 2007)
- Major non-ICH systemic hemorrhage
- Clinically relevant systemic hemorrhage

- AE
- SAE
- UPs

8.5 72 Hour Visit / Discharge (+/-12 hrs.) (whatever comes first)

- NIHSS
- Standard of Care anti-thrombotics started by end of day 2

8.6 Hospital discharge

- Concomitant antithrombotic medications during hospital course
- Anticoagulant deep vein thrombosis (DVT) prevention therapy during hospitalization
- Vascular medications at discharge (anticoagulants, antiplatelets, antihypertensives, statins, antidiabetics, cholesterol reducers)
- Stroke mechanism – TOAST criteria

    1) large-artery atherosclerosis

    2) cardioembolism

    3) small-vessel occlusion

    4) stroke of other determined etiology

    5) stroke of undetermined etiology

- Discharge destination (home, other acute hospital, acute rehab, skilled nursing facility, hospice, expired)
- Ambulatory status at discharge (independent, with assistance, unable)
- mRS at discharge
- AEs, SAEs, and UPs

8.7 Day 30 (±5) Visit

- mRS
- NIHSS

- Discharge Summary
- Concomitant medicines
- AEs, SAEs, and UPs

8.8 Day 90 (±7) Visit

- mRS
- NIHSS
- Concomitant meds
- Laboratory studies
    - Pharmacokinetic profile of TS23 and evaluation of anti-drug antibodies to TS23 at 90 (±7) days. Specimens are processed and frozen at site for every 6-month batch shipping to core lab.
    - (CBC, CMP, and Lipid profile if collected as standard of care at 90 (±7) days).
- AEs, SAEs, and UPs

9 SAFETY ASSESMENTS

9.1 Primary safety endpoint

ANY ICH visualized on the 30 (±4) h CT scan is the primary study endpoint for SISTER. This will be determined on the 30 (±4) h CT scan uploaded in the central imaging databank by local study personnel, by a trained neuroradiologist who is blinded to the participant's study group assignment.

9.2 Secondary safety endpoints

1. Incidence of symptomatic ICH within 30 (±4) h of study drug administration (SITS-MOST definition). (Wahlgren, Ahmed et al. 2007) The presence of type-2 parenchymal hematoma will be determined by a central, blinded, neuroradiologist and a worsening in NIHSS will determined by the NIHSS reported on the study CRF by the local, certified, blinded assessors.

2. Incidence of non-ICH major or clinically relevant non-major bleeding within 30 days of study drug administration.

   Non-ICH major bleeding is defined as clinically overt bleeding, that is not ICH, with one or more of the following:

    - causing a fall in hemoglobin of 2 g/dL or more,
    - or leading to a transfusion of 2 or more units of packed red blood cells or whole blood,

- symptomatic and occurring in a critical site: intracranial, intraspinal, intraocular, pericardial, intra- articular, intramuscular with compartment syndrome, retroperitoneal,
- contributing to death.

Clinically relevant non-major (CRNM) bleeding is defined as overt bleeding not meeting the criteria for major bleeding that requires medical attention or is associated with discomfort for the subject such as pain, or impairment of activities of daily life.

All other overt bleeding episodes not meeting the criteria for major or CRNM bleeding will be classified as nuisance bleeding.

Symptomatic ICH, non-ICH major bleeding, and CRNM will be adjudicated by the study independent medical monitor (IMM)

3. Non-bleeding, SAEs within 90 (±7) days will be determined by direct interview during all study visits.

4. Incidence of stroke-related and all-cause deaths within 90 (±7) days will be determined by direct interview.

5. Plasma fibrinogen levels at 3 (+/-1) h after completion of TS23 therapy will be determined by laboratory testing of study-specific blood draw.

9.3 Adverse Events and Serious Adverse Events

9.3.1 Definition of Adverse Events (AEs)

An AE is any untoward medical occurrence in a subject administered a pharmaceutical product that does not necessarily have to have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product (ICH E2A Guideline. Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, Oct 1994).

It is the responsibility of Investigators, based on their knowledge and experience, to determine those circumstances or abnormal laboratory findings which should be considered AEs.

9.3.2 Definition of Serious Adverse Events (SAEs)

An SAE is any untoward medical occurrence that at any dose:

- Results in death,
- Is life-threatening,
- Requires inpatient hospitalization or prolongation of existing hospitalization,

- Results in persistent or significant disability/incapacity,
- Is a congenital anomaly/birth defect, or
- Is an important medical event.

Note: The term "life-threatening" in the definition of "serious" refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe (ICH E2A Guideline. Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, Oct 1994).Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above. Examples include allergic bronchospasm, convulsions, and blood dyscrasias or development of drug dependency or drug abuse.

Note:

- Procedures are not AEs or SAEs, but the reason for the procedure may be an AE or SAE.
- Pre-planned (prior to signing the Informed Consent Form) procedures or treatments requiring hospitalizations for pre-existing conditions that do not worsen in severity are not SAEs.

9.3.3 Classification of an Adverse Event

9.3.3.1 Severity of Event

The severity of AEs and SAEs will be reported using the grading system outlined in the NCI Common Terminology Criteria for Adverse Events Version 5.0 (CTCAE). The CTCAE provides a grading (severity) scale for each AE term and AEs are listed alphabetically within categories based on anatomy or pathophysiology. The CTCAE (v5.0) displays Grades 1-5 with unique clinical descriptions of severity for each AE based on this general guidance:

CTCAE Severity Grading Summary	
Grade 1:	Mild AE
Grade 2:	Moderate AE
Grade 3:	Severe or Disabling AE
Grade 4:	Life-Threatening AE
Grade 5:	Death related to AE

The complete definitions of these grades are:

- Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Grade 2: Moderate; minimal, local, or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.).
- Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living (bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden).
- Grade 4: Life-threatening consequences; urgent intervention indicated.
- Grade 5: Death related to AE.

9.3.3.2 Relationship to Study INTERVENTION

The Investigator should assess causal relationship between an adverse event and the study drug on the basis of his/her clinical judgment and the following definitions. The causality assessment must be made based on the available information and can be updated as new information becomes available.

- Related:
  - The AE follows a reasonable temporal sequence from study drug administration and cannot be reasonably explained by the subject's clinical state or other factors (e.g., disease under study, concurrent diseases, and concomitant medications).

or

  - The AE follows a reasonable temporal sequence from study drug administration and is a known reaction to the drug under study or its chemical group or is predicted by known pharmacology.

- Not Related:
  - The AE does not follow a reasonable sequence from study drug administration or can be reasonably explained by the subject's clinical state or other factors (e.g., disease under study, concurrent diseases, and concomitant medications).

9.3.3.3 Expectedness

The IMM will be responsible for determining whether an SAE is expected or unexpected. An SAE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

9.3.3.4 Action Taken Regarding Study Drug(s)

- Dose Not Changed: No change in study drug dosage was made.

- Drug Withdrawn: The study drug was permanently stopped.
- Dose Increased: The dosage of study drug was increased.
- Not Applicable: Subject died, study treatment had been completed prior to reaction/event, or reaction/event occurred prior to start of treatment.

9.3.3.5 Other Actions Taken for Event

- None
  - No treatment was required.
- Medication required.
  - Prescription and/or over the counter (OTC) medication was required to treat the adverse event.
- Hospitalization or prolongation of hospitalization required.
  - Hospitalization was required or prolonged due to the AE, whether or not medication was required.
- Other.

9.3.3.6 Adverse Event Outcome

- Recovered/Resolved
  - The subject fully recovered from the AE with no residual effect observed.
- Recovering/Resolving
  - The AE improved but has not fully resolved.
- Not Recovered/Not Resolved
  - The AE itself is still present and observable.
- Recovered/Resolved with Sequelae
  - The residual effects of the AE are still present and observable.
  - Include sequelae/residual effects.
- Fatal
  - Fatal should be used when death is a direct outcome of the AE.

- Unknown

9.3.4 Time Period and Frequency for Event Assessment and Follow-Up

The occurrence of an AE or SAE may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate CRF. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

The study coordinator will record all reportable events with start dates occurring any time after informed consent is obtained until the patient completes their 90-day visit. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

9.3.5 Adverse Event Reporting

All reporting is done in accordance with StrokeNet SOPs for Safety Monitoring and Reporting as outlined in the administrative documents available on the website. Clinical sites will be responsible for submitting event reports in WebDCU and responding to queries for additional information in a timely manner. The MPIs will be responsible for ensuring that AE data is assessed and recorded appropriately following GCP guidelines.

9.3.6 Notifying Regulatory Authorities, Investigators, and Institutional Review Board

The SISTER MPIs will inform Investigators, IRB/ECs, and regulatory authorities of any Suspected Unexpected Serious Adverse Reactions (SUSARs) occurring in other study sites or related to other studies of the investigational drug, as appropriate per local reporting requirements.

In the U.S., upon receipt of the Sponsor's notification of SUSARs that occurred with the study drug, unless delegated to the Sponsor, it is the Investigator's responsibility to inform the IRB per Sponsor's instruction.

9.3.7 Adverse Events of Special Interest

Adverse Events of Special Interest should always be reported to the Sponsor as soon as possible following the procedures outlined in Section 9.3.5 for Adverse Event reporting, with the Investigator's assessment of seriousness, causality, and a detailed narrative.

These include:

- Combined elevations of aminotransferases and bilirubin, either serious or nonserious and whether or not causally related, meeting the criteria of a potential Hy's Law case (total bilirubin level ≥ 2 × upper limit of normal (ULN) with simultaneously ALT or AST ≥ 3 × ULN).

- Symptomatic ICH as defined above.

9.3.8 Reporting of Pregnancy

The MPIs must be notified of any subject who becomes pregnant while receiving study medication after the last dose of study medication. If a female subject partner becomes pregnant within 7 days after the subject receives study medication, the pregnant female partner will be consented so that the pregnancy can be followed through 7 days after birth of the infant.

Although pregnancy is not technically an AE, all pregnancies must be followed to conclusion, 7 days after birth, to determine their outcome. This information is important for both drug safety and public health concerns. It is the responsibility of the Investigator, or designee, to report any pregnancy in a female subject or female partners of male participants using the Exposure In Utero (EIU) Reporting form. Please contact your StrokeNet National Coordinating Center Project Manager to receive the EIU Reporting Form upon learning of a pregnancy. The Investigator should make every effort to follow the subject until completion of the pregnancy and complete the EIU Reporting Form with complete pregnancy outcome information, including normal delivery and induced abortion. The adverse pregnancy outcome, either serious or non- serious, should be reported in accordance with study procedures. In case of pregnancy in the female partner of a male patient, the outcome of the pregnancy should be obtained if the female partner agrees. If the outcome of the pregnancy meets the criteria for immediate classification as an SAE (i.e., post-partum complications, spontaneous or induced abortion, stillbirth, neonatal death, or congenital anomaly, including that in an aborted fetus), the Investigator should follow the procedures for reporting SAEs outlined in Section 9.3.5.

9.4 Unanticipated Problems

9.4.1 Definition of Unanticipated Problems (UPs)

The Office for Human Research Protections (OHRP) considers UPs involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;

Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and

Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

This definition could include any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, the drug, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with the drug that relates to the rights, safety, or welfare of participants (21 CFR 812.3(s)).

9.4.2 Unanticipated Problem Reporting

The investigator will report UPs to the reviewing IRB/EC and to the NCC/NDMC. The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

An investigator will be required to submit to the sponsor and to the reviewing IRB/EC a report of any UPs occurring during an investigation as soon as possible, but in no event later than 10 working days, after the investigator first learns of the effect.

9.4.3 Emergency Contact

All investigators will have access to a 24-hour study hotline, staffed by SISTER PIs, in case of emergency questions. The study PIs and project managers will be available via email for non-emergent study questions.

9.5 Interim Monitoring of Events

The Independent Medical Monitor (IMM), who is independent from any involvement with the study, will monitor the study with regard to safety on an ongoing basis to identify any safety concerns. The

IMM also will review all SAEs and determine whether they are related to study intervention (as described above) and will communicate with the investigators for any questions or clarifications regarding an event. Periodically throughout the study, the Executive Committee and the IMM will review reports on the incidence rates of all reported AEs, whether serious or not. Should such monitoring uncover issues that may threaten subject safety (e.g., unexpectedly high rate of AEs), the study statistician and PIs will prepare a report to be submitted to the DSMB for their review proposing further actions to be taken, if any. In addition to monthly Safety Reports, a comprehensive DSMB reports will be generated semi-annually by the NDMC: an open report to be distributed to the Executive Committee and IMM, and a closed report to be distributed only to the DSMB. Each semi-annual report will provide cumulative summary statistics on enrollment, subject status in the study, baseline characteristics, protocol violations, safety data (including a summary of the most frequent and most serious AEs, a summary of all MedWatch Reports, and a listing of all participants who were terminated from the study and the reason for termination), and data management/quality information. The open report statistics will be provided for the overall study with no separation of treatment groups. The closed report will provide cumulative summary statistics by partially blinded treatment group to DSMB members, the NIH liaison, and the project's unblinded statistician. All people with access to the closed reports will be fully independent from trial operation, and have no impact on patient recruitment, treatment, and assessment. If the DSMB wishes to be completely unblinded for these reports, a sealed identification envelope will be provided to the DSMB liaison; this envelope can be opened at the discretion of the DSMB. An annual report will be submitted to the FDA.

10 STATISTICAL CONSIDERATIONS

10.1 General Approach

This study is Phase IIa, Bayesian, adaptive, dose-finding trial. This study was designed and all simulations were performed using the Fixed and Adaptive Clinical Trial Simulator FACTS™ v6.4 Core Design software developed by Berry Consultants.

10.2 Primary Analysis

The primary analysis will be Bayesian. We will test whether the best dose of TS23 has at least 25% Utility ("Promising Zone"). The trial is a success if the best dose has utility of 0.25 or higher with at least 80% probability. Such a utility can be achieved through various combinations of 24-hour NIHSS improvement and ICH rate.

10.2.1 Dose-Response Models

For each endpoint, we model the dose-response relationship using a Bayesian, normal dynamic linear model (NDLM). This type of model is appropriate for a variety of dose-response shapes including non-monotonic. The NDLM is a method of smoothing between neighboring groups and was proposed for dose-finding trials by Berry in 2002. Both NDLM models assume weakly informative priors (for baseline NIHSS and control group) or non-informative priors (for smoothing parameters). The model details are provided in the SAP.

10.2.2 Utility Function

We define a utility function based the combination of the primary safety outcome, U1, and the primary efficacy outcome, U2, as U1xU2 where 1 is perfect utility and zero is no utility. This utility score

provides an integrated assessment of safety (any radiographic ICH on follow-up CT) and preliminary efficacy (improvement in 24-hour NIHSS) and is used to define our "promising zone".

For the safety outcome (ICH), U1=1 if the ICH response rate for that dose is ≤40% and U1=0 if the ICH rate is 70% or greater and takes a quadratic function between 40%-70%. This was based on a clinical discussion of what would be an acceptable rate of any ICH.

For the primary efficacy outcome (NIHSS), the utility equal

is defined based on the mean difference from control adjusted for baseline NIHSS. U2=1 if the dose is 2 points better than control and U2=0 if it is no different from control with a quadratic function in between 0 and 1.

10.2.3 Quantities of Interest

We define quantities that will be used to make decisions during the trial.

10.2.3.1 Probability of Being Target

The U_{max} is the dose with the greatest utility (target dose). For each dose, we will calculate the probability of being the U_{max}.

10.2.3.2 Posterior Probabilities for Safety

For each dose we will calculate the probability that the ICH rate is greater than 60%. The expected ICH rate is 0.40 and 0.60 is the upper bound on the acceptable rate. We will use the probability to establish safety stopping rule.

10.2.3.3 Posterior Probabilities for Utility

For each dose we will calculate the probability that the Utility (U1*U2) is greater than 0.25.

10.2.4 Final Evaluation Criteria

At the final analysis, criteria for trial to be a success ('Go'):

Pr(Utility >0.25| Greatest U_{max}) >80%

The trial is a success if the best dose has 0.25 utility or higher with at least 80% probability.

10.3 Sample Size Determination

Simulations (5000 replications) under 11 scenarios, which take the adaptive design and model assumptions into account, indicate that the probability of trial success ("power") is 84% - 95% for all safe scenarios. A 2-point difference in NIHSS is clinically meaningful and feasibly detectible according to our simulations.

The type I error rate is shown in Table 3. Because this is an early phase trial, we opted for higher Type I error rate since this is not a pivotal trial. Under the complete null case ('Null Safe") all doses are the same as control on NIHSS and ICH, the probability of trial success is 0.188 (type I error rate) but was smaller if there were safety concerns. The trial stopped early in 78% of simulations when the ICH rate was unsafe ("Null All Doses Unsafe").

While higher than the 0.05 type I error rate in Phase III trials, a false positive rate of 10-20% is typically accepted in early Phase II trials; any dose arm meeting the criteria for success will be tested in a Phase III trial where the type I error rate can be tightly controlled. In early development trials, such as SISTER, the more serious error is the False Negative error and this is well controlled.

Table 3. Design Simulation Results (Fixed and Adaptive Clinical Trial Simulator FACTSTM v6.4)

	True Mean 24-hour NIHSS (SD=5.5)					True Proportion ICH					Proportion of Trial Success "Power"	Proportion of Trial Stop Early for Safety
Scenario	Placebo	TS23 3mg	5mg	7mg	10mg	Placebo	TS23 3mg	5mg	7mg	10mg		
Safe and Effective												
3 Good Doses	7	6	5	5	5	0.4	0.4	0.4	0.4	0.4	0.95	0
2 Good Doses	7	6.5	6	5	5	0.4	0.4	0.4	0.4	0.4	0.93	0
1 Good Dose	7	7	6	6	5	0.4	0.4	0.4	0.4	0.4	0.87	0
Umbrella	7	6	5	6	7	0.4	0.4	0.4	0.4	0.4	0.84	0
Unsafe but Effective												
Umbrella High Doses Unsafe	7	6	5	6	7	0.4	0.5	0.6	0.7	0.7	0.58	0
Bouncy High Doses Unsafe	7	5	7	5	7	0.4	0.5	0.6	0.7	0.7	0.86	0
Weak High Doses Unsafe	7	6.5	6	5	5	0.4	0.5	0.6	0.7	0.7	0.29	0.03
Weakest High Doses Unsafe	7	7	6	6	5	0.4	0.5	0.6	0.7	0.7	0.17	0.04
Null											**Type I Error**	
Null Safe	7	7	7	7	7	0.4	0.4	0.4	0.4	0.4	0.188	0
Null High Doses Unsafe	7	7	7	7	7	0.4	0.5	0.6	0.7	0.7	0.069	0
Null All Doses Unsafe	7	7	7	7	7	0.4	0.7	0.7	0.7	0.7	0.002	0.78

10.4 Populations for Analyses

The primary analysis will be analyzed under the mITT principle. Under this principle, the evaluable sample will include all participants who are randomized for whom the study drug infusion was started. The Per-Protocol sample includes participants who had no eligibility violations (e.g., enrolled in error) and received their assigned study dose.

10.5 Conventions for Missing Data

At any analysis, some participants may have missing data. The missing data could result from the subject dropping out of the study, or because the subject simply has not yet reached the final visit.

For any subject whose endpoint is unknown due to drop out, the final outcome will be multiply imputed from the Bayesian model. However, if a patient dies before 24 hours and their death is a neurological death (potentially informative missingness), we will impute their missing NIHSS based on the worst observed NIHSS value and as an event for the ICH outcome.

10.6 Baseline Descriptive Statistics

A comparison of patient characteristics and at a baseline assessment between the treatment groups will be done. Baseline descriptive statistics will be provided for the following items: demographics, NIHSS, age, time from onset, prior medications, medical history, visualized occlusion status (yes/no).

10.7 Planned Interim Analyses

The trial may stop early for safety (futility). No early stopping criteria for success have been defined for this trial.

10.8 Analysis of the Secondary Endpoints

Secondary analyses will compare all doses to control by graphing the raw and model-fitted means and 95% CI. Group means (adjusted for baseline NIHSS and age) will be plotted with 95% confidence intervals around the mean for each dose and control. No formal hypothesis testing will be performed and no p-values reported for secondary efficacy outcomes.

10.9 Sub-Group Analyses

Recruitment and retention of females and minorities will be monitored by the DSMB and will be provided in the Final Report. Although we do not anticipate differential treatment effects based on sex, race, or ethnicity, our analyses will explore clinically important differences due to sex/race/ethnicity.

A table/forest plot will be created which shows the following by Subgroup level:

Subgroup/ Number of Patients/Adjusted Mean (SD) NIHSS at 30 (±4) hours for all treatment group/Adjusted Mean difference from control (95% CI) for each active arm.

10.10 Sensitivity Analysis

As a sensitivity analysis the primary analysis will be repeated with the Per-protocol sample. If these analyses are not consistent with the primary Intention-To-Treat (ITT) analysis, then the trial findings will be interpreted with caution.

11 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

11.1 Regulatory, Ethical, and Study Oversight Considerations

11.1.1 Informed Consent Process

11.1.1.1 Consent/assent and Other Informational Documents Provided to participants

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant if they are able to provide informed consent or their legally authorized representative as soon as the study team is able to contact them.

11.1.1.2 Consent Procedures and Documentation

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. SISTER trial will primarily utilize electronic consent forms. Paper consent forms will be available per site request. Consent forms will be IRB-approved and the participant or their surrogate healthcare decision maker will be asked to read and review the document. The investigator will explain the research study to the participant or their surrogate healthcare decision maker and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's or their surrogate healthcare decision maker's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants or their surrogate healthcare decision makers will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants or their surrogate healthcare decision makers will have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. Participants and their surrogate healthcare decision makers will be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document, either physical or electronic, will be given to the participants for their records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

11.1.2 Study Discontinuation and Closure

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants and funding agency. If the study is prematurely terminated or suspended, the PI will promptly inform study participants, the IRB, and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Insufficient compliance to protocol requirements

Study may resume once concerns about safety, protocol compliance, and data quality are addressed and satisfy the IRB.

11.1.3 Confidentiality and Privacy

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor in accordance with StrokeNet SOP GCP 05. This confidentiality is extended to cover clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated is held in strict confidence. No written documents related to the study (e.g., protocol, Manual of Procedures (MOP), Investigator's Brochure (IB)) or data may be released outside of the usual operations of the SISTER without the express permission of the leadership.

In accordance with subsection 301(d) of the Public Health Service Act, this research is covered by a Certificate of Confidentiality (COC), and therefore the investigators shall not disclose or provide, in any Federal, State, or local civil, criminal, administrative, legislative, or other proceeding, the name of a participant or any such information, document, or data that contains identifiable, sensitive information about a participant and that was created or compiled for purposes of the research, unless such disclosure or use is made with the consent of the individual to whom the information, document, or data pertains.

All research activities will be conducted in as private a setting as possible, within limitations sometimes imposed by the medical environment encountered as a result of acute stroke.

The medical safety monitors, DSMB, or Central Institutional Review Board (cIRB); study PIs, NCC, NDMC, or their authorized representatives; or relevant regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and supply records for the participants in this study.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location by performance sites and by the NDMC in accordance with the StrokeNet SOP GCP 12, or longer if required by the cIRB or local site regulations.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the NDMC at the Medical University of South Carolina (MUSC). This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by the NDMC research staff will be secured and password protected. At the end of the study, all study databases – already de-identified as above – will be archived at the NDMC (as well as archived in data repositories as described in 10.1.14). Data are collected electronically in WebDCU™, the data management system managed by the NDMC of StrokeNet.

11.1.4 Future Use of Stored Specimens and Data

Data collected for this study is analyzed and stored at the NDMC and images collected for this study are analyzed and stored at the SISTER Imaging Core at the University of Cincinnati. After the study is completed, the archived LDS data and imaging will be transmitted to and stored in the NINDS Data Repository, for use by other researchers including those outside of the study, per StrokeNet SOP ADM 04 and in accordance with de-identification procedures of GCP 05.

Specimens will be used only for purposes related to research described in this protocol, i.e., understanding the causes and optimal prevention, treatment, and outcomes of unexplained strokes. The Executive Committee reviews will ensure that uses of subject specimens are consistent with the informed consent obtained at the time the specimen was collected.

11.1.5 Key Roles and Study Governance

Role	Name, Degree, Title	Contact Information	Mailing Address
Principal Investigator	Pooja Khatri, MD MSc Professor of Neurology	pooja.khatri@uc.edu (513) 558-6411	University of Cincinnati 260 Stetson St, ML 0525, Cincinnati, OH 45267
Principal Investigator	Guy Reed, MD MS Dean, College of Medicine	guyreed@arizona.edu (602) 827-2701	University of Arizona Suite 1060, BSPB 475 N. 5th St Phoenix, AZ 85004
Principal Investigator	Eva A. Mistry, MBBS, MSCI Associate Professor of Neurology	mistryea@ucmail.uc.edu (513) 558-1291	University of Cincinnati 260 Stetson St, Suite 2310 Cincinnati OH 45219
Principal Investigator	Jordan Elm, PhD	elmj@musc.edu Tel 843-876-1605	Medical University of South Carolina 135 Cannon Street Ste 303, MSC835 Charleston SC 29425-8350
Independent Medical Monitor	Wade Smith, MD	wade.smith@ucsf.edu (415) 353-8897	UCSF 400 Parnassus Ave Eighth Floor, San Francisco, CA 94143

The study Executive Committee will include the multiple principal investigators: Pooja Khatri, Jordan Elm, Eva Mistry, and Guy Reed. They will oversee all study operations (in conjunction with the NCC, NDMC, and NINDS project officer), interact with the cIRB and the DSMB. The executive committee will also regularly interact with the FDA and the primary contact will be Dr. Reed, who will also be the primary IND holder.

The Operations Committee consists of key study investigators and personnel, including NINDS program officer, the PIs of the NCC and NDMC, primary project managers and lead coordinators. Operations committee will have the overall responsibility for the direction of the study. The committee will convene weekly to oversee and review the progress of the study, discuss, and recommend to the executive any major changes in study procedures or direction, review protocol adherence, and data collection and analyses.

11.1.5.1 Safety Oversight

Safety oversight is under the direction of the NINDS-appointed DSMB, composed of individuals with the appropriate expertise, including expertise in stroke and biostatistics. Members of the DSMB are independent from the study conduct and free of conflict of interest, or measures should be in place to minimize perceived conflict of interest. The DMSB operates under the rules of an approved charter to be written prior to, and reviewed at, the organizational meeting of the

DSMB. The DSMB provides its input to NINDS/NIH staff, as well as the PIs of the SISTER trial. The NIH StrokeNet DSMB-IRB SOP can be found at https://www.nihstrokenet.org/docs/default-source/default-document-library/strokenet-cirb-and-dsmb-joint-operating-procedure-9-15-2020.pdf?sfvrsn=0.

Changes in the constitution of the DSMB will not trigger a cIRB protocol amendment. Additionally, SISTER MPIs have named an IMM, who will review safety events including SAEs on an ongoing basis.

11.1.6 Clinical Monitoring

Clinical site monitoring is conducted to ensure that the rights and well-being of human participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with GCP, with applicable FDA regulations (21 CFR 312), and with the FDA's "Guidance for Industry Oversight of Clinical Investigations — A Risk-Based Approach to Monitoring."

- Monitoring for the trial will be performed by the NDMC centrally, on site, and remotely.

- Per the study's monitoring plan, monitoring will include a combination of on-site monitoring (to verify data entered into the WebDCU™ database against source documents and query inaccuracies between the source documents and WebDCU™ database), remote monitoring (source document verification, including verification of written consent, may be performed remotely by reviewing source documents that have been uploaded into WebDCU™ or via remote access to electronic medical records), and central monitoring (using web-based data validation rules, data manager review of entered data, statistical analysis, and on-going review of site metrics).

- The NDMC, study PIs, project managers, and the appropriate site PIs will be provided copies of monitoring reports within 30 days of site visits.

- In an effort to review informed consent forms in a timely manner, enrolling sites will upload a pdf of the signed informed consent form, into the password protected clinical trial management system, WebDCU™. The PDF file will be linked to the subject ID but will be stored on a secure server separate from the study's CRF data. The secure server on which these files are stored is not backed up to prevent copies of files containing individually identifiable health information from being copied and stored on non-NDMC back up servers. The files on these servers can only be accessed by designated NDMC study personnel. NDMC staff will remotely monitor the informed consent forms and issues identified will be relayed to the clinical site for corrective and preventative action. After remote monitoring is complete, the PDF file containing the informed consent form will be permanently deleted from the secure server. If a subject must be re-consented, the process will repeat itself.

11.1.7 Quality Assurance (QA) and Quality Control (QC)

Each clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation, and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written SOPs, the monitors will verify that the clinical trial is conducted, and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, ICH GCP, and applicable regulatory requirements.

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

11.1.8 Data Handling and Record Keeping

11.1.8.1 Data Collection and Management Responsibilities

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Black ink is required to ensure clarity of reproduced copies. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. **DO NOT ERASE, OVERWRITE, OR USE CORRECTION FLUID OR TAPE ON THE ORIGINAL**.

Copies of the electronic CRF (eCRF) are provided for use as source documents and maintained for recording data for each participant enrolled in the study. Data reported in the eCRF derived from source documents should be consistent with the source documents or the discrepancies should be explained and captured in a progress note and maintained in the participant's official electronic study record.

Clinical data (including AEs, concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into WebDCU™ a 21 CFR Part 11-compliant data capture system provided by the NDMC. The WebDCU™ data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate.

11.1.8.2 Study Records Retention

Study documents should be retained for the duration specified by the StrokeNet SOP or for a longer period if required by local regulations.

11.1.9 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, ICH GCP, or MOP requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH GCP:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site investigator to use continuous vigilance to identify and report deviations within 15 working days of identification of the protocol deviation. All deviations must be addressed in study source documents, reported to NINDS Program Official and NIDMC. Protocol deviations must be sent to the reviewing IRB per their policies. The site investigator is responsible for knowing and adhering to the reviewing IRB requirements. Further details about the handling of protocol deviations will be included in the MOP.

11.1.10 Publication and Data Sharing Policy

The SISTER trial will comply with the NIH Public Access Policy, which ensures that the public has access to the published results of NIH funded research, and the StrokeNet SOP (ADM 03) regarding results publication. Manuscripts and abstracts that use data from SISTER require approval from the Publication Committee of an original proposal before the concept may proceed. All publications will include this acknowledgement: “Research reported in this publication was supported by the National Institute of Neurological Disorders and Stroke of the National Institutes of Health under Award Number [*to be determined*]. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.”

11.1.11 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the relevant device industry, is critical. Therefore, any actual conflict of interest for persons who have a role in the design, conduct, analysis, publication, or any aspect of SISTER will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and

conduct of this study. The study leadership in conjunction with the NINDS has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

11.2 Additional Considerations

NA

11.3 Abbreviations

a2AP	Alpha-2 Antiplasmin
a2AP-I	Alpha-2 Antiplasmin Inactivating Antibody (Inhibitor)
AE	Adverse Event
ANCOVA	Analysis of Covariance
CFR	Code of Federal Regulations
cGMP	Current Good Manufacturing Practice
cIRB	Central Institutional Review Board
CNS	Central Nervous System
COC	Certificate of Confidentiality
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
CRNM	Clinically relevant non-major
CT	Computed tomography
CTA	CT angiography
CTCAE	Common Terminology Criteria for Adverse Events
CTP	CT perfusion
DSMB	Data Safety Monitoring Board
DVT	Deep Vein Thrombosis
EC	Ethics Committee
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EIU	Exposure in Utero
EVT	Endovascular Therapy
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
ICH	Intracranial Hemorrhage
IgG	Immunoglobulin G
IMM	Independent Medical Monitor
IND	Investigational New Drug Application
INR	International Normalized Ratio

IRB	Institutional Review Board
ITT	Intention-To-Treat
IV	Intravenous
KO	Knock-out
LKW	Last known well
LVO	Large Vessel Occlusion
MCA	Middle Cerebral Artery
mITT	modified Intent-to-Treat
MMP-9	Matrix metalloproteinase-9
MOP	Manual of Procedures
MPI	Multiple Principal Investigator team
MRA	MR Angiography
MRI	Magnetic Resonance Imaging
mRS	Modified Rankin Scale
MUSC	Medical University of South Carolina
NCC	National Clinical Coordinating Center
NCT	National Clinical Trial
NDLM	Normal Dynamic Linear Model
NDMC	National Data Management Center
NIH	National Institutes of Health
NIHSS	National Institutes of Health Stroke Scale
NMDA	N-methyl-D-aspartate receptor
OHRP	Office for Human Research Protections
OSH	Outside Hospital
OTC	Over the counter
PD	Pharmacodynamics
PE	Pulmonary Embolism
PHI	Protected Health Information
PI	Principal Investigator
PK	Pharmacokinetics
PWI	Perfusion Weighted Imaging
QA	Quality Assurance
QC	Quality Control
RFA	Rankin Focused Assessment

RFA-A	Rankin Focused Assessment-Ambulatory
rtPA	Recombinant Tissue Plasminogen Activator (alteplase)
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SFO	Symptoms First Observed
sICH	Symptomatic Intracranial Hemorrhage
SISTER	Strategy for Improving Stroke Treatment Response
SoA	Schedule of Activities
SOP	Standard Operating Procedure
STAIR	Stroke Academic Industry Roundtable
SUSAR	Suspected Unexpected Serious Adverse Reactions
TEAE	Treatment Emergent Adverse Events
TNKase	Tenecteplase
tPA	Tissue Plasminogen Activator
ULN	Upper Limit of Normal
UP	Unanticipated Problem
uPA	Urokinase-type Plasminogen Activator
U.S.	United States

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