SUPPLEMENTAL MATERIAL

Randomized, Multicenter Trial of Argatroban with Recombinant Tissue Plasminogen Activator for Acute Stroke Study (ARTSS-2)

Table I. ARTSS-2 Trial Eligibility Criteria

Inclusion Criteria

1	Disabling Ischemic stroke symptoms with onset < 3 hours treated with IV rt-PA by local standards*. * or ≤ 4.5 hours according to local standard of care. Symptoms must be distinguished from another ischemic event such as syncope, seizure, migraine, subarachnoid hemorrhage and hypoglycemia. If the patient reports awakening with the event, the time of onset should be considered as the last time the patient (or a witness to the patient's condition) considered herself/himself normal.
2	Age ≥18.
3	NIHSS ≥ 10* or any NIHSS with an intracranial clot should be demonstrated on neurovascular imaging (TCD or CTA) in any one of the following areas: distal ICA, MCA (M1 or M2), PCA (P1 or P2), distal vertebral or basilar artery. - TCD criteria: TIBI 0, 1, 2 or 3 - CT-Angiogram: TIMI 0 or 1 * NIHSS ≥ 10, demonstration of clot on neuroimaging is not necessary (i.e., enrollment can proceed with non-contrast head CT alone), but if performed, a clot must be demonstrated.
4	For those patients who will undergo repeat CT-Angiogram at 2-3 hours, estimated glomerular filtration rate (eGFR) must be ≥ 60 mL/min/1.73m^2.
5	Females of childbearing potential must have a negative serum pregnancy test (HCG) prior to the administration of trial medication.
6	Signed (written) informed consent by the patient or the patient's legal representative and/or guardian.

Exclusion Criteria

1	Patients whom the treating physician is planning (or could plan) to treat with intra-arterial thrombolysis or other endovascular procedures (i.e., mechanical clot retrieval) aimed at recanalization.
2	Evidence of intracranial hemorrhage (ICH) on baseline CT scan or diagnosis of a non-vascular cause of neurologic deficit.
3	NIHSS Level of Consciousness score (1a) ≥ 2.
4	Pre-existing disability with mRS ≥ 2.
5	CT scan findings of hypoattenuation of the x-ray signal (hypodensity) involving ≥ 1/3 of the MCA territory.
6	Any evidence of clinically significant bleeding, or known coagulopathy.
7	INR >1.5
8	Patients with an elevated aPTT greater than the upper limit of normal (test can be repeated if investigator suspects a falsely elevated value such as when the collection tube is not completely filled).
9	Patients currently, or within the previous 24 hours, on an oral direct thrombin inhibitor (i.e., dabigatran).
10	Heparin flush required for an IV line. Line flushes with saline only.
11	Any history of intra-cranial hemorrhage, known ateriovenous-malformation or unsecured cerebral aneurysms.
12	Significant bleeding episode [e.g. gastrointestinal (GI) or urinary tract] within the 3 weeks before study enrollment.

13	Significant bleeding episode [e.g. gastrointestinal (GI) or urinary tract] within the 3 weeks before study enrollment.
14	Major surgery or serious trauma in last 2 weeks.
15	Patients who have had an arterial puncture at a non-compressible site, biopsy of parenchymal organ, or lumbar puncture within the last 2 weeks.
16	Previous stroke, myocardial infarction (MI), post myocardial infarction pericarditis, intracranial surgery, or significant head trauma within 3 months.
17	Uncontrolled hypertension (SBP > 185 mmHg or DBP >110 mmHg) that does not respond to intravenous anti-hypertensive agents.
18	Surgical intervention (any reason) anticipated within the next 48 hours.
19	Known history of clinically significant hepatic dysfunction or liver disease – including a current history of alcohol abuse.
20	Abnormal blood glucose <50 mg/dL (2.7 mmol/L).
21	History of primary or metastatic brain tumor.
22	Current platelet count < 100,000/mm^3.
23	Life expectancy < 3 months.
24	Patients who, in the judgment of the investigator, needs to be on concomitant (i.e., during the Argatroban infusion) anticoagulants other than Argatroban, including any form of heparin, UFH, LMWH, defibrinogenating agent, dextran, other direct thrombin inhibitors or thrombolytic agents, GPIIb/IIIa inhibitor or warfarin. [*Caveat: However, if in the judgment of the investigator a patient needs to be anticoagulated, **but** this can be deferred for 48 hours, then they could be included.]
25	Currently participating or has participated in any investigational drug or device study within 30 days before the first dose of study medication.
26	Known hypersensitivity to Argatroban or its agents.
27	Additional exclusion criteria if patient receives IV rt-PA between 3-4.5 hours of last seen normal: - Age >80; Currently taking oral anticoagulants (regardless of INR); - History of stroke and diabetes; NIHSS > 25.

BAYESIAN PRIMER & ARTSS-2 BAYESIAN IMPLEMENTATION

Background Information

Bayesian inference focuses on the probability of hypothesized treatment effects given the observed data. In contrast, classical frequentist statistics focuses on the likelihood that the observed (or more extreme) data would be obtained assuming that the null hypothesis is true. Frequentist statistics address the question: What is the likelihood that this excess or a larger difference between groups would have occurred if the null hypothesis—e.g., that treatment has no effect on the outcome--is correct (i.e., $Pr(data|H_0)$)? Bayesian statistics addresses a fundamentally different question that is more clinically relevant: What is the probability that the intervention has no effect on the outcome (or conversely, the probability that it does), given the data obtained in the trial and any prior evidence (i.e., $Pr(H_0|data)$). Bayesian analyses provide direct estimates of treatment effect that are not obtainable from conventional frequentist analyses.[1,2] They also provide direct estimates of the probability of a treatment effect of a specific magnitude, which may be quite important in assessing the probability that the benefits outweigh the hazards or that there is at least a minimum clinically important difference.

In a Bayesian analysis the probability of treatment effect, either benefit or harm, is estimated by combining prior evidence with data from the current study. The result referred to as the posterior probability indicates the likelihood of benefit or harm from the treatment being studied. In contrast to Bayesian analyses, conventional frequentist analyses do not explicitly incorporate evidence from other relevant studies or skepticism about implausible large treatment effects. The reader must informally incorporate external evidence on her own when interpreting results from a study.

Concerns about Bayesian analyses have largely been related to choosing an overly optimistic prior probability (or a prior based on weak evidence), thus producing overly optimistic posterior probabilities of treatment benefit. This concern did not apply to this trial because we utilized a neutral estimate of treatment effect (RR = 1.0). This use of a prior RR of 1.0 in Bayesian analyses "shrinks" the RR estimate at the study conclusion closer to the null, resulting in more conservative estimates of the treatment effect than with conventional frequentist estimates. Further, our choice of the 95% prior interval allows for considerable uncertainty within a range that encompasses the RR observed for virtually all therapies between those that are quite beneficial (RR=0.3) or quite harmful (RR=3.3).[1,2]

In their statement on p-values, the American Statistical Association (ASA) states that studies should not simply rely on a p-value or statistical significance since neither is a good measure of evidence of benefit.[3] They state that where appropriate, results should be supplemented with other approaches including Bayesian methods. These methods are uncontested for evaluation of diagnostic tests and have been recommended by the FDA for studies of medical devices.25 Use of Bayesian methods in oncology[4] is widespread, and have also been adopted in NIH funded neurological trials (with FDA oversight), including an interventional trial for status epilepticus.[5]

Description of Bayesian analyses and implementation details

We used Bayesian Poisson to analyze the primary outcome mRS at 90 days. The Poisson models used a binary outcome (mRS 0-1 at 90-days: yes/no) whereas the ordinal model used a categorical outcome (mRS of 0-6 with scores 5 and 6 combined). Primary analyses were adjusted for country (US/UK), presence of terminal ICA occlusion, and HAT score. Separate analyses were conducted comparing argatroban with rt-PA alone and in combination (low+high argatroban + rt-PA vs. rt-PA alone). Secondary analyses additionally adjusted for age and NIHSS total score at baseline.

For the group terms (i.e., argatroban (yes/no) or argatroban dose (low, high), we used Normal(0, 0.56^2) neutral priors in the log relative risk (RR) scale, which have a 95% prior interval of 0.33–3.0 in the RR scale. This prior is based on the largest likely effect size identified for major outcomes in randomized trials. See figure on next page.

For the intercept term, we used a Normal(0,102) prior and a weakly informative Normal(0,1) priors for all other parameters in the model (excludes RR >7 or <0.14).

Secondary outcomes were analyzed using similar primary and secondary analyses as follows:

sICH. Poisson models were used using two sets of priors. The first was centered at RR of 1.0 with a 95% prior interval of 0.33-3.0. Since we expected escalating doses of Argatroban to somewhat increase the risk for ICH, we also chose a conservative prior centered at RR of 1.5 with a 95% prior interval 1.16 to 2.50 for the low dose group. For the high dose group, the prior was centered at RR of 2.0 with 95% prior interval of 1.33-4.0. For sICH, a RR of >1 indicates the increased risk of experiencing an sICH. Conversely, a RR of <1 indicates a decreased risk for developing sICH.

Degree and completeness of arterial recanalization. We conducted two separate analyses using Poisson regression. First analyses used complete or partial recanalization as the binary outcome, and the second used complete recanalization as the outcome. In both analyses, a neutral prior centered at RR of 1.0 with 95% prior interval of 0.75-1.75 for both Argatroban groups was used.

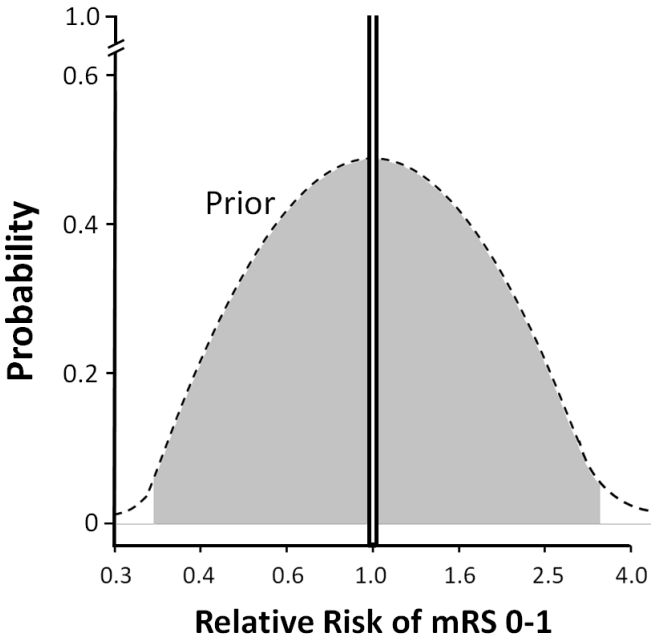

Figure. Graphical depiction of the ARTSS-2 prior. The double vertical line, centered at a neutral relative risk (RR) of 1.0, assumes no benefit or harm of combination treatment compared to rt-PA alone. The gray shaded area represents the 2.5th and 97.5th percentile bounds of the prior (e.g., 95% prior interval). In other words, the area under the curve to the right and left of no benefit/harm (RR=1.0) means there is a 50:50 chance of combination treatment superiority or inferiority compared to rt-PA alone. Note: x-axis is set to logarithmic scale.

NIHSS total score at 2 hours, 24 hours, 48 hours post rt-PA bolus, Day 7 (or discharge, whichever comes first) and day 90. Negative binomial regression models were used for these analyses. For the NIHSS at 2, 24, and 48 hours, a prior centered at RR of 1.0 with 95% prior interval of 0.33-3.0 was used for both Argatroban groups. For the NIHSS at day 7 and day 90, a neutral prior with a wider 95% prior interval (RR: 0.25-4.0) was used for both Argatroban groups.

Methodology of Markov Chain Monte Carlo (MCMC) simulations.

We fitted all Bayesian models via Markov Chain Monte Carlo methods (MCMC) using Stan (2.14.1)[6] through R (version 3.3.2)[7] package *rstanarm* (version 2.13.1).[8] For each analysis we ran 3 MCMC chains with starting values randomly drawn from the estimated parameters from a frequentist Poisson model. A burn-in of 3,000 iterations was used, with sampling from a further 10,000 iterations for each chain. To monitor convergence, trace plots and the Gelman-Rubin convergence diagnostic (Rhat) were used for all parameters. All point estimates reported are posterior medians.

For all analyses, the trace plots showed good mixing of the 3 chains with Rhat < 1.01 for all parameters, indicating convergence.

References

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2. Berry DA. Bayesian clinical trials. Nat Rev Drug Discov 2006;5:27-36.
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Table II. Adverse events by body system.

	Control rt-PA-alone N=29	**Low-Dose** Argatroban + rt-PA N=30	**High-Dose** Argatroban + rt-PA N=31
Adverse events (AE) n, mean per patient	65, 2.2	112, 3.7	88, 2.9
Probably related to argatroban, n	-	3	2
Possibly related to argatroban, n	-	27	32
Patients with ≥1 AE n (%)	20 (69)	27 (90)	25 (81)
No. of patients with ≥1 SAE n (%)	14 (48)	15 (50)	15 (48)
Serious adverse events (SAE) n	22	29	24
Probably related to argatroban, n	-	0	0
Possibly related to argatroban, n	-	5	5
Not related to argatroban, n	-	24	19
Serious adverse events n, mean/patient	22, 0.8	29, 1.0	24, 0.8
Probably related to argatroban	0	0	0
Possibly related to argatroban	0	5	5
Body System			
Cardiac Disorders	1	0	2
Gastrointestinal disorders	1	1	1
Infectious Disease	1	3	1
Pulmonary Disorders	3	5	5
Nervous System Disorders	9	11	10
Skin Disorders	0	3	0
Electrolyte Imbalances	5	5	3
Musculoskeletal Disorders	1	1	0
Genitourinary Disorders	0	0	1
Blood-Circulation Disorders	1	0	0

All non-sICH bleeding events. Abbreviations: PH-2–Parenchymal Hematoma Type-2; PH-1–Parenchymal Hematoma Type 1; HT-2–Hemorrhagic Transformation Type-2; HT-1–Hemorrhagic Transformation Type-1.

	Control rt-PA-alone N=29	**Low-Dose** Argatroban + rt-PA N=30	**High-Dose** Argatroban + rt-PA N=31	**Low + High-Dose** Argatroban + rt-PA N=61
***Major systemic bleeding** n (%)	1 (3)	1 (3)	0	1 (2)
PH-2 n (%), 95% CI	3 (10), 2.2-27.4	1 (3), 0.1-17.2	2 (7), 0.8-21.4	3 (5), 1.0-13.7
PH-1 n (%), 95% CI	1 (4), 0.1-17.8	3 (10), 2.1-26.5	0 (0)	3 (5), 1.0-13.7
HT-2 n (%), 95% CI	4 (14), 3.9-31.7	4 (13), 3.8-30.7	3 (10), 2.0-25.8	7 (12), 4.7-22.2
HT-1 n (%), 95% CI	2 (7), 0.9-22.8	7 (23), 10-42.3	3 (10), 2.0-25.8	10 (16), 8.2-28.1

* rt-PA alone case - Enrollment: 7/1/13; Bleed date: 8/1/13; Diverticulosis of colon with hemorrhage. Patient had ≥2g/dL drop in hemoglobin and transfused ≥2 units of blood.

* Low-dose Argatroban + rt-PA case - Enrollment: 5/19/14; Bleed date: 7/3/14; Traumatic Gastric Ulcer. Patient had ≥2g/dL drop in hemoglobin and transfused ≥2 units of blood. Event adjudicated as not related to argatroban by the independent medical monitor.

Table III. Stroke etiology by trial arm.

Stroke Etiology n (%)	**Control** rt-PA-alone N=29	**Low-Dose** Argatroban + rt-PA N=30	**High-Dose** Argatroban + rt-PA N=31	**P-value**
Cardio embolism	13 (45)	14 (47)	11 (36)	0.49*
Large artery atherosclerosis	5 (17)	6 (20)	5 (16)	
Small artery disease	2 (7)	0 (0)	0 (0)	
Other determined etiology	1 (4)	2 (7)	6 (19)	
Cryptogenic	8 (27)	8 (27)	9 (29)	
* Fischer's exact test.				

Table IV. **Unadjusted** results of primary, secondary and post-hoc outcomes. Abbreviations: CreI – Credible Interval; PrI – Prior Interval; CI – Confidence Interval.

	Control rt-PA-alone N=29	**Low-Dose** Argatroban + rt-PA N=30	**High-Dose** Argatroban + rt-PA N=31	**Low + High-Dose** Argatroban + rt-PA N=61
PRIMARY OUTCOME				
MODIFIED RANKIN SCALE SCORE 0-1 AT 90-DAYS N (%)	6 (21)	9 (30)	10 (32)	19 (31)
RR (95% CrI), probability RR>1.0	-	1.15 (0.56, 2.34), 0.65	1.23 (0.61, 2.51), 0.71	1.29 (0.65, 2.56), 0.76
SECONDARY OUTCOMES				
SYMPTOMATIC INTRACEREBRAL HEMORRHAGE N (%)	3 (10)	4 (13)	2 (7)	6 (10)
RR (95% CrI), probability RR>1.0	-	1.55 (1.06, 2.25), 0.99	1.67 (0.93, 3.01), 0.96	-
RECANALIZATION AT 2-3 HOURS	N=11	N=20	N=15	N=35
N (%); RR (95% CrI), probability RR>1.0				
Complete	2 (18); -	3 (15); 0.96 (0.61, 1.52), 0.43	4 (27); 1.06 (0.67, 1.68), 0.59	7 (20); 1.01 (0.63, 1.62), 0.52
Complete + Partial	6 (55); -	4 (20); 0.85 (0.54, 1.33), 0.24	7 (47); 1.06 (0.69, 1.64), 0.59	11 (31); 0.90 (0.58, 1.40), 0.32
NEUROLOGICAL IMPROVEMENT *(NIHSS)*, median (IQR) RR (95% CrI), probability RR>1.0				
2-hours	11.0 (6.0, 19.0) N=29	14.5 (5.0, 18.0) N=28	11.0 (4.0, 16.0) N=31	13.0 (5.0, 18.0) N=59
	-	1.07 (0.73, 1.56), 0.63	0.95 (0.65, 1.37), 0.38	1.0 (0.71, 1.39), 0.49
24-hours	10.0 (5.0, 18.0) N=29	14.0 (3.0, 20.0) N=29	9.0 (4.0, 16.0) N=31	11.5 (3.5, 18.0) N=60
	-	1.13 (0.73, 1.75), 0.71	0.90 (0.58, 1.38), 0.31	1.06 (0.67, 1.6), 0.51
48-hours	8.0 (3.0, 18.0) N=27	10.0 (4.0, 21.0) N=29	10.0 (2.0, 17.0) N=31	10.0 (2.5, 20.0) N=60
	-	1.15 (0.72, 1.83), 0.72	0.96 (0.60, 1.52), 0.43	1.06 (0.69, 1.6), 0.61
Day 7	5.0 (3.0, 14.0) N=27	10.5 (3.0, 18.0) N=30	6.0 (2.0, 14.0) N=29	9.0 (2.0, 16.0) N=59
	-	1.22 (0.72, 2.04), 0.77	0.93 (0.55, 1.57), 0.40	1.08 (0.67, 1.71), 0.63
Day 90	2.5 (1.0, 8.0) N=24	5.5 (1.5, 12.5) N=24	2.0 (1.0, 7.0) N=25	3.0 (1.0, 12.0) N=49
	-	1.37 (0.75, 2.44), 0.85	0.99 (0.54, 1.81), 0.49	1.19 (0.68, 2.03), 0.74
POST HOC ANALYSES				
PRIMARY OUTCOME (mRS 0-1 at 90-days)				
1) *Ordinal Logistic Regression OR (95% CI), p-value	-	0.98 (0.40, 2.41), 0.96	1.68 (0.68, 4.12), 0.26	1.29 (0.59, 2.81), 0.53
2) *Poisson Regression RR (95% CI), p-value	-	1.45 (0.59, 3.56), 0.42	1.56 (0.65, 3.75), 0.32	1.51 (0.67, 3.37), 0.32
SYMPTOMATIC INTRACEREBRAL HEMORRHAGE				
3) *Intention To Treat* (ITT) N (%), 95% CI	3 (10), 2.2-27.4	4 (13), 3.8-30.7	2 (7), 0.8-21.4	6 (10), 3.7-20.2
*RR (95% CI), p-value	-	1.29 (0.32-5.3), 0.72	0.62 (0.11-3.47), 0.59	0.95 (0.26-3.54), 0.94
As Treated N (%), 95% CI	4 (13), 3.8-30.7	4 (13), 3.8-30.7	1 (3), 0.1-17.2	5 (8), 2.8-18.4
*RR (95% CI), p-value	-	1.0 (0.26-3.63), 1.00	0.25 (0.03-2.11), 0.20	0.63 (0.18-2.16), 0.46
4) †Neutral Prior Bayesian RR (95% CrI), probability RR>1.0	-	1.17 (0.48, 2.77), 0.65	0.83 (0.34, 2.0), 0.34	0.99 (0.43, 2.39), 0.49

* Frequentist Poisson Regression.
† Prior: RR=1.0 and 95% Prior Interval (PrI) of 0.33-3.0.

Table V. Frequentist Analysis of the primary clinical outcome (mRS 0-1 at 90-days): relative risk versus odds ratios. Relative risk was calculated using a Poisson regression with robust error variance and odds ratios were generated using logistic regression. Analyses are Intention To Treat. Reference: rt-PA alone. Abbreviations: aRR – adjusted Relative Risk; aOR – adjusted Odds Ratio; CI – Confidence Interval.

	Low-Dose Argatroban + rt-PA N=30	**High-Dose** Argatroban + rt-PA N=31	**Low + High-Dose** Argatroban + rt-PA N=61
FREQUENTIST ANALYSIS			
mRS 0-1 at 90 days			
aRR, 95% CI; p-value	**1.50**, 0.64-3.49; 0.35	**1.63**, 0.72-3.72; 0.24	**1.57**, 0.74-3.33; 0.24
aOR, 95% CI; p-value	**1.73**, 0.51-5.85; 0.38	**1.97**, 0.59-6.52; 0.27	**1.83**, 0.63-5.33; 0.27
OR overestimation of RR	13%	17%	14%
Increase of 95% CI, OR vs. RR	35%	38%	32%

Figure I. ARTSS-2 Trial Time-Flow. ®- Randomization; CT- computed tomography; rt-PA – tissue plasminogen activator; CTA- computed tomography angiogram; TCD- transcranial Doppler ultrasound; NIHSS- National Institutes of Health Stroke Scale; mRS- modified Rankin Scale score; QOL- quality of life.

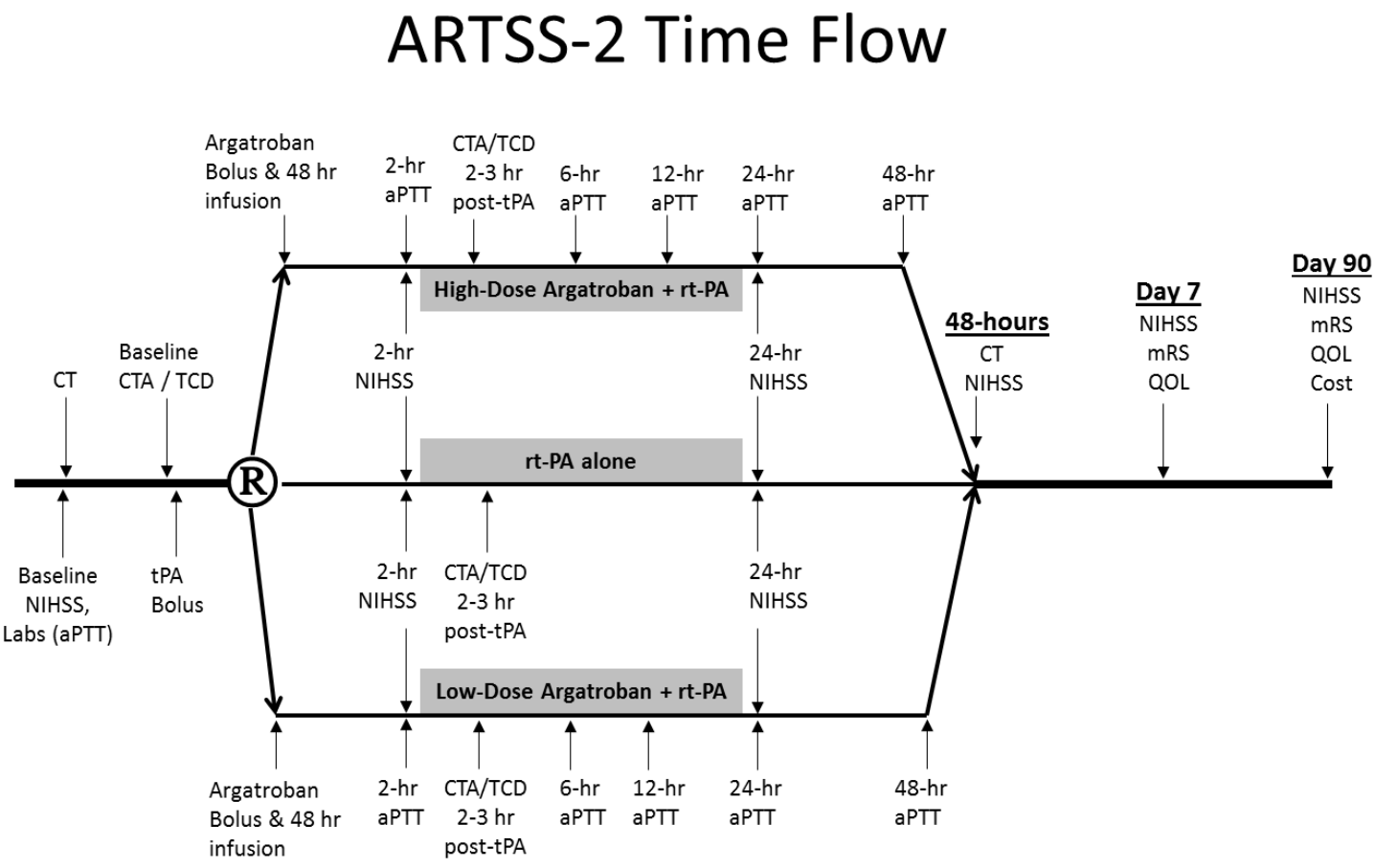
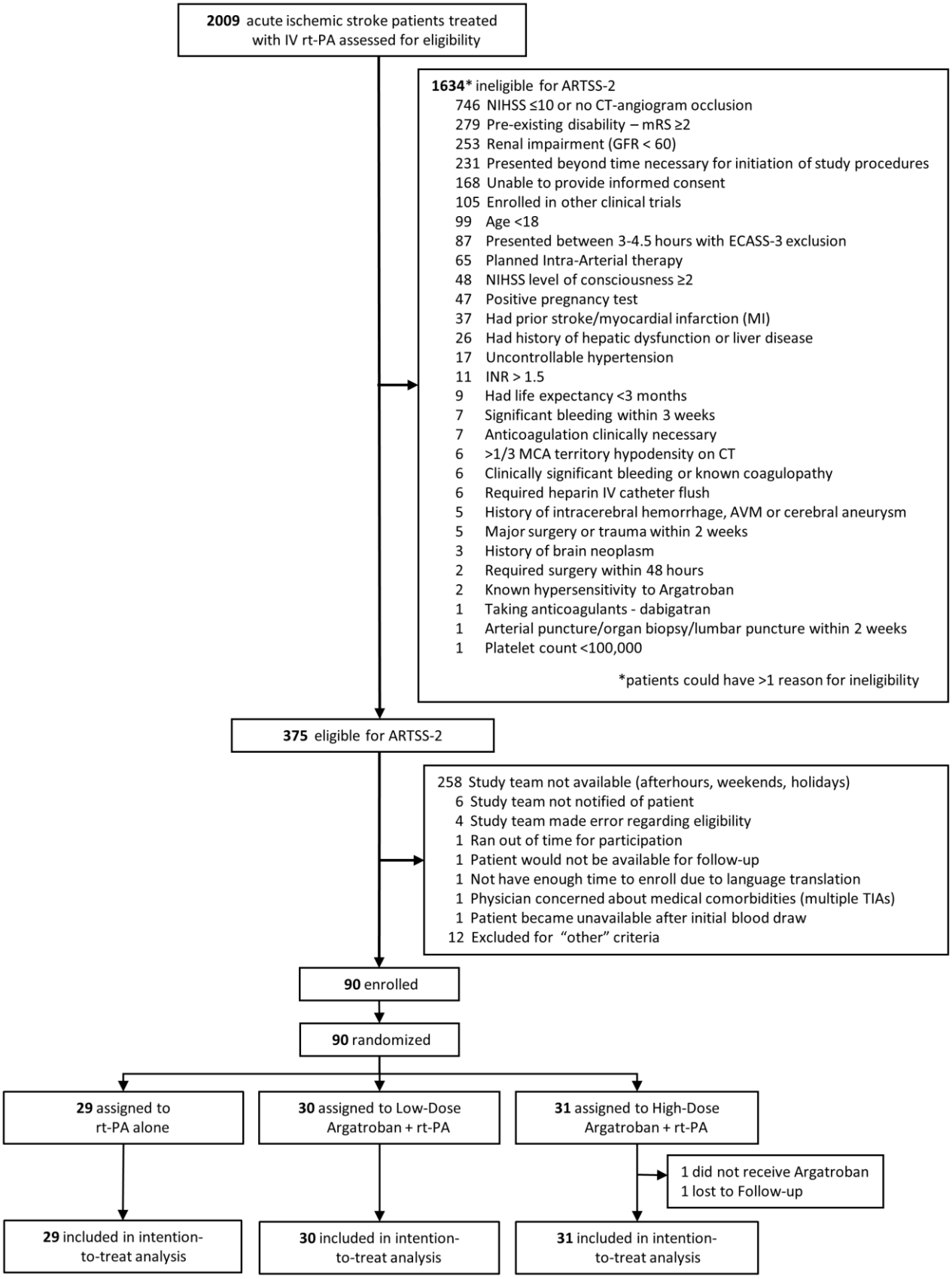


Figure II. Trial profile: CONSORT patient flow diagram with complete list of eligibility exclusions.



Appendix I. Trial protocol including Safety Stopping Rules

See attached.

Clinical Protocol

ARTSS-2: A pilot, phase IIb, randomized, multi-center trial of Argatroban in combination with recombinant tissue plasminogen activator for acute stroke

Phase IIb

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Version 1.0 June 12, 2011
Version 1.1 updated August 5, 2011
Version 1.11 updated Sept 26, 2011
Version 1.2 updated Feb 28, 2012
Version 1.3 updated Jan 11, 2013

PROTOCOL SYNOPSIS

Title	**ARTSS-2**: A pilot, phase IIb, randomized, multi-center trial of Argatroban in combination with recombinant tissue plasminogen activator for acute stroke
Study Purposes	**Background:** Recombinant tissue plasminogen activator (rt-PA), the only proven treatment for acute ischemic stroke, fails to reperfuse brain in most patients with large thrombi. In our Phase IIa low-dose safety study (n=65), the two drugs appear safe when delivered concomitantly and recanalization rates were greater than with historical controls. This study will provide evidence-based hypotheses and data needed to design a larger definitive trial. **Primary Objective**: To estimate overall treatment benefit (improvement in disability) among stroke patients treated with rt-PA who are randomized to also receive either low-dose Argatroban, high-dose Argatroban or neither. **Secondary Objectives***:* 1) To help verify the safety (as measured by incidence of intracranial hemorrhage) of low-dose combination Argatroban and rt-PA and test the safety of high-dose combination treatment; 2) To assess rates of early recanalization for use in assessing mechanisms of treatment effect and in predicting outcome of the drug combination.
Design	**P**rospective **R**andomized **O**utcome **B**linded **E**ndpoint - **PROBE**. Multicenter phase IIb trial with unblinded caregivers but blinded assessors.
Study Population	105 total ischemic stroke patients all treated with rt-PA (0-3 hour or 0-4.5 hour according to each site's local standard); age ≥18 years; proximal (intracranial) artery occlusion as imaged by either transcranial Doppler ultrasound (TCD) or CT-angiogram (CTA), or clinically suspected occlusion with NIHSS ≥10.
Treatment	Three treatment arms (n=35 each) will be enrolled: 1) Low-dose Argatroban* (1.0μg/kg/min continuous infusion of Argatroban, preceded by a 100 μg/kg bolus administered over 3-5 minutes Infusion will be titrated to achieve an aPTT of 1.75 times baseline - not to exceed 10 μg/kg/min) + usual care IV-rt-PA; 2) High-dose Argatroban* 3.0μg/kg/min continuous infusion of Argatroban, preceded by a 100 μg/kg bolus administered over 3-5 minutes Infusion will be titrated to achieve an aPTT of 2.25 times baseline - not to exceed 10 μg/kg/min) + usual care IV-rt-PA; 3) Intravenous-rt-PA alone (usual care). *Argatroban infusions will continue for a maximum of 48 hours.
Primary Outcome	Excellent functional outcome as measured by the percentage of patients with a 0 or 1 on the modified Rankin Scale (mRS) at day 90 as assessed by study personnel blinded to treatment.

Secondary Outcomes	1) Safety as measured by the incidence of: a) Symptomatic intracranial hemorrhage (sICH); b) Parenchymal Hemorrhage 2 (PH-2); c) Major systemic hemorrhage. 2) Rates and completeness of arterial recanalization assessed at baseline and 2-3 hours by Transcranial Doppler ultrasound (TCD) or CT-Angiogram (CTA). 3) Neurological deficits improvement from baseline to 2 hours, 24 hours, end of Argatroban infusion, Day 7/discharge and day 90 as measured by NIHSS. 4) Quality of Life – obtained by standard gamble, time-trade-off method and visual analogue scale (VAS). 5) Cost and cost-effectiveness analysis ▪ Medical costs associated with each treatment ▪ Incremental cost-effectiveness ratio (change in cost divided by quality of life gained)
Assessments Baseline	History & physical exam, vital signs, *CBC, liver function tests, *PT/INR, *PTT, non-contrast head CT, TCD or CTA (unless NIHSS ≥10), NIHSS, mRS, concomitant medications. *Laboratory results must be reported before study drug administration.
0-24 hours	Vital signs, aPTT (scheduled 2, 6, 12, 24 hours), NIHSS (2 and 24-hours), repeat TCD or CTA at 2-3 hours post rt-PA bolus.
24-48 hours	Vital signs, aPTT (scheduled 48 hours)
End of Argatroban Infusion (or 48 hours post tPA bolus)	Non-contrast head CT, vital signs, physical examination, lab work (same as baseline), NIHSS
Day 7 / or discharge (whichever occurs first)	Vital signs, physical examination, mRS, NIHSS, ACE, MoCA (Montreal Cognitive Assessment), Quality of life assessments (standard gamble, EQ-5D and VAS)
Day 90	mRS; NIHSS; ACE, MoCA; Quality of Life assessments (standard gamble, EQ-5D and VAS), economic substudy cost data collection.
Significance	Data generated will be used to: 1) Compare with Phase IIa study safety and recanalization results. 2) Design a Phase 3 efficacy study of 1 or 2 doses of Argatroban + rt-PA compared to usual care (rt-PA alone).

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List of Abbreviations

ACE	Aid to Capacity Evaluation
ACT	Activated clotting time
AE	Adverse event
A/G ratio	Albumin to globulin ratio
ALT (SGPT)	Alanine transaminase
ASA	Aspirin
AST (SGOT)	Aspartate transaminase
AT-III	Antithrombin III
aPTT	Activated partial thromboplastin time
AUC	Area under curve
BUN	Blood urea nitrogen
^0C	Celcius
CABG	Coronary artery bypass graft
CBC	Complete blood count
CFR	Code of Federal Regulations
CK	Creatine kinase
Clcr	Creatinine clearance
cm/s	Centimeter per second
Cmax	Maximum concentration of drug
CO_2	Carbon dioxide
CRF	Case Report Form
CT	Computed tomography
CTA	Computed tomography-Angiogram
CVA	Cerebrovascular accident
DBP	Diastolic blood pressure
ECG	Electrocardiogram
eGFR	Estimated Glomerular Filtration Rate
EQ-5D	European Quality of Life-5 Dimensions
^0F	Fahrenheit
FDA	Food and Drug Administration
g/dL	Grams per deciliter
GGT	Gamma-glutamyl transferase
GI	Gastrointestinal
GPIIb/IIIa	Glycoprotein IIb/IIIa
H, h or hr, hrs	Hour(s)
$_\beta$HCG	beta-human chorionic gonadotropin
HIPA	Heparin-induced platelet antibody
HIT	Heparin-induced thrombocytopenia
HITTS	Heparin-induced thrombocytopenia and thrombosis syndrome
ICA	Internal carotid artery
ICH	Intracranial hemorrhage
	International Conference on Harmonization
IEC	Independent Ethics Committee
IND	Investigational New Drug
INR	International Normalized Ratio
IRB	Institutional Review Board
IV or iv	Intravenous
K_i	Inhibitory constant
kg	Kilogram

L/kg/hr	Liters per kilogram per hour
LDH	Lactic dehydrogenase
LFTs	Liver function tests
LMWH	Low molecular weight heparin
MFV	Mean flow velocity
MoCA	Montreal Cognitive Assessment
μg/kg	Microgram per kilogram
μg/kg/min	Microgram per kilogram per minute
μM	Micromolar
mg	Milligram
mg/dL	Milligram per deciliter
mg/kg	Milligram per kilogram
mg/mL	Milligram per milliliter
mL	Milliliter
mL/kg/min	Milliliter per kilogram per minute
mmHg	Millimeters of mercury
MAP	Mean arterial pressure
MCA	Middle cerebral artery
MI	Myocardial infarction
MoCA	Montreal Cognitive Assessment
mRS	Modified Rankin Scale
NAP	Not applicable
NAV	Not available
NCR	No carbon required
ND	Not done
NDA	New Drug Application
NIHSS	National Institute of Health Stroke Scale
NLV	Normal Lab Value
NS	Normal saline
PCA	Posterior cerebral artery
PD	Pharmacodynamics
pH	Hydrogen ion concentration
PICU	Pediatric intensive care unit
PK	Pharmacokinetics
PSG	Paper Standard Gamble
PT	Prothrombin time
PTCA	Percutaneous transluminal coronary angioplasty
Q or q	Every
rt-PA	Recombinant tissue plasminogen activator
SAE	Serious adverse event
SBP	Systolic blood pressure
SE	Standard error
SGOT (AST)	Serum glutamic oxaloacetic transaminase
SGPT (ALT)	Serum glutamic pyruvic transaminase
$t_{1/2}$	Half-life
TBC	Texas Biotechnology Corporation
TCD	Transcranial Doppler
TIA	Transient ischemic attack
TIBI	Thrombolysis In Brain Ischemia
TIMI	Thrombolysis In Myocardial Ischemia
TT	Thrombin time
UFH	Unfractionated heparin

U/kg	Units per kilogram
VAS	Visual Analogue Scale
<	Less than
≤	Less than or equal to
>	Greater than
≥	Greater than or equal to
+	Plus

1.0 INTRODUCTION

Argatroban is a synthetic direct thrombin inhibitor derived from L-arginine. The chemical name for Argatroban is 1-[5-[(aminoiminomethyl)amino]-1-oxo-2-[[(1,2,3,4-tetrahydro-3-methyl-8-quinolinyl) sulfonyl]amino]pentyl]-4-methyl-2-piperidinecarboxylic acid, monohydrate. Argatroban has 4 asymmetric carbons. One of the asymmetric carbons has an *R* configuration (stereoisomer Type I) and an *S* configuration (stereoisomer Type II). Argatroban consists of a mixture of *R* and *S* stereoisomers at a ratio of approximately 65:35 (± 2%).

The molecular formula of Argatroban is $C_{23}H_{36}N_6O_5S \bullet H_2O$. Its molecular weight is 526.66.[1]

1.1 Mechanism of Action

Argatroban is highly selective for thrombin with an inhibitory constant (K_i) of 0.04 μM. At therapeutic concentrations, Argatroban has no or minimal effect on related serine proteases (trypsin, factor Xa, plasmin, and kallikrein).[2] It is well known that thrombin plays a pivotal role in thrombosis. Argatroban is capable of inhibiting the action of both free and clot-associated thrombin.[2]

In rat middle cerebral artery occlusion models, Matsuo, et al, reported observation of microthrombi in the infarcted and penumbral areas.[3] Using the same model, Kawai, et al, demonstrated that Argatroban could significantly reduce the size of an infarct and the number of microthrombi.[4] Results of studies by Tanaka, et al, suggest that Argatroban directly inhibits secondary microthrombus formation in acute stages of ischemic stroke.[5] An explanation offered by Tamao and Kikumoto describes the mode of action of Argatroban in cerebral thrombosis as an inhibition of the local thrombin formed due to ischemic tissue damage. Blocking of this local thrombin inhibits fibrin formation and platelet aggregation that lead to formation of thrombi in the microcirculation in and around the core zone of ischemia. By preventing subsequent thrombus formation in the microcirculation, the hypothesis is that improved blood flow to the peri-ischemic/penumbra would rescue neuronal cells at risk.[6]

Because the action of local thrombin inhibition halts and possibly reverses microthrombi occlusion, Argatroban could potentially prove to be of benefit in the treatment of strokes classified as large-artery atherosclerotic, acute cardioembolic, and small-artery occlusion (lacunar infarcts).[7]

1.2 Rationale for Combining rt-PA with Argatroban

rt-PA is highly effective if patients can be treated within 3 hours of symptom onset and has recently been shown to be effective out to 4.5 hours.[8] However, there is room for improvement. Only about one third of patients receiving rt-PA completely recover by 3 months. The benefit of rt-PA has been linked to clot lysis, however earlier drug administration and clot lysis results in better outcome. Only 20-30% of patients with documented arterial occlusion who receive rt-PA will have complete recanalization; up to 60% will have partial recanalization[9]. Furthermore, clinical deterioration, perhaps due to reocclusion occurs in at least 15% of those treated. Therefore, it is possible that Argatroban given along with rt-PA may improve results obtained with rt-PA alone by several mechanisms; improving re-flow in the microcirculation, increasing the speed and completeness of recanalization, and preventing reocclusion.

1.3 Pharmacodynamics

Argatroban is 54% bound to human serum proteins, with binding to albumin and α_1-acid glycoprotein being 20% and 34%, respectively.

The main route of Argatroban metabolism is hydroxylation and aromatization of the 3-methyltetrahydroquinoline ring in the liver. The formation of each of the four known metabolites is catalyzed *in vitro* by the human liver microsomal cytochrome P450 enzymes CYP3A4/5. The primary metabolite (M1) exerts 3 to 5-fold weaker anticoagulant effects than Argatroban. Unchanged Argatroban is the major component in plasma. The plasma concentrations of M1 range between 0 – 20% of that of the parent drug. The other metabolites (M2 – 4) are found only in extremely low quantities in the urine and have not been detected in plasma or feces. This data, together with the lack of effect of erythromycin (a potent CYP3A4/5 inhibitor) on Argatroban pharmacokinetics suggest that CYP3A4/5 mediated metabolism is not an important elimination pathway *in vivo*.[1]

Total body clearance is approximately 5.1 mL/kg/min (0.31 L/kg/hr) for infusion doses up to 40 μg/kg/min. The terminal elimination half-life ($t_{1/2}$) of Argatroban ranges between 39 and 51 minutes.[1]

When Argatroban is administered by continuous infusion, anticoagulant effects and plasma concentrations of Argatroban follow similar, predictable temporal response profiles, with low intersubject variability. Immediately upon initiation of Argatroban infusion, anticoagulant effects are

produced as plasma Argatroban concentrations begin to rise. Steady-state levels of both drug and anticoagulant effect are typically attained within 1-3 hours (0.5 – 1 hour if a loading bolus is administered) and are maintained until the infusion is discontinued or the dosage adjusted. Steady-state plasma Argatroban concentrations increase proportionally with dose (for infusion doses up to 40 μg/kg/min in healthy subjects) and are well correlated with steady-state anticoagulant effects. For infusion doses up to 40 μg/kg/min (and for bolus doses up to 350 μg/kg), Argatroban increases in a dose-dependent fashion, the activated partial thromboplastin time (aPTT), the activated clotting time (ACT), the prothrombin time (PT) and International Normalized Ratio (INR), and the thrombin time (TT). These effects occurred in healthy volunteers and patients undergoing interventional procedures such as percutaneous transluminal coronary angioplasty (PTCA), stent placement or atherectomy.

Argatroban has been tested concurrently with aspirin, acetaminophen, lidocaine, and erythromycin. In the aspirin trial, the TT and aPTT effect of Argatroban were unaffected by concomitant administration of aspirin. There was a positive, linear correlation between Argatroban concentration aPTT and TT. Multiple-dose oral administration of acetaminophen did not significantly alter the pharmacokinetic parameters of continuously infused Argatroban. The pharmacokinetics were likewise unaffected in concomitant use of acetaminophen. Co-administration of Argatroban and lidocaine resulted in slightly lower aPTT values than Argatroban alone, consistent with the slightly lower plasma Argatroban concentrations occurring during co-administration. In the erythromycin and Argatroban co-administration trial, there were no effects of the pharmacokinetic and pharmacodynamic profiles. Co-administration with CYP3A4/5 inhibitors should not require modification in the dosage of Argatroban.[1] In light of these findings, concurrent administration of Argatroban with aspirin, acetaminophen, lidocaine and erythromycin is acceptable.

1.4 Preclinical Thrombolysis Studies

The effects of heparin (IV infusion of 200 U/kg over 60 min), Argatroban (IV infusion of 100 μg/kg/min over 60 min), and aspirin (IV bolus injection of 17 mg/kg), alone and in combination, on thrombolysis with rt-PA were studied in a rabbit arterial thrombosis model. Argatroban was effective with respect to acceleration of arterial recanalization with rt-PA and for the prevention of reocclusion. Recanalization was more extensive and more stable in the presence of Argatroban (see Table 1).[10]

Table 1 Effects of Heparin, Argatroban, Aspirin, and rt-PA on Rabbit Femoral Arterial Patency Status						
Number of Animals						
Infusion Protocol	**PO**	**RR**	**CR**	**PP**	**Total**	**Time to Reflow (min)**
rt-PA + ASA	6	1	0	0	7	47
rt-PA + Hep	2	2	1	2	7	37 ± 9
rt-PA + ASA + Hep	2	3	0	2	7	33 ± 13
rt-PA + Arg	1	0	2	3	6	12 ± 7
rt-PA + ASA + Arg	0	0	1	5	6	15 ± 8
rt-PA, recombinant tissue-type plasminogen activator; ASA, aspirin; Hep, heparin; Arg, Argatroban; PO, persistent occlusion; RR, reocclusion after reflow; CR, cyclic reflow; PP, persistent patency						

The effect of Argatroban on the *in vivo* thrombolysis was studied on the arterial thrombosis generated by the endothelial cell injury of the rabbit carotid artery by acetic acid. An infusion of rt-PA at 0.96 mg/kg over 2 hours dissolved the thrombi without a significant activation of a systemic fibrinolysis. At a dose of 0.48 mg/kg rt-PA the thrombi were not dissolved, but the combined use of Argatroban at 1.2 mg/kg over 2 hours effectively dissolved the thrombi. Thus, the combination of Argatroban with plasminogen activators accelerated thrombolysis of experimental thrombosis in rabbits.[11]

1.5 Human clinical trials of Argatroban in myocardial infarction and monotherapy stroke treatment (no rt-PA)

Texas Biotechnology Corporation (TBC) investigated Argatroban in patients with heparin-induced thrombocytopenia (HIT); 568 adult patients were treated with Argatroban and 193 adult patients made up a historical control group. Patients were required to have a clinical diagnosis of HIT, either without thrombosis or with thrombotic complications (HITTS). The initial dose of Argatroban was 2 μg/kg/min (not to exceed 10 μg/kg/min) until the steady-state aPTT was 1.5 to 3 times the baseline value, not to exceed 100 seconds.[12] The proportion of patients who remained free of death, amputation or new thrombosis indicated that Argatroban was superior to the historical control group.

Argatroban has been administered to 810 patients with acute myocardial infarction (see figure 1). Eighty-five were given one of two doses of Argatroban (1.0 or 3.0 μg/kg/min) as an adjunct to rt-PA. This study showed that patients receiving Argatroban, started more than 3 hours following the onset of symptoms, had improved recanalization (i.e. achievement of thrombolysis in myocardial ischemia (TIMI)

flow grade 3.[13] Argatroban was given as an adjunct to streptokinase in 725 of those patients. Argatroban has been administered in combination with aspirin to 91 HIT patients undergoing coronary interventions including PTCA, coronary stent placement or atherectomy. Although not statistically significant, the combined incidence of major and minor bleeding was lower in high-dose argatroban patients as compared with heparin patients: 59.6% versus 77.5% (p = 0.07). Despite these results, the effectiveness and dosing regimen of routine use of Argatroban in cardiac patients has not been established.

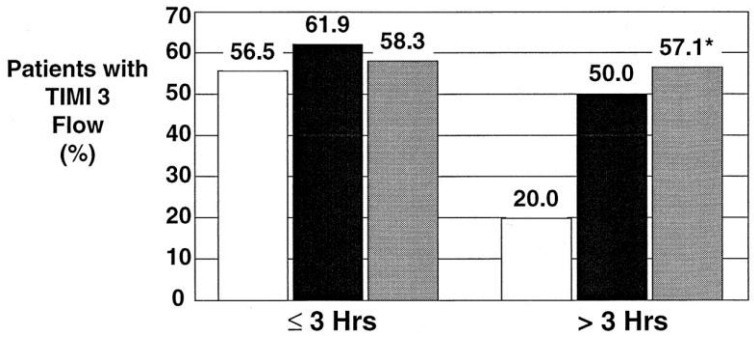

Figure1. **White**: tPA+heparin; **Black**: tPA+low-dose (1μg/kg/min) Argatroban; **Gray**: tPA + high-dose (3μg/kg/min) Argatroban.

However, Argatroban does have a FDA labeling indication for anticoagulation in patients with or at risk for HIT undergoing percutaneous coronary intervention.

Kobayashi and Tazaki reported treatment of 60 patients with Argatroban in a placebo-controlled clinical trial involving 119 patients with acute thrombotic stroke. In 43.7% of Argatroban patients, treatment was started within 48 hours of onset of stroke. Of those, Argatroban demonstrated a statistically significant improvement over placebo in neurologic deficits and symptoms at 30 days.[14] Intracranial bleeding occurred in 1 Argatroban patient and 2 placebo patients.

A randomized, placebo-controlled trial by Lamonte et. al., evaluated two doses of Argatroban as monotherapy in 171 patients with acute ischemic stroke presenting within a 0-12 hour treatment window (ARGIS-1 trial).[15] Safety and efficacy of the two doses (1.0 and 3.0 μg/kg/min) both preceded by a 100 μg/kg bolus over 3-5 minutes were evaluated. The mean time to treatment from symptom onset was 9 ± 2 hours. Rates of sICH were 5.1% high-dose, 3.4% low-dose and 0% placebo, P≥0.18. However, 90 day functional outcomes (% of patients with mRS of 0-2) did not suggest a treatment effect (51% high-dose, 45% low-dose and 54% placebo). The authors suggested that perhaps earlier timing of direct thrombin inhibition (such as proposed in the current protocol) could prevent infarction extension and improve collateral pathways.

Adverse Events

The most frequently occurring non-hemorrhagic adverse events reported in ≥ 6% of patients (regardless of relationship to treatment) in the Argatroban studies in HIT/HITTS patients were dyspnea (8.1%), hypotension (7.2%), fever (6.9%), diarrhea (6.2%) and sepsis (6.0%). The rates for these AEs were: 8.8%, 2.6%, 2.1%, 1.6% and 12.4% in historical control patients. None of these differences were statistically significant.

Plasma from 12 healthy volunteers treated with Argatroban over 6 days showed no evidence of neutralizing antibodies. Repeated administration of Argatroban to more than 40 patients in clinical trials in the USA was tolerated with no loss of anticoagulant activity.[1]

Bleeding

In previously noted experiences in the HIT population in 754 patients, the most commonly reported major hemorrhagic event in Argatroban patients was gastrointestinal bleeding (2.3% compared with 1.6% in historical controls). There were no intracranial hemorrhages. In the Japanese trial noted above,[14] one hemorrhagic transformation during the ischemic event was reported in the Argatroban-treated group and two in placebo-treated patients. In the Japanese trial, bleeding disorders (ischemic hemorrhagic transformations and hematuria) were reported in 7 patients (1.7%).[16] In TBC conducted studies, intracranial bleeding only occurred in patients with acute myocardial infarction who were started on both Argatroban and streptokinase and occurred at a rate similar to placebo or lytic alone. This potentially life-threatening complication occurred in 1% (8 of 810) of patients receiving both Argatroban and thrombolytic therapy (streptokinase or tissue plasminogen activator). Intracranial hemorrhage occurred in 0.7% (2/306) of patients given a lytic and placebo. Intracranial bleeding was not observed in 317 patients experiencing a myocardial infarction (MI) or patients who did not receive concomitant thrombolysis. Intracranial bleeding was also not noted in patients receiving argatroban in combination with rt-PA.

1.6 Pre-Clinical Argatroban + rt-PA Stroke Studies

Morris, et al, conducted an additional study in rats subjected to embolic focal cerebral ischemia by placement of an embolus at the origin of the MCA.[17] The purpose was to test whether administration of both Argatroban + rt-PA 4 hours after stroke onset would reduce lesion size without increasing gross cerebral hemorrhage. The combination therapy of Argatroban + rt-PA had the smallest mean lesion size

(17.1% ± 10.4) compared to untreated controls, and rats treated with Argatroban alone and rt-PA alone. In addition, the gross cerebral hemorrhage rate was equal in the Argatroban + rt-PA and Argatroban alone groups (both 17%), which was a lower rate than both the control (20%) and rt-PA alone (33%) groups. It was concluded that combination therapy of Argatroban + rt-PA significantly reduced ($p \leq 0.05$) mean ischemic lesion size without increasing the rate of gross cerebral hemorrhage when administered at 4 hours following stroke onset.

1.7 Human Clinical Studies of Argatroban + rt-PA Stroke Studies

We completed a multi-centered, pilot safety study of the combination Argatroban +rt-PA (ARTSS-1 study). The first 15 patients were published in 2006.[18] While receiving the rt-PA infusion, each patient received a 100µm/kg IV bolus over 3-5 minutes followed by an infusion started at 1.0µg/kg/min and adjusted to maintain a PTT 1.75 times his/her baseline PTT value. The Argatroban infusion was continued for 48 hours. All patients had middle cerebral artery occlusion with a median NIHSS score of 14 (range 3-25). NIHSS at baseline was used to limit very severe stroke inclusion (we excluded left hemisphere NIHSS >22 and right >17). Two of the 15 patients (13%) experienced a significant ICH (95 CI, 4-48%). Despite the hemorrhage rate, the rate of complete recanalization within two hours was double than that of pre-specified historical controls who received IV-rt-PA alone (40% vs. 18%). Historical controls were from the randomized Phase II, CLOTBUST trial [19] which utilized the exact inclusion/exclusion criteria as our study except for the upper-limit NIHSS cutoff.

The FDA asked the study sponsors to enroll an additional 50 patients at the same dosage in order to obtain a better estimate of safety in a larger cohort. This became important as the original design was to have a low-dose arm and then a high-dose arm (n=15 for each arm). We obtained ARRA (American Recovery and Reinvestment Act of 2009) funding, added 5 centers and completed the study in August 2010 [www.clinical trials.gov Identifier: NCT00268762]. The study was to be stopped for safety reasons if the lower limit of the 80% confidence limit for >10% significant ICH (symptomatic or parenchymal hemorrhage type-2) was exceeded. The study completed and never approached this limit - only 4 significant ICHs occurred (6.2%, 95% CI: 1.7-15). Results are pending manuscript decision and were presented at the 2011 International Stroke Conference (Feb 10, Los Angeles, California). Significant ICH was defined as symptomatic ICH or parenchymal hematoma type-2 (PH-2). Three of these four cases met criteria for symptomatic ICH (4.6%, 95% CI: 1-12.9). Complete recanalization exceeded that of historical controls of rt-PA alone (40% versus 18%, *P*=0.007) from the CLOTBUST study (see figure2).

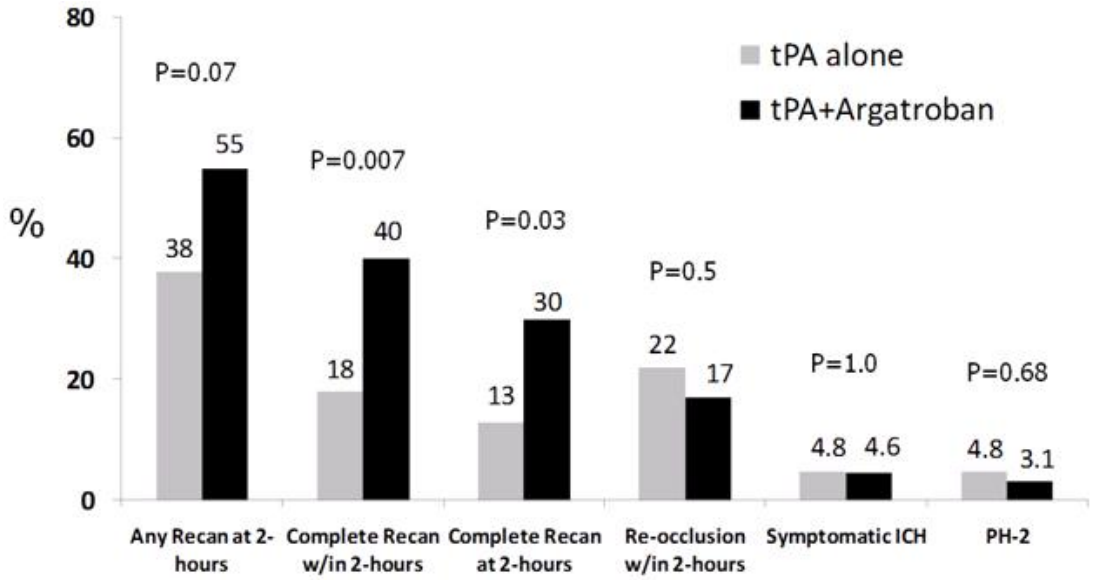

Figure 2. Recanalization data from the ARTSS-1 study. rt-PA alone patients were historical controls from the CLOTBUST study[19] which enrolled a similar patient population.

Table 2 on the next page summarizes human clinical trials of Argatroban.

Study	Patient population	Concomitant therapy	Argatroban dosing	Benefit	Harm	Comments
TBC [12]	**HIT and HITTS** *N=761* (568 Argatroban treated and 198 historical controls)	None	2 μg/kg/min initial rate (no bolus). Titrated to aPTT 1.5-3 times baseline value	Reduced % of death, amputation or new thrombosis: 34.2% vs. 43%. HIT: p=0.007 HITTS: p=0.018	None	Uncontrolled design with historical control comparison (n=193).
Jang [13]	**AMI** *N=85*	rt-PA	1.0 μg/kg/min (low-dose) or 3.0 μg/kg/min (high-dose)	Improved coronary reperfusion (TIMI 3) in >3 hours treated patients (57.1% in high-dose, 50% in low-dose and 20% in heparin controls.	None 59.6% Major and minor bleeding (77.5% in heparin controls).	
Kobayashi [14]	**Stroke** Treatment started within 48 hours of symptom onset. (60 Argatroban and 59 placebo). *N=119*	None	Infusion (total of 60mg/day) x 2 days, then 10mg twice daily x 5 days.	Global clinical symptom improvement scale: 54.2% Argatroban vs. 23.7% placebo.	None 1 mild HT in Argatroban patients and 2 in placebo	Infusion rate not specified in manuscript. No data for PTT levels.
Lamonte [15]	**Stroke** Treatment started within 12 hours (59 high-dose Argatroban, 59 low-dose Argatroban and 54 placebo). *N=171*	none	100 μg/kg bolus followed by either: 1μg/kg/min (low-dose) Or 3μg/kg/min (high-dose)	No difference among groups with regards to 90 day mRS, BI or NIHSS improvement.	Rates of Symptomatic ICH were not significantly different between groups: 5.1% high-dose; 5.1% low-dose and 3.4% placebo, P≥0.18.	Safety study not powered for clinical efficacy endpoints. Low-dose arm titrated to a goal PTT 1.75 times baseline; high dose: 2.25 times baseline.
Sugg [18]	**Stroke** Treatment started within 1 hour of IV-tPA. *N=15*	IV-tPA (standard, 0.9mg/kg dosing)	100 μg/kg bolus followed by 1μg/kg/min infusion for 48 hours	Improved rates of 2-hours complete recanalization compared with historical controls (40% vs. 18%, P=0.25).	13% (95 CI 4-48%) Significant ICH (defined as symptomatic or PH-2)	Uncontrolled safety study. Argatroban titrated to a goal PTT 1.75 times baseline.
Barreto* 2011 ISC Abstract.	**Stroke** Treatment started within 1 hour of IV-tPA. *N=65*	IV-tPA (standard, 0.9mg/kg dosing)	100 μg/kg bolus followed by 1μg/kg/min infusion for 48hrs	Improved rates of 2-hours complete recanalization compared with historical controls (40% vs. 18%, P=0.007).	6.2% (95 CI 1.7-15%) Significant ICH (defined symptomatic or PH-2)	Uncontrolled safety study. Argatroban titrated to a goal PTT 1.75 times baseline. Submitted for publication.
* Includes the 15 patients from Sugg et al.[18]						

Table 2. Summary of human studies of Argatroban. Abbreviations: TBC – Texas Biotechnology Coporation; HIT – Heparin-Induced Thrombocytopenia; HITTS - Heparin-Induced Thrombocytopenia with Thrombosis; AMI – Acute Myocardial Infarction; TIMI- Thrombolysis In Myocardial Infarction; HT-Hemorrhagic Transformation; BI – Barthel Index; mRS – modified Rankin Scale; NIHSS – National Institute of Health Stroke Scale; ICH – Intracerebral Hemorrhage; PH-2 – Parenchymal Hematoma-Type 2; ISC – International Stroke Conference.

1.8 Thrombolysis In Brain Ischemia (TIBI) and TCD Data

Previously, perfusion and degree of thrombus of cerebral vessels were monitored using angiography assessed using a modified version of the grading scale called Thrombolysis In Myocardial Ischemia (TIMI). Burgin, et al, showed that either complete recanalization or complete occlusion in the MCA could be accurately predicted by TCD. The study compared TCD measurement after rt-PA administration to angiographic measurement at the same time point. TCD accurately predicted angiographic findings in complete MCA recanalization and partial signal improvement on TCD corresponded with persistent occlusion on angiography.[20]

Alexandrov and Grotta found that early arterial re-occlusion occurs in 27% of these patients within the first 2 hours after rt-PA bolus for M1-M2 occlusions. No concurrent anticoagulation was used.[9]

Upon establishing a correlation between the angiographic grading system (TIMI) and the TCD sonographic results, Demchuk et al, classified TIBI (thrombolysis in brain ischemic) as follows: 0= absent, 1=minimal, 2=blunted, 3=dampened, 4=stenotic and 5=normal. A correlation between the TIBI grading system and stroke severity and outcome in patients treated with iv rt-PA, was established by examining both TIBI and NIHSS prior to and post rt-PA administration. This study concluded that emergent TCD TIBI classification correlates with initial stroke severity, clinical recovery and mortality in stroke patients treated with IV rt-PA.[21]

Further investigation have shown a clear relationship between the speed of recanalization, measured by TCD and short-term improvement, measured by NIHSS.[22] Measurements were taken at baseline (prior to rt-PA bolus) and at 24 hours. The following classification system was developed for recanalization: sudden (abrupt appearance of a normal or stenotic low-resistance signal), stepwise (flow improvement over 1-29 minutes), or slow (flow improvement over $\geq$ 30 minutes). Based on this classification system, this trial showed that faster recanalization (i.e. sudden and stepwise), as well as the completeness of the recanalization both predicted better short-term improvement as measured by NIHSS at 24-hours in stroke patients treated with rt-PA.[22]

1.9 CT-Angiogram Data

Although not recommended in current practice guidelines, a substantial number of acute ischemic stroke patients treated at dedicated stroke centers receive a baseline (in the Emergency Department) vessel imaging study. In the ED, this is predominantly a CT-angiogram of the head and neck. Typically, a follow-up vessel image study occurs in the stroke unit and is either an MRA or CTA. A CT-angiogram involves an intravenous injection of iodinated contrast while a spiral CT image is obtained. A computer then reconstructs each slice to create a 2-dimensional image of the blood vessels that have been filled with the contrast. The clot can be visualized by demonstrating an abrupt cut-off of the vessel.

Frequently, a follow-up CT-angiogram (or MR-angiogram) is used to assess the degree of recanalization after thrombolysis. The amount of recanalization is important to ascertain as directly impacts clinical outcomes. For instance, a patient who has suffered a proximal MCA or terminal ICA occlusion has a high-likelihood of their stroke worsening and developing into a "malignant pattern". A malignant pattern is caused by cerebral edema which can rapidly (on the order of 1-3 days) progress to brain herniation and death. The presence of a CTA-determined arterial occlusion correlates with resultant stroke disability.[23] Conversely, recanalization is associated with smaller territory infarction and lower risk of a malignant pattern. The degree of recanalization has treatment and management implications such as consulting neurosurgery for hemicraniectomy (surgery to relieve the pressure within the brain caused by a malignant stroke). The radiographic determination of the presence of a large vessel thrombus on CTA has been shown to have excellent inter-rater reliability and accuracy. For example, one series reported an accuracy of 99%.[24] The degree of accuracy of TCD compared to CTA is very good with published rates. Tsivgoulis et al found a sensitivity of 79.1%, specificity of 94.3%, PPV of 87.2 and NPV of 90.3% when comparing TCD to CTA.[25] Their overall accuracy was 89.4%.

There has been wide exposure to Argatroban in the Americas, Europe, Japan, Korea and China in a number of indications. Its safety profile weighed against placebo has been comparable. As a direct thrombin inhibitor, Argatroban has been used in practice for a number of years to treat acute ischemic stroke in Japan. Research of previous publications reporting the findings of animal studies and clinical trials indicate that further investigation of Argatroban in this setting is reasonable and compelling. Our center has completed the first-ever pilot, safety study of the combination Argatroban + rt-PA which appeared to be safe and was associated with improved arterial recanalization. The purpose of this randomized, controlled Phase2b study is to confirm safety of low-dose Argatroban + rt-PA, explore

safety of a high-dose Argatroban + rt-PA, and explore a signal of efficacy with concurrent controls (rt-PA alone) in acute ischemic stroke.

2.0 STUDY OBJECTIVES

The next step in the Argatroban program is to test the efficacy of Argatroban + rt-PA. The current trial is designed with similar inclusion/exclusion criteria and treatment algorithm used in the phase IIa trial (ARTSS-1) to complement data already obtained and to help inform the design of a definitive efficacy trial.

Primary Objective

To develop an unbiased estimate of the probability that low and high dose Argatroban increase the probability of an excellent functional recovery (mRS of 0 or 1 at 90 days as evaluated by a blinded assessor). Data obtained will help develop an evidence-based hypothesis for testing in a larger definitive trial.

Secondary Objectives

1) Safety Endpoints

a) Intracranial hemorrhage:

i. incidence of symptomatic ICH (sICH) as defined as any evidence of bleeding on CT scan that in the opinion of the treating physician and/or an independent safety monitor is associated with a clinically significant neurological worsening,
(a 4 or more point increase in the NIHSS score from baseline (or last score obtained prior to blood found on CT scan) to subsequent CT scan at the time of potential worsening can be used as a guide by the clinical investigator or safety monitor for what represents a significant worsening in neurologic status but sICH can include any worsening deemed significant by the clinical investigator or independent safety monitor)

ii. Parenchymal Hemorrhage 2 (PH-2); evidence of confluent hemorrhage on

CT scan that occupies > 30% of the volume of the infarct and produces significant mass effect will be strongly considered an sICH. The presence of PH2 will also be adjudicated by the independent physician safety monitor.

b) Major systemic hemorrhage
   i. any bleeding associated with a fall in hemoglobin of ≥2 g/dL that results in 2 or more units of blood transfusion.

2) Clinical and Radiographic Activity Endpoints

a) Arterial recanalization at 2-3 hours post-rt-PA as measured by TCD or CT-Angiogram.
   a. Low and high-dose argatroban + rt-PA will be directly compared with control (rt-PA alone) as well as a pooled (low + high dose) versus control.

b) Neurological deficits improvement as measured by NIHSS at 2 hours (± 30 min), 24 hours (± 4 hours), end of Argatroban infusion (± 4 hours), and Day 7 or discharge whichever comes first.
   A. Percentage of patients who experience early neurological improvement (ENI) at 2-hours will be compared among study groups. ENI is defined as a NIHSS improvement of at least 20% and has been shown to be the best predictor of recanalization and 3-month functional outcomes.[26]

c) Quality of life at discharge and day 90 (+/- 10 days) as measured by:
   i. Paper standard gamble (PSG)
   ii. Visual analogue scale (VAS)
   iii. EuroQol-5D (EQ-5D)

   In cases where the patient is unable to perform the above assessments (as determined by a passing score on the Aid to Capacity Evaluation (ACE) questionnaire, then their closest next of kin should complete the forms.

b) Cognitive assessment utilizing the Montreal Cognitive Assessment tool (MoCA)

c) Cost Effectiveness Analysis allowing a calculation of incremental cost-utility per intervention and Quality Adjusted Life Years (QALYs)

3.0 TRIAL ETHICS AND REGULATION

3.1 Investigators and Study Site

Sites will be selected after review of a 3-page questionnaire concerning: a history of treating at least 10 stroke patients per year with IV rt-PA and the presence of a very active and coordinated multidisciplinary stroke team who have experience with participating in clinical trials.

The investigator and study site agree to conduct this protocol in accordance with the FDA Regulations 21 CFR Parts 50.20-50.27, 56.107-56.115, 312.50-312.70 (US sites), Good Clinical Practices, local guidelines and the principles of the Declaration of Helsinki.

3.2 Institutional Review Board Approval

An Institutional Review Board (IRB) will approve the conduct of this clinical study, together with the Investigator's informed consent document, prior to the study initiation. All participating sites will have IRB approval from their own site or where allowed, from another institutional review board.

3.3 Informed Consent

Informed consent from each patient enrolled will be obtained in accordance with the U.S. Food and Drug Administration (FDA) regulations 21 CFR Parts 50.20 – 50.27 or the current version of the Declaration of Helsinki and the laws and regulations of the country in which the investigation is being conducted.

The IRB for each institution will approve the Informed Consent document to be used for patients in that site. Informed consent will be obtained from the patient or his/her guardian or legal representative before any activity or treatment is undertaken which is not part of any routine care. This includes, but is not limited to, the performance of diagnostic or therapeutic procedures and the administration of the first dose of study medication.

4.0 STUDY DESIGN

This is a 3-arm, randomized and controlled study of Argatroban Injection in combination with rt-PA in acute ischemic stroke patients. During the course of the treatment, patients will be evaluated via TCD, CTA, CT scans (as indicated), vital signs, laboratory measurements, and neurological and functional

outcomes. Patients will also be evaluated at 24 hours following the onset of the stroke, Day 7 or discharge whichever comes first and at day 90.

4.1 Study Population

A total of 105 patients with acute ischemic stroke receiving intravenous rt-PA (0.9mg/kg, maximum 90mg) are planned to be enrolled (n=35 per arm). Eligibility for IV rt-PA will be determined by either local standard of practice as guided by national guidelines/position statements or according to regulatory and license labeling.[8, 27, 28]

4.2 Subject Enrollment

The data-core from University of Texas-Houston will develop a web-based randomization program that will be accessed through a secure web address. The webpage will ask for key baseline variables: the HAT score (Hemorrhage after thrombolysis) and presence of terminal ICA occlusion. The HAT score is a validated 5-point scale tool that predicts brain hemorrhage after IV-rt-PA.[29] Patients are assigned points as to the presence of diabetes/elevated glucose, severity of stroke and presence of hypodensity on CT scan. The rate of symptomatic ICH was greater in patients with more HAT points: 2% (0 points), 5% (1 point), 10% (2 points), 15% (3 points), and 44% (>3 points). Terminal ICA occlusions are more resistant to IV-thrombolysis and thus have worse prognoses.

A randomize button will assign the treatment (1:1:1 assignment) arm using an adaptive covariate procedure. The randomization will balance on 3 factors: 1) low (HAT 0-2) versus high-bleeding risk patients (HAT 3-5); 2) presence of terminal ICA occlusion; and 3) by clinical site among the groups. The probability of being assigned to a group decreases if the group is over-represented and increases if the group is under-represented.

A total of 70 patients will receive Argatroban infusion for 48 hours. The low-dose arm (1.0 µg/kg/min) will be titrated for a target aPTT of 1.75 times baseline. The high-dose arm (3.0 µg/kg/min) will be target a aPTT of 2.25 times the patient's baseline. In the event of an increased rate of bleeding, safety halting rules have been developed (Appendix # 4) to assist the DSMB. These rules serve to balance the risk of early hemorrhage with the potential for improved clinical outcomes at 90 days (net clinical benefit). Any

AEs related to intracranial bleeding (any intracranial bleeding is an AE) will be reviewed by an independent safety monitor and the DSMB.

4.3 Description of Clinical Supplies

Argatroban is a white, odorless crystalline powder that is freely soluble in glacial acetic acid, slightly soluble in ethanol, and insoluble in acetone, ethyl acetate and ether. Argatroban Injection is a sterile clear, colorless to pale yellow, slightly viscous solution. Argatroban is available in 250 mg (in 2.5 mL) single-use amber vials, with gray flip-top caps at a concentration of 100 mg/mL. Each mL of sterile, nonpyrogenic solution contains 100 mg Argatroban. Inert ingredients: D-sorbitol, dehydrated alcohol. If the solution is cloudy, or if an insoluble precipitate is noted, the vial should be discarded (see Appendix 1.0).

Argatroban Injection is to be stored protected from light in the original cartons at room temperature [25^{o}C (77^{o}F) excursion permitted to 15-30^{o}C (59-86^{o}F)]. Do not freeze.

4.4 Labeling of Clinical Supplies

Vials from the commercial lot of Argatroban will be used for this trial. No specific label changes will be made to the commercial label attached to the vials except for the placement of a sticker indicating “For investigational use only”.

5.0 PATIENT SELECTION

5.1 Inclusion Criteria

1. Disabling Ischemic stroke symptoms with onset $\leq$ 3 hours treated with IV rt-PA by local standards*.

   * or ≤ 4.5 hours according to local standard of care. [8]

   Symptoms must be distinguished from another ischemic event such as syncope, seizure, migraine, subarachnoid hemorrhage and hypoglycemia. If the patient reports awakening with the event, the time of onset should be considered as the last time the patient (or a witness to the patient’s condition) considered herself/himself normal.

2. Age ≥18.
3. NIHSS ≥ 10* or any NIHSS with an intracranial clot should be demonstrated on neurovascular imaging (TCD or CTA) in any one of the following areas: distal ICA, MCA (M1 or M2), PCA (P1 or P2), distal vertebral or basilar artery.
   - TCD criteria: TIBI 0, 1, 2 or 3
   - CT-Angiogram: TIMI 0 or 1

   * NIHSS ≥ 10, demonstration of clot on neuroimaging is not necessary (i.e., enrollment can proceed with non-contrast head CT alone), but <u>if performed</u>, a clot must be demonstrated.
4. For those patients who will undergo repeat CT-Angiogram at 2-3 hours, estimated glomerular filtration rate (eGFR) must be ≥ 60 mL/min/1.73m^2.
5. Females of childbearing potential must have a negative serum pregnancy test ($_\beta$HCG) prior to the administration of trial medication.
6. Signed (written) informed consent by the patient or the patient's legal representative and/or guardian.

5.2 Exclusion Criteria

1. Patients whom the treating physician is planning (or could plan) to treat with intra-arterial thrombolysis or other endovascular procedures (i.e., mechanical clot retrieval) aimed at recanalization.
2. Evidence of intracranial hemorrhage (ICH) on baseline CT scan or diagnosis of a non-vascular cause of neurologic deficit.
3. NIHSS Level of Consciousness score (1a) ≥ 2.
4. Pre-existing disability with mRS ≥ 2.
5. CT scan findings of hypoattenuation of the x-ray signal (hypodensity) involving ≥ 1/3 of the MCA territory.
6. Any evidence of clinically significant bleeding, or known coagulopathy.
7. INR >1.5.
8. Patients with an elevated aPTT greater than the upper limit of normal (test can be repeated if investigator suspects a falsely elevated value such as when the collection tube is not completely filled).

9. Patients currently, or within the previous 24 hours, on an oral direct thrombin inhibitor (i.e., dabigatran).
10. Heparin flush required for an IV line. Line flushes with saline only.
11. Any history of intra-cranial hemorrhage, known ateriovenous-malformation or unsecured cerebral aneurysms.
12. Significant bleeding episode [e.g. gastrointestinal (GI) or urinary tract] within the 3 weeks before study enrollment.
13. Major surgery or serious trauma in last 2 weeks.
14. Patients who have had an arterial puncture at a non-compressible site, biopsy of parenchymal organ, or lumbar puncture within the last 2 weeks.
15. Previous stroke, myocardial infarction (MI), post myocardial infarction pericarditis, intracranial surgery, or significant head trauma within 3 months.
16. Uncontrolled hypertension (SBP > 185 mmHg or DBP >110 mmHg) that does not respond to intravenous anti-hypertensive agents.
17. Surgical intervention (any reason) anticipated within the next 48 hours.
18. Known history of clinically significant hepatic dysfunction or liver disease – including a current history of alcohol abuse.
19. Abnormal blood glucose <50 mg/dL (2.7 mmol/L).
20. History of primary or metastatic brain tumor.
21. Current platelet count < 100,000/mm^3.
22. Life expectancy < 3 months.
23. Patients who, in the judgment of the investigator, needs to be on concomitant (i.e., during the Argatroban infusion) anticoagulants other than Argatroban, including any form of heparin, UFH, LMWH, defibrinogenating agent, dextran, other direct thrombin inhibitors or thrombolytic agents, GPIIb/IIIa inhibitor or warfarin. [*Caveat: However, if in the judgment of the investigator a patient needs to be anticoagulated, **but** this can be deferred for 48 hours, then they could be included.]
24. Currently participating or has participated in any investigational drug or device study within 30 days before the first dose of study medication.
25. Known hypersensitivity to Argatroban or its agents.
26. **Additional exclusion criteria if patient presents between 3-4.5 hours**:
    a) Age >80

b) Currently taking oral anticoagulants (regardless of INR)

c) A history of stroke and diabetes.

d) NIHSS > 25.

6.0 STUDY PROCEDURES

6.1 Schedule of Observations and Procedures

Study Flow Chart (See Table 3 in section 10.0).

6.2 Pre-treatment Period

6.2.1 Medical History and Physical Examination

Patients will have a complete Medical History completed before initiating treatment. This history will include at minimum: date of birth, gender, race, smoking/alcohol history, current medical diagnoses, any medications taken within the 14 days prior to the initiation of study treatment, and clinically significant past medical history including cerebrovascular, cardiovascular, surgical, traumatic, neurologic and psychiatric history. A pre-stroke modified Rankin Scale score will be obtained.

A complete physical examination including cardiovascular and neurologic examination (including NIHSS) as well as weight (estimated if not known) and vital signs (heart rate, blood pressure, respiration rate, and temperature) will be completed prior to initiating treatment.

6.2.2 Pre-treatment Diagnostic and Clinical Tests

The following diagnostic and clinical tests will be completed before initiating treatment infusion. The results of the platelet count, aPTT and INR will be known before starting the Argatroban infusion.

1. Laboratory Tests:
    - Hematology: Complete blood count (CBC), aPTT, PT and INR.
    - Chemistry Panel: ALT (SGPT), AST (SGOT), alkaline phosphatase, bilirubin (direct, indirect, total), BUN, calcium, chloride, creatinine, glucose, sodium, potassium, carbon dioxide (CO_2).
    - Serum βHCG: for females of childbearing potential.
1. ECG: a 12-lead ECG will be performed prior (as close as possible) to beginning infusion per routine stroke evaluation.

2. Head CT Scan: a CT scan of the head will be completed prior to the initiation of rt-PA and the Argatroban infusion.
3. Intracranial vessel imaging, if available (either):
   - CT-Angiogram: patients who have a TIMI 0 or 1 occlusion will be eligible for enrollment.
   - Transcranial Doppler Ultrasound: TCD examination will be performed and the residual flow signals at occlusion location will be identified with the TIBI system (TIBI score 0-3).

   Vessel imaging studies can be performed either before or immediately after IV-tPA bolus (but before Argatroban bolus). If vessel imaging is planned, a patient should not be randomized until after the CTA or TCD demonstrates occlusion.

6.2.3 Admission of Patients

After screening, eligible patients will be given a site and patient number. In addition, they will be identified by the first initial and first two letters of the last name. Example: John Smith- JSM.

6.3 Treatment Period

6.3.1 Selection of Treatments Administered

This is a randomized clinical study consisting of a bolus of Argatroban followed by a continuous IV infusion of Argatroban in combination with rt-PA. Three treatment arms will be enrolled:
1) Usual care IV-rt-PA (0.9mg/kg - 90mg maximum. 10% given as 1 minute bolus and remainder over 1 hour);
2) IV-rt-PA + low-dose Argatroban x 48 hours (1.0µg/kg/min - goal aPTT 1.75 x baseline);
3) IV-rt-PA + high-dose Argatroban x 48 hours (3.0µg/kg/min – goal aPTT 2.25 x baseline).

A member of the treatment team will enter minimal baseline data into a secured website which will randomize the patient into one of the three treatment arms (*see section 4.2*).

6.3.2 Identity of Investigational Product

The molecular formula of Argatroban is $C_{23}H_{36}N_6O_5S \bullet H_2O$. Its molecular weight is 526.66. The study drug will be obtained from the commercial lot of Argatroban Injection which is manufactured by Abbott Laboratories. The diluent (0.9% Sodium Chloride Injection) required for dilution of the Argatroban will be provided on site.

6.3.3 Patient Status and Dosing Schedule

All patients randomized to one of the two Argatroban treatment arms will receive the assigned dosing regimen within the timeframes previously described. Patients will remain on the desired therapeutic dose of Argatroban + rt-PA for a maximum of 48 hours. The titration of the Argatroban infusion will follow a strict protocol (see Appendix 2.0).

6.3.4 Study Medication Administration

The start of rt-PA therapy will not be delayed because of participation in this trial. Argatroban and rt-PA administration should occur concurrently. Should a delay in Argatroban preparation occur, rt-PA should be initiated and Argatroban treatment MUST be initiated within 1 hour, i.e. before the rt-PA infusion is completed. All patients will receive rt-PA given in the following manner via a separate infusion line (not to exceed a total dose of 90 mg): 0.9 mg/kg infused over 60 minutes with 10% of the total dose administered as a bolus over 1 minute.

Patients randomized to the low-dose Argatroban arm will receive a 100 μg/kg bolus of Argatroban over 3-5 minutes followed by a continuous iv infusion of 1.0 μg/kg/min infusion for a maximum of 48 hours. Argatroban will be titrated (not to exceed 10 μg/kg/min) during the infusion period to achieve an aPTT of 1.75 times baseline (± 10%). High-dose Argatroban randomized patients will also receive a 100 μg/kg bolus of Argatroban over 3-5 minutes followed by a continuous iv infusion of 3.0 μg/kg/min infusion for 48 hours. Argatroban will be titrated (not to exceed 10 μg/kg/min) during the infusion period to achieve an aPTT of 2.25 times baseline (± 10%).

Every effort will be made by treating physician and nursing staff to not divulge the treatment arm. This is especially true for patients enrolled into the Argatroban arms. Patients and family members will not be told whether they are receiving the high or low-dose regimen until study completion.

The suggested dosing algorithm is provided in Appendix 2.0.

6.3.5 Concomitant Medications

Concomitant medications can be administered as clinically indicated provided that all doses and times are recorded with the exception of the following:

Warfarin, defibrinogenating agents, UFH, LMWH, GPIIb/IIIa inhibitors, other direct thrombin inhibitors (i.e., dabigatran), other thrombolytic agents, dextran, vitamin K antagonists, and platelet function alteration drugs (i.e., aspirin, clopidogrel, prasugrel, dipyridamole, cilostazol).

Therefore, patients randomized to one of the Argatroban arms will begin their antiplatelet therapy at 48 hours post rt-PA.

For those patients needing warfarin after discontinuation of study medication, transition from Argatroban to warfarin will be done according to the Package Insert (see Appendix 1.0). However, no co-administration of Argatroban and warfarin is allowed during the 48 hours of Argatroban infusion. After the 48 hours, if the patient's clinical findings require therapy with oral anticoagulation, co-administration of Argatroban and warfarin will be permitted. Guidelines in Appendix 1.0 will be followed.

All concomitant medications will be collected from prior to the initiation of treatment through the 90-day (± 10 days) follow-up period.

6.3.6 Vitals / Laboratory Tests / Specialized Testing

1. Vessel Monitoring:

Patients enrolled using TCD or CTA will undergo a repeat TCD or CTA (same modality as baseline) to assess completeness of recanalization. This will occur at 2 hours from the rt-PA bolus. Timing of the repeat imaging is between 2-3 hours from bolus.

2. Vital Signs:

Vital signs will be collected as follows throughout the treatment period:

- Blood pressure and heart rate:
  - First 24 hours post IV-tPA: standard-of-care post tPA (every 15 minutes for 2 hours; then every 30 minutes for 6 hours; then every 1 hour until 24 hours from tPA bolus).
  - Second 24 hours: every 4 ± 1 hours.

In addition, all vital signs will be obtained as clinically indicated throughout the study period.

3. Laboratory Tests:

Laboratory tests (hematology and chemistries), consisting of the same parameters as baseline testing, will be performed as clinically indicated throughout the treatment period and within 24 hours of final discontinuation of the Argatroban infusion.

The aPTT will be checked at: baseline (prior to the rt-PA and Argatroban bolus dose); 2 hours (±30 min); 6 hours (± 30 min); 12 hours (± 30 min); 24 hours (± 30 min) and 48 hours (± 30 min). In addition, a PTT will be drawn **every 2-4 hours after each dose change** (unless the scheduled pTT is due within 30 minutes). An aPTT will also be obtained in the event of a major bleed.

4. ECG:

ECG results will be obtained and recorded at as clinically indicated throughout the treatment period. Every stroke patient upon admission does receive a baseline ECG considered standard-of-care.

5. CT Scans:

A CT scan will be performed at 48 hours post the rt-PA bolus (± 4 hours) and whenever neurologic deterioration occurs and is associated with a clinically significant increase in the NIHSS score (≥ 4 points is a guide) compared to baseline. MRI brain imaging can substitute for CT scan, but only if no hemorrhage is present on the MRI sequences. If any hemorrhage is present on MRI imaging, a non-contrast head CT must be performed within 4 hours of the MRI to confirm extent of blood and allow the use of CT-based definitions of hemorrhage (i.e., hemorrhagic transformation type 1 or 2 and parenchymal hematoma type 1 or 2).

A baseline CT-Angiogram may have been obtained as part of a stroke center's routine standard-of-care practice. Those patients who undergo enrollment using the CTA and have an eGFR of ≥ 60 will have a repeat study at 2-3 hours post tPA bolus to evaluate the extent of any recanalization.

6. NIHSS:

NIHSS assessments will be performed at 2 hours (± 30 min), 24 hours (± 4 hours), and end of Argatroban infusion (± 4 hours) / 48 hours post rt-PA bolus (± 4 hours). In addition, NIHSS assessments will be made

whenever neurologic deterioration occurs or with any unscheduled CT scan. Post-randomization NIHSS scores will be obtained (whenever possible) by a blinded study member.

6.3.7 Criteria for Discontinuation

The Argatroban infusion will be *held* in the event of a suspected intracranial hemorrhage. If ICH is not confirmed on CT scan of head, the infusion can be restarted. Infusion of the drug will be *terminated* immediately if any of the following events occur:

1. Major bleeding defined as any symptomatic ICH, bleeding into a major prosthetic joint or bleeding into a retroperitoneal area.
    - A major bleed may further be defined as overt bleeding associated with a fall in hemoglobin of $\geq$ 2 g/dL and transfusion of $\geq$ 2 units of blood. At the discretion of the Investigator, a patient may be discontinued if the patient has a decrease in hemoglobin of 2-5 g/dL.
2. Symptomatic ICH - defined as:

    any evidence of bleeding on CT scan that in the opinion of the clinical investigator or independent safety monitor is associated with a clinically significant neurological worsening.
3. Parenchymal Hematoma Type-2 (without neurological worsening).
4. Clinically significant bleeding unresponsive to usual clinical interventions.
5. The infusion of Argatroban will be discontinued at least 30 minutes (up to 2 hours is recommended) prior to any surgical procedure. The infusion may be reinstituted post operatively as soon as hemostatic control is achieved. **Patients will NOT undergo intra-arterial thrombolysis or other endovascular procedures aimed at recanalization, percutaneous coronary artery angioplasty, coronary artery bypass graft (CABG), or other surgery while on Argatroban.**

If the infusion is interrupted for $\geq$ 4 consecutive hours, the study drug will be discontinued permanently. (*** Caveat** – unless the infusion is held for safety purposes such as persistent critically high aPTT values. For any interruptions < 4 consecutive hours, the infusion will be re-initiated at the same rate prior to the interruption, and the aPTT will be checked at 2-4 hours after resuming the infusion. If the aPTT does not meet the target requirements for the dose given, the dose will be titrated and the aPTT will be re-checked every 2-4 hours as needed until the target aPTTs is achieved or the infusion is completed.

6.3.8 Withdrawal of a Patient Prior to Study Completion

If for any reason a patient is withdrawn before completing the study, the reason for withdrawal will be entered on the Study Completion Form and other appropriate CRF pages will be completed.

6.3.9 Grading Recanalization

All neuroimaging will be anonymized. CT-Angiogram images will be reviewed by the imaging core – the University of Calgary imaging center. TCD waveforms will be read by a separate neurosonology expert. Physicians who have extensive experience with both TCD and CTA will read the images blinded to treatment assignment using the rules below.

Assessments of the speed of intracranial clot lysis as well as the completeness of clot lysis will be determined at baseline (can be pre-rt-PA or pre-Argatroban depending on timing of the study) and 2 (up to 3 hours) hours. The assessments will be characterized as follows:

- TCD - TIBI Flow Grades defined as complete, partial, or none based on the scale below:
  I. **Grade 0**: absent – absent flow signals are defined by the lack of regular pulsatile flow signals despite varying degrees of background noise.
  II. **Grade 1**: minimal – systolic spikes of variable velocity and duration; absent diastolic flow during all cardiac cycles based on a visual interpretation of period of no flow during end diastoli. Reverberating flow is a type of minimal flow.
  III. **Grade 2**: blunted – flattened systolic flow acceleration of variable duration compared to control; positive end diastolic velocity and pulsatility index <1.2.
  IV. **Grade 3**: dampened – normal systolic flow acceleration; positive end diastolic velocity; decreased mean flow velocities (MFV) by >30% compared to control.
  V. **Grade 4**: stenotic – MFV of >80 cm/s AND velocity difference of >30% compared to the control aide; or if both affected and comparison sides have MFV <80 cm/s due to low end-diastolic velocities, MFV >30% compared to the control aide AND signs of turbulence.
  VI. **Grade 5**: normal - <30% mean velocity difference compared to control; similar waveform shapes compared to control.

Recanalization definitions:

TCD

1. Increase in TIBI flow by 1 grade and TIBI ≥ 2
2. Partial = improvement of flow to grade 2 or 3
3. Complete = improvement of flow to grade 4 or 5

CT-Angiogram

1. Complete Recanalization
    i. Flow signal of normal intensity was detected on CTA (*TIMI=3*).
    ii. TIMI 0 or 1 → 3
2. Partial Recanalization
    i. TIMI=0 or 1 → 2
3. No Recanalization
    i. TIMI 0 or 1

6.4 Follow-up Period

NIHSS, mRS at day 7 or discharge (whichever comes first). A day 90 day (± 10 days) modified Rankin Scale score and a NIHSS will be collected in-person by a blinded study team member. Health utilities (standard gamble, VAS and EQ-5D) and MoCA will be collected at day 7 or discharge as well as day 90. Due to the encephalopathy or language dysfunction that can occur with stroke, complex questions regarding assessment of quality of life may not be adequately obtained from the patient. In this event, the patient's proxy (closest next-of-kin relative) will be asked to assess their loved one's health state as if they were the patient.

In order to determine if aphasia or mental status would impair these questions, selected items in the patient's NIHSS will be reviewed. In addition, if the patient is intubated, the proxy would answer the quality of life questions – see table below. If "Yes" is selected for any item, then quality of life questions will be obtained from the proxy. In this event, a MoCA will not be obtained.

Assessments	Yes	No
NIHSS: 1a-LOC score ≥2	☐	☐
NIHSS: 1b-LOC questions score =2	☐	☐
NIHSS: 1c-LOC commands score =2	☐	☐
NIHSS: Best Language/Aphasia score ≥2	☐	☐
NIHSS: Neglect/Inattention score=2	☐	☐
Intubated	☐	☐

If all answers to above table are "No", it is still possible that the patient's capacity might be impaired by their stroke. Therefore, the Aid to Capacity Evaluation (ACE) questionnaire will be performed (see Appendix 5.0). In the event that the patient passes the ACE, then the patient will complete the quality of life forms as well as the MoCA. However, if the patient fails the ACE questionnaire, then the proxy will complete the quality of life forms (and in this case, no MoCA will be obtained).

In the event of being lost to follow-up, day 7 mRS, MoCA and health utilities will be carried forward to day 90 values.

7.0 SAFETY

7.1 Safety Monitoring and Determination of Symptomatic ICH

A physician will be assigned by the Principal Investigator to serve as the independent safety medical monitor for this trial. In the event of any intracranial bleeding, CT scans and a clinical summary will be provided to the safety monitor for review. Importantly, for determining ICH, treatment assignment will not be given to the safety monitor (i.e., Argatroban arm versus rt-PA alone). In addition, all SAEs will be reviewed by the safety monitor. The initial report will submitted to the University of Texas-Houston data core which will alert both the study PI as well as the independent physician safety monitor. The medical monitor will complete a monitoring report and return it to the data-core/PI. Depending on the outcome of the safety monitor's report, the data-core/study PI will notify the local site, the coordinating site IRB, DSMB and/or FDA where applicable. The DSMB committee will use the halting rules as guidance in determining whether to halt or terminate a study arm or the entire study (see appendix #4).

In the case of any symptomatic ICH, a standard protocol will be followed for reversing the effects of rt-PA and Argatroban (see next section). An AE form will be completed for any ICH. A hemorrhage can be labeled as symptomatic by <u>either</u> the local principal investigator or the safety monitor.

7.2 Management of Symptomatic ICH

1. Discontinue rt-PA and Argatroban infusions;

2. Type and cross;
3. Check fibrinogen level stat and every 6 hours;
4. Give 10-20 units cryoprecipitate;
5. Repeat cryoprecipitate (cryo) to bring fibrinogen > 100 mg/dl (1 unit cryo raises fibrinogen 5-10 mg/dl);
6. May give fresh frozen plasma in case of no cryo (1 unit of cryo is made from 1 bag of FFP);
7. Give platelet concentrate if platelet count is low.

7.3 Adverse Events (AEs)

The Investigator will provide appropriate information concerning any findings that suggest a significant hazard, contraindication, side effect, or precaution pertinent to the safety of Argatroban.

7.3.1 Types of Adverse Events

The term adverse event could include any of the following events, which develop or increase in severity during the course of the study:

a. Any signs or symptoms whether thought to be related or unrelated to the condition under study;
b. Any clinically significant laboratory abnormality;
c. Any abnormality detected during physical examination.

These data will be recorded on the appropriate CRFs, regardless of whether they are thought to be associated with the study or the drug under investigation, (associated with the use of the drug means that there is a reasonable possibility that the event may have been caused by the drug).

Signs or Symptoms will be graded by the Investigator as mild, moderate or severe according to the following definitions:

Grade:	Definition
Mild:	Causing no limitation of usual activities
Moderate:	Causing some limitations of usual activities
Severe:	Causing inability to carry out usual activities.

7.3.2 Serious Adverse Events (SAEs)

A serious adverse event is defined as any event that suggests a significant hazard, contraindication, side effect or precaution. A serious adverse event includes any event that:

- Results in Death;
- Is life threatening;
- Results in inpatient hospitalization or prolongation of existing hospitalization;
- Results in persistent or significant disability/incapacity;
- Is a congenital anomaly or birth defect;
- Important or significant medical events that require medical or surgical intervention, based upon appropriate clinical judgment.

An unexpected event is any adverse event that is not identified in nature, severity or frequency in the Investigational Brochure and/or the Product Package Insert.

7.3.3 Reporting of Serious Adverse Events

Each individual study site will follow their internal policies for reporting SAEs to their IRB. In addition, sites will alert the UT-Houston data-core within 24-hours of any suspected SAE. This alert will be relayed to the study PI and the independent physician safety monitor for review. If an SAE is confirmed, the coordinating site (UT-Houston) will collect these SAEs for every-10 enrollments and report to the DSMB. DSMB reports will be forwarded to all participating clinical sites for IRB submission. The coordinating site will also report all SAEs to the study drug supplier, Glaxo-Smith-Kline, Inc. If the SAE is unexpected and associated with Argatroban dispensing, then the FDA will be alerted. The IRB will also be promptly informed of any serious adverse event or unexpected adverse event that is considered possibly related to the study drug. SAEs will be reported during the entire 90 day study period. See Appendix #3 for further details regarding SAE submission.

7.3.4 Follow-Up of Adverse Events

All serious adverse events will be followed with appropriate medical management until resolved. For adverse events, a rechallenge may be conducted if considered both safe and ethical.

7.4 Data and Safety Monitoring Board (DSMB)

A three person DSMB is established to monitor safety concerns which arise during the study. The DSMB meets and discusses enrollment or safety concerns after each 10 enrollments or if additional safety concerns arise. In addition, if a particular safety concern arises before the 10 enrollments, the DSMB reserves the right to meet earlier according to their ratified charter.

In the event that treatment causes is associated with both increased sICH (secondary endpoint) and a lower percentage of excellent functional outcomes at 90 days, the DSMB may choose to continue enrollment or terminate the treatment arm. The probabilities of abandoning a potential efficacious treatment have been calculated using Bayesian methodology. To avoid abandoning a truly beneficial therapy for a very common condition based on chance findings in a small number of patients, stopping enrollment in either argatroban treatment arm would not be considered unless the lower limit of the 95% confidence interval for the hemorrhage rate is >10% and the probability of any increase in excellent clinical outcomes in that arm is ≤20% (see appendix 4).

Sites will submit a screening log in order to monitor the speed of recruitment and tracking of adverse events.

8.0 STATISTICAL CONSIDERATIONS

All data will be analyzed using intention-to-treat analyses. Baseline variables will be analyzed for treatment group differences using descriptive statistics. Demographics, vital signs, laboratory variables, stroke type and duration from onset of symptoms will be summarized for each treatment arm. Vital signs taken during the treatment period will be displayed graphically. Pre-treatment vs. 48 hour (end of treatment) displays will be provided for laboratory values. Changes from baseline for aPTT values will be summarized for each time period. In addition to ICH, other medical events will be tabulated by body system and severity. If patients are lost to follow-up at the 90 day end of study evaluation, the worst-case scenario will be entered into the database (i.e., mRS = 6). However, if the percent of patients lost to follow-up exceeds 5% then we plan a multiple imputation technique. This technique quantifies the uncertainty due to the missing data.

8.1 Determination of Sample Size

Sample size is based on medical criteria balancing patient exposure with the objective of gaining useful preliminary safety information. Since the current study is a pilot study, 99 patients will be enrolled in a 1:1:1 fashion (33 per arm). Centers will be chosen with track-records of excellent patient follow-up, thus we expect very few lost-to-follow-up cases (~ 5 %). Therefore, a total of 105 (35 per patient arm) will be enrolled.

Safety (incidence of sICH or PH-2) will be assessed using a binomial confidence interval approach. Using 95% confidence intervals, we will be able to determine if the true rate of ICH is >10%. See Appendix #4 for halting / stopping rules.

8.2 Primary Outcome

The primary outcome is an excellent functional outcome measured by a modified Rankin scale score of 0 or 1 at 90 days. Power to detect a statistically significant difference will be low due to our small numbers. However, the study size is appropriate for pilot studies such as this trial. Kraemer and Frank point out that randomized clinical trials have over-relied on statistical significance. They point out that all that a p-value <0.05 usually means is that that the sample size was large enough to detect a non-random effect. Assessment of the clinical significance of a treatment effect requires a clinically interpretable effect size in addition to the p-value.[30] We plan a Bayesian analysis (with either skeptical or neutral priors) to estimate the probability (with 95% credible intervals) of treatment effect. Conventional (frequentist) analyses do not allow the probability of benefit or harm from treatment to be calculated which is an important advantage of the Bayesian approach.[31, 32] Concerns about Bayesian analyses have largely been related to choosing an overly optimistic prior probability (or a prior based on weak evidence), thus producing overly optimistic posterior probabilities of treatment benefit. This concern does not apply to the current protocol since we plan to utilize neutral or skeptical (conservative) prior probabilities only.

The primary outcome will be analyzed with a robust Poisson regression model with two dummy variables (using control group as the referent category) representing the three groups (low dose/high dose/control), and interaction terms of the group dummy variables with HAT score, terminal ICA occlusion and clinical sites as covariates. This model will provide estimates of adjusted relative risks

(RR). **A RR of >1 indicates the "risk" of an <u>improved</u> outcome.** Conversely, a RR of <1 would indicate worse outcomes (e.g., less patients achieving a mRS of 0-1). Due to limited same size and planned stratification of the HAT score and terminal ICA occlusion, the analysis will not be adjusted for site. We will assume a neutral prior centered at a relative risk (RR) of 1.0 with 95% credible interval (CrI) of 0.33 to 3.0 for both Argatroban groups.

Secondary analyses of the primary outcome will include an unadjusted analysis using an extension of Fisher's exact test. Another secondary analysis will use a robust Poisson regression with the two dummy variables representing the three groups, while controlling for potential confounding variables (e.g., age and NIHSS) and potential effect modifications by HAT score, and terminal ICA occlusion (interactions between the study group dummy variables and HAT score, and terminal ICA occlusion and country of enrollment (U.S./United Kingdom)). For all analyses, we will report point estimates (posterior medians) as well as 95% CrI's and posterior probabilities of benefit/harm.

8.3 Secondary Outcomes

Secondary outcomes will be analyzed similarly using a Poisson regression for binary outcomes and a linear regression for continuous variables with the same covariates used in the primary analysis. Secondary analyses will also be performed (unadjusted and adjusted for age, NIHSS, and country).

The secondary outcomes are:

1) **Incidence of symptomatic ICH (sICH)**:
   any evidence of bleeding on CT scan that in the opinion of the treating physician and/or an independent safety monitor is associated with a clinically significant neurological. We would expect escalating doses of Argatroban to somewhat increase the risk for ICH and have therefore chosen a conservative prior. However, we consider these priors as neutral even though the RR is >1. We will use a RR of 1.5 with 95% CrI of 1.16 to 2.50 for the low dose group. For the high dose group, the prior will be centered at RR of 2.0 with 95% CrI of 1.33-4.0. **For sICH, a RR of >1 indicates the increased risk of experiencing an sICH.** Conversely, a RR of <1 indicates a decreased risk for developing sICH.

   - Incidence of other safety parameters will also be collected and descriptive statistics will be calculated (i.e., Chi-square) including:

- Parenchymal Hemorrhage 2 (PH-2) - evidence of confluent hemorrhage on CT scan that occupies >30% of the volume of the infarct and produces significant mass effect.
- Major systemic hemorrhage resulting in a hemoglobin reduction of ≥2 g/dL that results in 2 or more units of blood transfusion.

2) **Degree and completeness of arterial recanalization.** Defined as baseline arterial imaging (TCD or CTA) compared with the follow-up – 2-3 hour study. Recanalization will be displayed graphically with means and standard deviations. Completeness of arterial recanalization (complete, partial, none) will be tabulated. The start time of the Argatroban bolus will be correlated with these measurements. We will use a neutral prior centered at RR of 1.0 with 95% CrI of 0.75-1.75 for both Argatroban groups.

3) **Neurological improvement as measured by NIHSS** at 2 hours (± 30 min), 24 hours (± 4 hours), end of Argatroban infusion / 48 hours post rt-PA bolus (± 4 hours), Day 7 (or discharge, whichever comes first) and day 90 (± 10 days). For the NIHSS at 2, 24, and 48 hours, a neutral prior will be used centered at RR of 1.0 with 95% CrI of 0.33-3.0 for both Argatroban groups. For the NIHSS at day 7 and day 90, a neutral prior with wider 95% CrI (RR: 0.25-4.0) will be used for both Argatroban groups. The wider CrI at days 7 and 90 reflect the increased uncertainty of treatment hazards at these later timepoints.

4) **Health related quality of life** as measured utilizing health utility scores obtained by the standard gamble technique, VAS and EQ-5D (see appendix #5). The mean ± SD and median (IQR) of the utilities and MoCA scores for each treatment arm will be calculated for all three methods and compared using one-way ANOVA or non-parametric tests where appropriate.

5) **Cost and cost effectiveness analysis** – (see appendix #5). The mean ± SD and median (IQR) of costs for each treatment arm will be calculated for all three methods and compared using descriptive statistics such as one-way ANOVA or non-parametric tests where appropriate.

8.4 Interim Analysis

One interim analysis is planned after data is obtained from 75 enrolled patients. The purpose of this analysis is to prepare a grant proposal for a larger trial. The primary outcome of the interim analysis will be to compare the rates of 2-3 hour recanalization between Argatroban groups and controls. Rates from this trial will be compared with those obtained from the ARTSS-1 trial. If Argatroban is associated with greater rates of recanalization, particularly if other available data show a trend toward improved 90-day mRS and minimal increase in sICH, then a grant proposal will be submitted for a pivotal efficacy trial. Otherwise, preparation will be deferred until after full recruitment and all outcomes are available.

9.0 STUDY MANAGEMENT

9.1 Clinical Laboratory Tests and Normal Laboratory Values (NLV)

Clinical Laboratory Tests will be performed by the same laboratory throughout the study. The laboratory is accredited by local and/or federal agencies as appropriate.

9.2 Disposition of Clinical Supplies

The Investigator will maintain adequate records showing the receipt, dispensing, return, or other disposition of the investigational drug, including the date, quantity, batch or code number, and identification of patients (number, initials) who received study drug.

When the investigation is discontinued or completed, unused supplies of drug will be disposed of, per the local Standard Operating Procedures.

9.3 Maintenance of Records

The Investigator will retain a copy of all study documents as per the polices mandated by the University of Texas or their local IRB.

9.4 Data Quality Assurance

Data quality will be maintained by establishing a written Data Management Plan describing all applicable aspects of the data management process including:

- Developing a database that meets all verification and validation requirements of FDA rule 21 CFR 11, including reference to all pertinent FDA-provided guidelines;

- Developing a Data Clarification Plan which describes
    - Edit check logic for all variables specified,
    - CRF pages referenced by edit check,
    - Types of query responses: manual, site notification and clinical query;
    - Query text;
- Establishing a data audit strategy and associated procedures.

The database will be frozen and data will be audited prior to database lock by the UT Statistical Center. Critical variables from the data will be identified and subjected to a 100% audit, with all errors corrected. Twenty percent (20%) of variables identified as non-critical will be audited, and if an error rate greater than 0.05 percent (0.05%) is found then 100 percent (100%) of the non-critical variables with the elevated error rates will be audited and all errors corrected.

9.5 Changes in Protocol

Changes to the protocol (after Signatures of Agreement are obtained) that affect the decision of the IRB (e.g., more extensive procedures, increased risk to patients, changes in the patient population, additional safety information, etc.) will be documented and are the responsibility of the Investigator, and will be approved by the IRB before they may be implemented. If the amendment is minor, or reduces the risk to the patient, the chairperson of the IRB alone may approve it. IRB approval is not necessary for protocol clarifications that consist of minor protocol changes such as correcting typographical errors, rewording for clarity, changes in monitoring personnel, or for other changes to the protocol that do not affect the conduct of the study, including changes in the plan for statistical analysis.

The only circumstances in which the amendment may be initiated without IRB Approval is where the change is necessary to eliminate apparent immediate hazards to the patients. In that event, the Investigator must notify the IRB in writing within five (5) working days after the implementation.

10.0 TABLES

TABLE 3. Study Flow Chart

PROCEDURES	Baseline	2-3 hours post tPA bolus	Day 1 (0-24 hrs)	Day 2 (24-48 hrs)	End of Infusion/Early withdrawal	7 Day follow-up (or discharge)	90 ± 10 days
I & E Criteria	X						
Consent Form	X						
Physical Exam[a]	X				X	X	
Medical History & Current Medications	X						
Vital Signs[b]	X		X	X	X	X	
Lab Work[c]	X		if clinically indicated		X		
aPTT[d]	X		X	X	X		
Serum HCG	X						
TIBI / TIMI Flow Grading[e]	X	X					
NIHSS[f]	X	X	X		X	X	X
Modified Rankin Scale[g]	X					X	X
Argatroban Bolus Dosing			X				
Infusion			X	X			
Head CT[h]	X			X			
ECG	X		if clinically indicated				
AE Reporting			X	X	X	X	X
Concomitant Medications	X		X	X	X	X	X
Health utility and cognitive assessment (MoCA)						X	X
Economic Substudy cost data collection							X

a. Physical exam at baseline, w/in 24 hrs of Argatroban discontinuation and Day 7 (or discharge whichever comes first).
b. Vital Signs (BP and HR) recorded at baseline (pre-tPA); first 24-hours: per standard-of-care routine post-tPA vitals; Second 24hours of admission: every 4 ± 1 hours; daily thereafter; end of study and Day 7 (± 3 days) or discharge whichever comes first.
c. Labs (Hematology & Chemistry): Pre-enrollment (baseline) and within 24 hrs of treatment discontinuation (or 48 hrs post rt-tPA bolus). During treatment period all clinically significant labs must be repeated.
d. aPTT will be checked at: baseline (immediately prior to the Argatroban bolus dose); 2 hours (±60 min); 6 hours (± 30 min); 12 hours (± 30 min); 24 hours (± 30 min) and 48 hours (± 30 min). In addition, a PTT will be drawn every 2-4 hours after each dose change (unless the scheduled PTT is due within ±30 minutes. An aPTT will also be obtained in the event of a major bleed (See Section 6.3.6).
e. TCD or CTA will be performed at baseline and at 2-3 hours. Note: not necessary if NIHSS ≥ 10.
f. NIHSS -baseline, 2 (± 30 min), 24 and 48 hrs post rt-PA bolus (± 4 hrs), Day 7 (or discharge), with any signs of neurologic deterioration and with any unscheduled CT Scan (± 4 hrs).
g. 90 day mRS (blinded assessment) will be performed in-person whenever possible. Telephone is allowed if only option.
h. CT scan : Baseline, 48 hrs post rt-PA infusion (± 4 hrs), and as indicated during treatment period. MRI brain imaging can substitute for CT scan, but only if no hemorrhage is present on the MRI sequences. If any hemorrhage is present on MRI imaging, a non-contrast head CT must be performed within 4 hours of the MRI to confirm extent of blood and allow the use of CT-based definitions of hemorrhage (i.e., hemorrhagic transformation type 1 or 2 and parenchymal hematoma type 1 or 2).

11.0 REFERENCES

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12.0 APPENDICES

Appendix 1.0	Argatroban Product Package Insert
Appendix 2.0	Dosing Algorithm
Appendix 3.0	Safety Reporting Procedures
Appendix 4.0	Safety Stopping Rules
Appendix 5.0	Health Related Quality of Life protocol and forms

Appendix 1.0 ARGATROBAN PRODUCT PACKAGE INSERT

ARGATROBAN

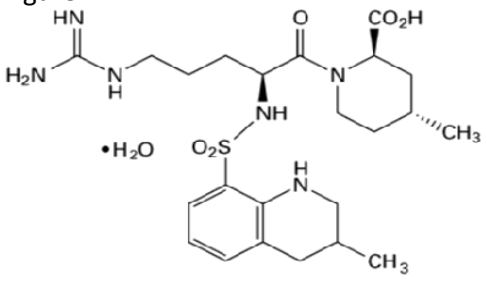
Injection

DESCRIPTION

Argatroban is a synthetic direct thrombin inhibitor derived from L-arginine. The chemical name for Argatroban is 1-[5-[(aminoiminomethyl)amino]-1-oxo-2-[[(1,2,3,4-tetrahydro-3-methyl-8-quinolinyl)sulfonyl]amino]pentyl]-4-methyl-2-piperidinecarboxylic acid, monohydrate. Argatroban has 4 asymmetric carbons. One of the asymmetric carbons has an R configuration (stereoisomer Type I) and an S configuration (stereoisomer Type II). Argatroban consists of a mixture of R and S stereoisomers at a ratio of approximately 65:35.

The molecular formula of Argatroban is C23H36N6O5S•H2O. Its molecular weight is 526.66. The structural formula is shown below:

Figure 1



Argatroban is a white, odorless crystalline powder that is freely soluble in glacial acetic acid, slightly soluble in ethanol, and insoluble in acetone, ethyl acetate and ether. Argatroban Injection is a sterile clear, colorless to pale yellow, slightly viscous solution. Argatroban is available in 250 mg (in 2.5 mL) single-use amber vials, with gray flip-top caps. Each mL of sterile, nonpyrogenic solution contains 100 mg Argatroban. Inert ingredients: 750 mg D-sorbitol, 1000 mg dehydrated alcohol.

CLINICAL PHARMACOLOGY

Mechanism of Action

Argatroban is a direct thrombin inhibitor that reversibly binds to the thrombin active site. Argatroban does not require the co-factor antithrombin III for antithrombotic activity. Argatroban exerts its anticoagulant effects by inhibiting thrombin-catalyzed or -induced reactions, including fibrin formation; activation of coagulation factors V, VIII, and XIII; activation of protein C; and platelet aggregation.

Argatroban is highly selective for thrombin with an inhibitory constant (Ki) of 0.04 μM. At therapeutic concentrations, Argatroban has little or no effect on related serine proteases (trypsin, factor Xa, plasmin, and kallikrein).

Argatroban is capable of inhibiting the action of both free and clot-associated thrombin.

Argatroban does not interact with heparin-induced antibodies. Evaluation of sera in 12 healthy subjects and 8 patients who received multiple doses of Argatroban did not reveal antibody formation to Argatroban (see **CLINICAL STUDIES**).

Pharmacokinetics

Distribution

Argatroban distributes mainly in the extracellular fluid as evidenced by an apparent steady-state volume of distribution of 174 mL/kg (12.18 L in a 70 kg adult). Argatroban is 54% bound to human serum proteins, with binding to albumin and α1-acid glycoprotein being 20% and 34%, respectively.

Metabolism

The main route of Argatroban metabolism is hydroxylation and aromatization of the 3-methyltetrahydroquinoline ring in the liver. The formation of each of the four known metabolites is catalyzed in vitro by the human liver microsomal cytochrome P450 enzymes CYP3A4/5. The primary metabolite (M1) exerts 3- to 5-fold weaker anticoagulant effects than Argatroban. Unchanged Argatroban is the major component in plasma. The plasma concentrations of M1 range between 0 – 20% of that of the parent drug. The other metabolites (M2 – 4) are found only in very low quantities in the urine and have not been detected in plasma or feces. These data, together with the lack of effect of erythromycin (a potent CYP3A4/5 inhibitor) on Argatroban pharmacokinetics suggest that CYP3A4/5 mediated metabolism is not an important elimination pathway in vivo.

Total body clearance is approximately 5.1 mL/kg/min (0.31 L/kg/hr) for infusion doses up to 40 μg/kg/min. The terminal elimination half-life of Argatroban ranges between 39 and 51 minutes.

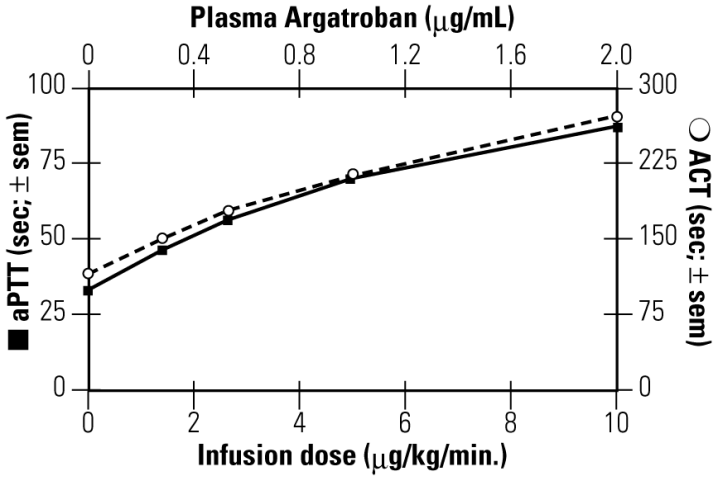
There is no interconversion of the 21–(R): 21–(S) diastereoisomers. The plasma ratio of these diastereoisomers is unchanged by metabolism or hepatic impairment, remaining constant at 65:35 (+2%).

Excretion

Argatroban is excreted primarily in the feces, presumably through biliary secretion. In a study in which 14C-Argatroban (5 μg/kg/min) was infused for 4 hours into healthy subjects, approximately 65% of the radioactivity was recovered in the feces within 6 days of the start of infusion with little or no radioactivity subsequently detected. Approximately 22% of the radioactivity appeared in the urine within 12 hours of the start of infusion. Little or no additional urinary radioactivity was subsequently detected. Average percent recovery of unchanged drug, relative to total dose, was 16% in urine and at least 14% in feces.

Pharmacokinetic/Pharmacodynamic Relationship
When Argatroban is administered by continuous infusion, anticoagulant effects and plasma concentrations of Argatroban follow similar, predictable temporal response profiles, with low intersubject variability. Immediately upon initiation of Argatroban infusion, anticoagulant effects are produced as plasma Argatroban concentrations begin to rise. Steady-state levels of both drug and anticoagulant effect are typically attained within 1-3 hours and are maintained until the infusion is discontinued or the dosage adjusted. Steady-state plasma Argatroban concentrations increase proportionally with dose (for infusion doses up to 40 μg/kg/min in healthy subjects) and are well correlated with steady-state anticoagulant effects. For infusion doses up to 40 μg/kg/min, Argatroban increases in a dose-dependent fashion, the activated partial thromboplastin time (aPTT), the activated clotting time (ACT), the prothrombin time (PT) and International Normalized Ratio (INR), and the thrombin time (TT) in healthy volunteers and cardiac patients. Representative steady-state plasma Argatroban concentrations and anticoagulant effects are shown below for Argatroban infusion doses up to 10 μg/kg/min (see Figure 2).

Figure 2. Relationship at Steady State between Argatroban Dose, Plasma Argatroban Concentration and Anticoagulant Effect



Effect on International Normalized Ratio (INR)
Because Argatroban is a direct thrombin inhibitor, co-administration of Argatroban and warfarin produces a combined effect on the laboratory measurement of the INR. However, concurrent therapy, compared to warfarin monotherapy, exerts no additional effect on vitamin K dependent factor Xa activity.

The relationship between INR on co-therapy and warfarin alone is dependent on both the dose of Argatroban and the thromboplastin reagent used. This relationship is influenced by the International Sensitivity Index (ISI) of the thromboplastin. Data for two commonly utilized thromboplastins with ISI values of 0.88 (Innovin, Dade) and 1.78 (Thromboplastin C Plus, Dade) are presented in Figure 3 for an Argatroban dose of 2 μg/kg/min. Thromboplastins with higher ISI values than shown result in higher INRs on combined therapy of warfarin and Argatroban. These data are based on results obtained in normal individuals (see **DOSAGE AND ADMINISTRATION, Conversion to Oral Anticoagulant Therapy**).

Figure 3. INR Relationship of Argatroban plus Warfarin Versus Warfarin Alone

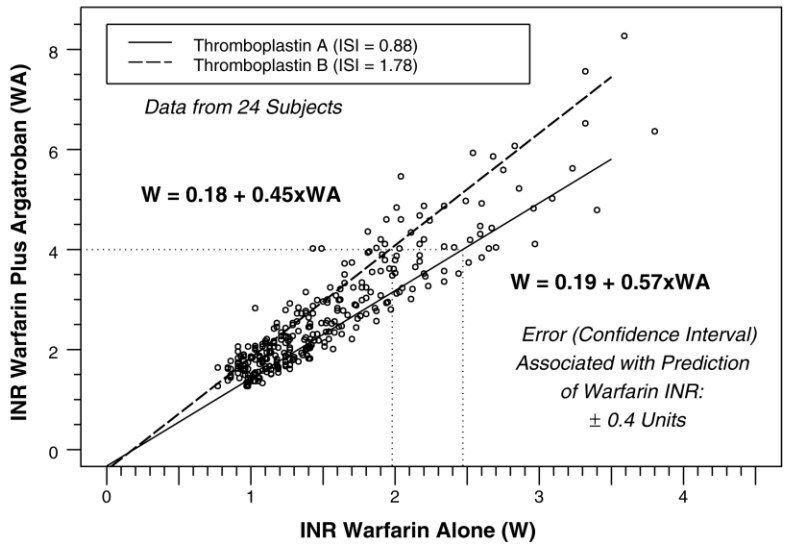


Figure 3 demonstrates the relationship between INR for warfarin alone and INR for warfarin co-administered with Argatroban at a dose of 2 μg/kg/min. To calculate INR for warfarin alone (INRW), based on INR for co-therapy of warfarin and Argatroban (INRWA), when the Argatroban dose is 2 μg/kg/min, use the equation next to the appropriate curve. Example: At a dose of 2 μg/kg/min and an INR performed with Thromboplastin A, the equation 0.19 + 0.57 (INRWA) = INRW would allow a prediction of the INR on warfarin alone (INRW). Thus, using an INRWA value of 4.0 obtained on combined therapy: INRW =0.19 +0.57 (4)=2.47 as the value for INR on warfarin alone. The error (confidence interval) associated with a prediction is ±0.4 units. Similar linear relationships and prediction errors exist for Argatroban at a dose of 1 μg/kg/min. Thus, for Argatroban doses of 1 or 2 μg/kg/min, INRW can be predicted from INRWA. For Argatroban doses greater than 2 μg/kg/min, the error associated with predicting INRW from INRWA is ±1. Thus, INRW cannot be reliably predicted from INRWA at doses greater than 2 μg/kg/min.

SPECIAL POPULATIONS

Renal Impairment

No dosage adjustment is necessary in patients with renal dysfunction. The effect of renal disease on the pharmacokinetics of Argatroban was studied in 6 subjects with normal renal function (mean Clcr = 95 + 16 mL/min) and in 18 subjects with mild (mean Clcr = 64 + 10 mL/min), moderate (mean Clcr = 41 + 5.8 mL/min), and severe (mean Clcr = 5 + 7 mL/min) renal impairment. The pharmacokinetics and pharmacodynamics of Argatroban at dosages up to 5 μg/kg/min were not significantly affected by renal dysfunction.

Hepatic Impairment
The dosage of Argatroban should be decreased in patients with hepatic impairment (see **PRECAUTIONS and DOSAGE AND ADMINISTRATION**). Patients with hepatic impairment were not studied in percutaneous coronary intervention (PCI) trials. At a dose of 2.5 μg/kg/min, hepatic impairment is associated with decreased clearance and increased elimination half-life of Argatroban (to 1.9 mL/kg/min and 181 minutes, respectively, for patients with a Child-Pugh score >6).

Age, Gender
There are no clinically significant effects of age or gender on the pharmacokinetics or pharmacodynamics (e.g., aPTT) of Argatroban.

Drug-Drug Interactions
Digoxin: In 12 healthy volunteers, intravenous infusion of Argatroban (2 μg/kg/min) over 5 days (study days 11-15) did not affect the steady-state pharmacokinetics of oral digoxin (0.375 mg daily for 15 days).

Erythromycin: In 10 healthy subjects, orally administered erythromycin (a potent inhibitor of CYP3A4/5) at 500 mg four times daily for 7 days had no effect on the pharmacokinetics of Argatroban at a dose of 1 μg/kg/min for 5 hours. These data suggest oxidative metabolism by CYP3A4/5 is not an important elimination pathway in vivo for Argatroban.

CLINICAL STUDIES
Heparin-Induced Thrombocytopenia
Heparin–induced thrombocytopenia (HIT) is a potentially serious, immune–mediated complication of heparin therapy that is strongly associated with subsequent venous and arterial thrombosis. Whereas initial treatment of HIT is to discontinue administration of all heparin, patients may require anticoagulation for prevention and treatment of thromboembolic events.

The conclusion that Argatroban is an effective treatment for heparin–induced thrombocytopenia (HIT) and heparin–induced thrombocytopenia and thrombosis syndrome (HITTS) is based upon the data from an historically controlled efficacy and safety study (Study 1) and a follow-on efficacy and safety study (Study 2). These studies were comparable with regard to study design, study objectives, dosing regimens as well as study outline, conduct and monitoring.

In these studies, 568 adult patients were treated with Argatroban and 193 adult patients made up the historical control group. Patients were required to have a clinical diagnosis of heparin-induced thrombocytopenia, either without thrombosis (HIT) or with thrombosis (HITTS) and be males or non-pregnant females between the age of 18 and 80 years old. HIT/HITTS was defined by a fall in platelet count to less than 100,000/μL or a 50% decrease in platelets after the initiation of heparin therapy with no apparent explanation other than HIT. Patients with HITTS also had presence of an arterial or venous thrombosis documented by appropriate imaging techniques or supported by clinical evidence such as acute myocardial infarction, stroke, pulmonary embolism, or other clinical indications of vascular occlusion. Patients who required anticoagulation with documented histories of positive HIT antibody test were also eligible in the absence of thrombocytopenia or heparin challenge (e.g., patients with latent disease).

Patients with documented unexplained aPTT >200% of control at baseline, documented coagulation disorder or bleeding diathesis unrelated to HITTS, a lumbar puncture within the past 7 days or a history

of previous aneurysm, hemorrhagic stroke, or recent thrombotic stroke, within the past 6 months, unrelated to HITTS were excluded from these studies.

The initial dose of Argatroban was 2 μg/kg/min not to exceed 10 μg/kg/min. Two hours after the start of the Argatroban infusion, an aPTT level was obtained and dose adjustments were made to achieve a steady-state aPTT value that was 1.5 to 3.0 times the baseline value, not to exceed 100 seconds. In Study 1, the mean aPTT level for HIT patients was 38 seconds prior to start of Argatroban infusion. At first assessment,* during the Argatroban infusion, mean aPTT level for HIT patients was 64 seconds. Overall the mean aPTT level during the Argatroban infusion for HIT patients was 62.5 seconds. In Study 1, the mean aPTT level for HITTS patients was 34 seconds prior to start of Argatroban infusion. At first assessment,* during the Argatroban infusion, mean aPTT level for HITTS patients was 70 seconds. Overall, the mean aPTT level during the Argatroban infusion for HITTS patients was 64.5 seconds (see **DOSAGE AND ADMINISTRATION**). (*First assessment was defined as occurring at least 2 hours post-infusion start time.)

The primary efficacy analysis was based on a comparison of event rates for a composite endpoint that included death (all causes), amputation (all causes) or new thrombosis during the treatment and follow-up period (study days 0 to 37). Secondary analyses included evaluation of the event rates for the components of the composite endpoint as well as time-to-event analyses.

In Study 1, 304 patients were enrolled having active HIT (129/304, 42%), active HITTS (144/304, 47%) or latent disease (31/304, 10%). Among the 193 historical controls, 139 (72%) had active HIT, 46 (24%) had active HITTS, and 8 (4%) had latent disease. Within each group, those with active HIT and those with latent disease were analyzed together. Positive laboratory confirmation of HIT/HITTS by the heparin-induced platelet aggregation test or serotonin release assay was demonstrated in 174 of 304 (57%) Argatroban-treated patients (i.e., in 80 with HIT or latent disease and 94 with HITTS) and in 149 of 193 (77%) historical controls (i.e., in 119 with HIT or latent disease and 30 with HITTS). The test results for the remainder of the patients and controls were either negative or not determined.

A categorical analysis showed a significant improvement in the composite outcome in patients with HIT and HITTS treated with Argatroban versus those in the historical control group (see Table 1). The components of the composite endpoint are shown in Table 2.

Table 1. Efficacy Results of Study 1:
Composite Endpoint*

	HIT		HITTS		HIT/HITTS	
Parameter, N (%)	Control n=147	Argatroban n=160	Control n=46	Argatroban n=144	Control n=193	Argatroban n=304
Composite Endpoint	57 (38.8)	41 (25.6)	26 (56.5)	63 (43.8)	83 (43.0)	104 (34.2)

*Death (all causes), amputation (all causes) or new thrombosis within 37-day study period.

Table 2. Efficacy Results of Study 1:
Components of the Composite Endpoint, Ranked by Severity*

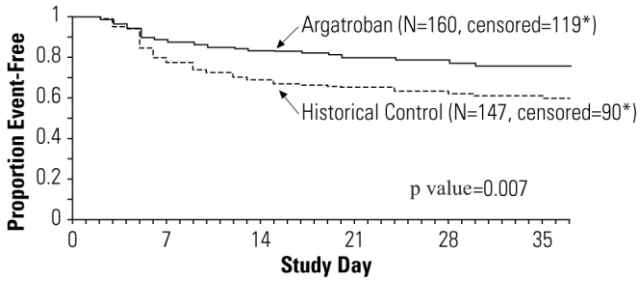
	HIT		HITTS		HIT/HITTS	
Parameter, N (%)	Control n=147	Argatroban n=160	Control n=46	Argatroban n=144	Control n=193	Argatroban n=304
Death	32 (21.8)	27 (16.9)	13 (28.3)	26 (18.1)	45 (23.3)	53 (17.4)
Amputation	3 (2.0)	3 (1.9)	4 (8.7)	16 (11.1)	7 (3.6)	19 (6.2)
New Thrombosis	22 (15.0)	11 (6.9)	9 (19.6)	21 (14.6)	31 (16.1)	32 (10.5)

*Reported as the most severe outcome among the components of composite endpoint (severity ranking: death > amputation > new thrombosis); patients may have had multiple outcomes.

Time-to-event analyses showed significant improvements in the time-to-first event in patients with HIT or HITTS treated with Argatroban versus those in the historical control group. The between-group differences in the proportion of patients who remained free of death, amputation or new thrombosis were statistically significant in favor of Argatroban by these analyses (p=0.007 in patients with HIT and p=0.018 in patients with HITTS, according to log-rank test).
A time-to-event analysis for the composite endpoint is shown in Figure 4 for patients with HIT and Figure 5 for patients with HITTS.

STUDY 1

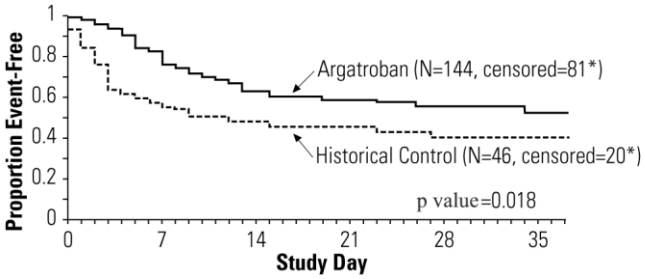
Figure 4. Time to First Event for the Composite Efficacy Endpoint: HIT Patients



*censored indicates no clinical endpoint (defined as death, amputation or new thrombosis) was observed during the follow-up period (maximum period of follow-up was 37 days).

STUDY 1

Figure 5. Time to First Event for the Composite Efficacy Endpoint: HITTS Patients



*censored indicates no clinical endpoint (defined as death, amputation or new thrombosis) was observed during the follow-up period (maximum period of follow-up was 37 days).

In Study 2, 264 patients were enrolled, having either HIT (125/264, 47.3%) or HITTS (139/264, 52.7%), and then treated with Argatroban. Categorical analysis demonstrated significant improvement in the composite efficacy outcome for Argatroban-treated patients, versus the same historical control group from Study 1, among patients having HIT (25.6% vs. 38.8%), patients having HITTS (41.0% vs. 56.5%), and patients having either HIT or HITTS (33.7% vs. 43.0%). Time-to-event analyses showed significant improvements in the time-to-first event in patients with HIT or HITTS treated with Argatroban versus those in the historical control group. The between-group differences in the proportion of patients who remained free of death, amputation or new thrombosis were statistically significant in favor of Argatroban.

Anticoagulant Effect: In Study 1, the mean (+SE) dose of Argatroban administered was 2.0 ± 0.1 μg/kg/min in the HIT arm and 1.9±0.1 μg/kg/min in the HITTS arm. Seventy-six percent of patients with HIT and 81% of patients with HITTS achieved a target aPTT at least 1.5-fold greater than the baseline aPTT at the first assessment occurring on average at 4.6 hours (HIT) and 3.9 hours (HITTS) following initiation of Argatroban therapy.

No enhancement of aPTT response was observed in subjects receiving repeated administration of Argatroban.

Platelet Count Recovery: In Study 1, the majority of patients, 53% of those with HIT and 58% of those with HITTS, had a recovery of platelet count by day 3. Platelet Count Recovery was defined as an increase in platelet count to >100,000/μL or to at least 1.5-fold greater than the baseline count (platelet count at study initiation) by day 3 of the study.

Percutaneous Coronary Intervention (PCI) in HIT/HITTS Patients

In three similarly designed trials, Argatroban was administered to 91 patients with current or previous clinical diagnosis of HIT/HITTS or heparin-dependent antibodies, who underwent a total of 112 percutaneous coronary interventions (PCIs) including percutaneous transluminal coronary angioplasty (PTCA), coronary stent placement or atherectomy.

Among the 91 patients undergoing their first PCI with Argatroban, notable ongoing or recent medical history included myocardial infarction (n=35), unstable angina (n=23), and chronic angina (n=34). There were 33 females and 58 males. The average age was 67.6 years (median 70.7, range 44-86), and the average weight was 82.5 kg (median 81.0 kg, range 49-141).

Due to the history or presence of the heparin-dependent antibody or HIT/HITTS, these patients required alternative anticoagulation. Twenty-one of the 91 patients had a repeat PCI using Argatroban an average of 150 days after their initial PCI. Seven of 91 patients received glycoprotein IIb/IIIa inhibitors. Safety and efficacy were assessed against historical control populations.

Per protocol, all patients received oral ± (325 mg) 2-24 hours prior to the interventional procedure. After venous or arterial sheaths were in place, anticoagulation was initiated with a bolus of Argatroban of 350 μg/kg via a large bore IV line or through the venous sheath over 3-5 minutes. Simultaneously, a maintenance infusion of 25 μg/kg/min was initiated to achieve a therapeutic activated clotting time (ACT) of 300-450 seconds. If necessary to achieve this therapeutic range, the maintenance infusion dose was titrated (15-40 μg/kg/min) and/or an additional bolus dose of 150 μg/kg could be given. Each patient's ACT was checked 5-10 minutes following the bolus dose. The ACT was checked as clinically indicated thereafter. Arterial and venous sheaths were removed no sooner than 2 hours after discontinuation of Argatroban and when the ACT was less than 160 seconds.

If a patient required anticoagulation after the procedure, Argatroban could be continued, but at a lower infusion dose between 2.5 and 5 μg/kg/min. An aPTT was drawn 2 hours after this dose reduction and the dose of Argatroban then adjusted as clinically indicated (not to exceed 10 μg/kg/min), to reach an aPTT between 1.5 and 3 times baseline value (not to exceed 100 seconds).

Ninety-one patients were treated with Argatroban on their first PCI, and 21 patients were reexposed to Argatroban on subsequent PCIs. In 92 of the 112 interventions (82%), the patient received the initial bolus of 350 μg/kg and an initial infusion dose of 25 μg/kg/min. The majority of patients did not require additional bolus dosing during the PCI procedure. The mean value for the initial ACT measurement after the start of dosing for all interventions was 379 sec (median 338 sec; 5th percentile-95th percentile 238-675 sec). The mean ACT value per intervention over all measurements taken during the procedure was 416 sec (median 390 sec; 5th percentile-95th percentile 261-698 sec). About 65% of patients had ACTs within the recommended range of 300 to 450 seconds throughout the procedure. The investigators did not achieve anticoagulation within the recommended range in about 23% of patients. However, in this small sample, patients with ACTs below 300 seconds did not have more coronary thrombotic events, and patients with ACTs over 450 seconds did not have higher bleeding rates.

Acute procedural success was defined as lack of death, emergent coronary artery bypass graft (CABG), or Q-wave myocardial infarction. Acute procedural success was reported in 98.2% of patients who underwent PCIs with Argatroban anticoagulation compared with 94.3% of historical control patients anticoagulated with heparin (p=NS). Among the 112 interventions, 2 patients had emergency CABGs, 3 had repeat PTCAs, 4 had non-Q-wave myocardial infarctions, 3 had myocardial ischemias, 1 had an abrupt closure, and 1 had an impending closure (some patients may have experienced more than one event). No patients died. Two patients had protocol-defined major bleeding one of which was retroperitoneal and the other gastrointestinal. Minor bleeding, defined as spontaneous and observed with hemoglobin decreasing >3g/dL or with no bleeding site and hemoglobin decreasing >4g/dL, occurred in 4.5% of interventions.

Additional Information

Cardiac Therapy: The safety and effectiveness of Argatroban for cardiac indications outside of percutaneous coronary intervention in patients with HIT have not been established.

Reexposure and Lack of Antibody Formation: Plasma from 12 healthy volunteers treated with Argatroban over 6 days showed no evidence of neutralizing antibodies. Repeated administration of Argatroban to more than 40 patients was tolerated with no loss of anticoagulant activity. No change in the dose is required.

INDICATIONS AND USAGE
Argatroban is indicated as an anticoagulant for prophylaxis or treatment of thrombosis in patients with heparin-induced thrombocytopenia.

Argatroban is indicated as an anticoagulant in patients with or at risk for heparin-induced thrombocytopenia undergoing percutaneous coronary intervention (PCI).

CONTRAINDICATIONS
Argatroban is contraindicated in patients with overt major bleeding, or in patients hypersensitive to this product or any of its components (see **WARNINGS**).

WARNINGS
Argatroban is intended for intravenous administration. All parenteral anticoagulants should be discontinued before administration of Argatroban.

Hemorrhage
Hemorrhage can occur at any site in the body in patients receiving Argatroban. An unexplained fall in hematocrit, fall in blood pressure, or any other unexplained symptom should lead to consideration of a hemorrhagic event. Argatroban should be used with extreme caution in disease states and other circumstances in which there is an increased danger of hemorrhage. These include severe hypertension; immediately following lumbar puncture; spinal anesthesia; major surgery, especially involving the brain, spinal cord, or eye; hematologic conditions associated with increased bleeding tendencies such as congenital or acquired bleeding disorders and gastrointestinal lesions such as ulcerations.

PRECAUTIONS

Hepatic Impairment

Caution should be exercised when administering Argatroban to patients with hepatic disease, by starting with a lower dose and carefully titrating until the desired level of anticoagulation is achieved. Also, upon cessation of Argatroban infusion in the hepatically impaired patient, full reversal of anticoagulant effects may require longer than 4 hours due to decreased clearance and increased elimination half-life of Argatroban (see **DOSAGE AND ADMINISTRATION**).

Use of high doses of Argatroban in PCI patients with clinically significant hepatic disease or AST/ALT levels >3 times the upper limit of normal should be avoided. Such patients were not studied in PCI trials.

Laboratory Tests

Anticoagulation effects associated with Argatroban infusion at doses up to 40 μg/kg/min correlate with increases of the activated partial thromboplastin time (aPTT).

Although other global clot-based tests including prothrombin time (PT), the International Normalized Ratio (INR) and thrombin time (TT) are affected by Argatroban, the therapeutic ranges for these tests have not been identified for Argatroban therapy. Plasma Argatroban concentrations also correlate well with anticoagulant effects (see **CLINICAL PHARMACOLOGY**).

In clinical trials in PCI, the activated clotting time (ACT) was used for monitoring Argatroban anticoagulant activity during the procedure.

The concomitant use of Argatroban and warfarin results in prolongation of the PT and INR beyond that produced by warfarin alone. Alternative approaches for monitoring concurrent Argatroban and warfarin therapy are described in a subsequent section (see **DOSAGE AND ADMINISTRATION**).

Drug Interactions

Heparin: Since heparin is contraindicated in patients with heparin-induced thrombocytopenia, the co-administration of Argatroban and heparin is unlikely for this indication. However, if Argatroban is to be initiated after cessation of heparin therapy, allow sufficient time for heparin's effect on the aPTT to decrease prior to initiation of Argatroban therapy.

Aspirin/Acetaminophen: Pharmacokinetic or pharmacodynamic drug-drug interactions have not been demonstrated between Argatroban and concomitantly administered aspirin (162.5 mg orally given 26 and 2 hours prior to initiation of Argatroban 1 μg/kg/min over 4 hours) or acetaminophen (1000 mg orally given 12, 6 and 0 hours prior to, and 6 and 12 hours subsequent to, initiation of Argatroban 1.5 μg/kg/min over 18 hours).

Oral anticoagulant agents: Pharmacokinetic drug-drug interactions between Argatroban and warfarin (7.5 mg single oral dose) have not been demonstrated. However, the concomitant use of Argatroban and warfarin (5-7.5 mg initial oral dose followed by 2.5-6 mg/day orally for 6-10 days) results in prolongation of the prothrombin time (PT) and International Normalized Ratio (INR) (see **CLINICAL PHARMACOLOGY and DOSAGE AND ADMINISTRATION**).

Thrombolytic agents: The safety and effectiveness of Argatroban with thrombolytic agents have not been established (see **ADVERSE REACTIONS, Intracranial Bleeding**).

Glycoprotein IIb/IIIa antagonists: The safety and effectiveness of Argatroban with glycoprotein IIb/IIIa antagonists have not been established.

Co-administration: Concomitant use of Argatroban with antiplatelet agents, thrombolytics, and other anticoagulants may increase the risk of bleeding (see **WARNINGS)**. Drug-drug interactions have not been observed between Argatroban and digoxin or erythromycin (see **CLINICAL PHARMACOLOGY, Drug-Drug Interactions**).

Carcinogenesis, Mutagenesis, Impairment of Fertility
No long-term studies in animals have been performed to evaluate the carcinogenic potential of Argatroban.

Argatroban was not genotoxic in the Ames test, the Chinese hamster ovary cell (CHO/HGPRT) forward mutation test, the Chinese hamster lung fibroblast chromosome aberration test, the rat hepatocyte and WI-38 human fetal lung cell unscheduled DNA synthesis (UDS) tests, or the mouse micronucleus test.

Argatroban at intravenous doses up to 27 mg/kg/day (0.3 times the recommended maximum human dose based on body surface area) was found to have no effect on fertility and reproductive performance of male and female rats.

Pregnancy. Teratogenic Effects. Pregnancy Category B.
Teratology studies have been performed in rats with intravenous doses up to 27 mg/kg/day (0.3 times the recommended maximum human dose based on body surface area) and rabbits at intravenous doses up to 10.8 mg/kg/day (0.2 times the recommended maximum human dose based on body surface area) and have revealed no evidence of impaired fertility or harm to the fetus due to Argatroban. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Nursing Mothers
Experiments in rats show that Argatroban is detected in milk. It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from Argatroban, a decision should be made whether to discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the mother.

Geriatric Use
In the clinical studies of adult patients with HIT or HITTS, the effectiveness of Argatroban was not affected by age.

Pediatric Use
The safety and effectiveness of Argatroban in patients below the age of 18 years have not been established.

ADVERSE REACTIONS
Adverse Events Reported in HIT/HITTS Patients
The following safety information is based on all 568 patients treated with Argatroban in Study 1 and Study 2. The safety profile of the patients from these studies is compared with that of 193 historical

controls in which the adverse events were collected retrospectively. The adverse events reported in this section include all events regardless of relationship to treatment. Adverse events are separated into hemorrhagic and non-hemorrhagic events.

Major bleeding was defined as bleeding that was overt and associated with a hemoglobin decrease >2 g/dL, that led to a transfusion of >2 units, or that was intracranial, retroperitoneal, or into a major prosthetic joint. Minor bleeding was overt bleeding that did not meet the criteria for major bleeding.

Table 3 gives an overview of the most frequently observed hemorrhagic events, presented separately by major and minor bleeding, sorted by decreasing occurrence among Argatroban-treated HIT/HITTS patients.

Table 3. Major and Minor Hemorrhagic Adverse Events in HIT/HITTS Patients

Major Hemorrhagic Events*		
	Argatroban-treated Patients (Study 1 and Study 2) (n=568) %	**Historical Control (n=193) %**
Overall Bleeding	5.3	6.7
Gastrointestinal	2.3	1.6
Genitourinary and hematuria	0.9	0.5
Decrease in hemoglobin and hematocrit	0.7	0
Multisystem hemorrhage and DIC	0.5	1
Limb and BKA stump	0.5	0
Intracranial hemorrhage	0†	0.5

Minor Hemorrhagic Events*		
	Argatroban-treated Patients (Study 1 and Study 2) (n=568) %	**Historical Control (n=193) %**
Gastrointestinal	14.4	18.1
Genitourinary and hematuria	11.6	0.8
Decrease in hemoglobin and hematocrit	10.4	0
Groin	5.4	3.1
Hemoptysis	2.9	0.8
Brachial	2.4	0.8

*Patients may have experienced more than one adverse event.
† One patient experienced intracranial hemorrhage 4 days after discontinuation of Argatroban and following therapy with urokinase and oral anticoagulation.
DIC = disseminated intravascular coagulation.
BKA = below the knee amputation.

Table 4 gives an overview of the most frequently observed non-hemorrhagic events sorted by decreasing frequency of occurrence (>2%) among argatroban-treated hit/hitts patients.

Table 4. Non-hemorrhagic Adverse Events in HIT/HITTS Patients*

	Argatroban-treated Patients) (Study 1 and Study 2) (n=568) %	Historical Control (n=193) %
Dyspnea	8.1	8.8
Hypotension	7.2	2.6
Fever	6.9	2.1
Diarrhea	6.2	1.6
Sepsis	6.0	12.4
Cardiac arrest	5.8	3.1
Nausea	4.8	0.5
Ventricular tachycardia	4.8	3.1
Pain	4.6	3.1
Urinary tract infection	4.6	5.2
Vomiting	4.2	0
Infection	3.7	3.6
Pneumonia	3.3	9.3
Atrial fibrillation	3.0	11.4
Coughing	2.8	1.6
Abnormal renal function	2.8	4.7
Abdominal pain	2.6	1.6
Cerebrovascular disorder	2.3	4.1

*Patients may have experienced more than one adverse event.

Adverse Events Reported in HIT/HITTS Patients Undergoing PCI

The following safety information is based on 91 patients initially treated with Argatroban and 21 patients subsequently re-exposed to Argatroban for a total of 112 PCIs with Argatroban anticoagulation. The adverse events reported in this section include all events regardless of relationship to treatment. Adverse events are separated into hemorrhagic (Table 5) and non-hemorrhagic (Table 6) events.

Major bleeding was defined as bleeding that was overt and associated with a hemoglobin decrease $\geq$5 g/dL, that led to a transfusion of $\geq$2 units, or that was intracranial, retroperitoneal, or into a major prosthetic joint.

The rate of major bleeding events and intracranial hemorrhage in the PCI trials was 1.8% and in the placebo arm of the EPILOG trial (placebo plus standard dose, weight-adjusted heparin) was 3.1%.

Table 5. Major and Minor Hemorrhagic Adverse Events in HIT/HITTS Patients Undergoing PCI

Major Hemorrhagic Events*	
	Argatroban-treated Patients (n=112)† %
Retroperitoneal	0.9
Gastrointestinal	0.9
Intracranial Hemorrhage	0

Minor Hemorrhagic Events*	
	Argatroban-treated Patients (n=112) † %
Groin (bleeding or hematoma)	3.6
Gastrointestinal (includes hematemesis)	2.6
Genitourinary (includes hematuria)	1.8
Decrease in hemoglobin and/or hematrocrit	1.8
CABG (coronary arteries)	1.8
Access site	0.9
Hemoptysis	0.9
Other	0.9

*Patients may have experienced more than one adverse event.
† 91 patients who underwent 112 interventions.
CABG = coronary artery bypass graft.

Table 6 gives an overview of the most frequently observed non-hemorrhagic events (>2%), sorted by decreasing frequency of occurrence among Argatroban-treated PCI patients.

Table 6. Non-hemorrhagic Adverse Events* in HIT/HITTS Patients Undergoing PCI

	Argatroban Procedures* (n=112)† %	Controls (n=2226)‡ %
Chest Pain	15.2	9.3
Hypotension	10.7	10.3
Back Pain	8.0	13.7
Nausea	7.1	11.5
Vomiting	6.3	6.8
Headache	5.4	5.5
Bradycardia	4.5	3.5
Abdominal Pain	3.6	2.2
Fever	3.6	<0.5
Myocardial Infarction	3.6	NR§

*Patients may have experienced more than one adverse event.
† 91 patients who underwent 112 interventions.
‡ Controls from EPIC (Evaluation of c7E3 Fab in the Prevention of Ischemic Complications), EPILOG (Evaluation in PTCA to Improve Long-Term Outcome with Abciximab GP IIb/IIIa Blockade Study) and CAPTURE (Chimeric 7E3 Antiplatelet Therapy in Unstable angina Refractory to standard treatment) trials.
Source: ReoPro® Prescribing Information.
§ NR=not reported.

There were 22 serious adverse events in 17 PCI patients (19.6% in 112 interventions). The types of events, which are listed regardless of relationship to treatment, are shown in Table 7. Table 7 lists the serious adverse events occurring in Argatroban-treated HIT/HITTS patients undergoing PCI.

Table 7. Serious Adverse Events in HIT/HITTS Patients Undergoing PCI*

Coded Term	Argatroban Procedures† (n=112)
Chest Pain	1 (0.9%)
Fever	1 (0.9%)
Retroperitoneal Hemorrhage	1 (0.9%)
Angina Pectoris	2 (1.8%)
Aortic Stenosis	1 (0.9%)
Coronary Thrombosis	2 (1.8%)
Arterial Thrombosis	1 (0.9%)
Myocardial Infarction	4 (3.5%)
Myocardial Ischemia	2 (1.8%)
Occlusion Coronary	2 (1.8%)
Gastrointestinal Hemorrhage	1 (0.9%)
Gastrointestinal Disorder (GERD)	1 (0.9%)
Cerebrovascular Disorder	1 (0.9%)
Lung Edema	1 (0.9%)
Vascular Disorder	1 (0.9%)

* Individual events may also have been reported elsewhere (see Table 5 and 6).
† 91 patients underwent 112 procedures. Some patients may have experienced more than one event.

Adverse Events Reported in Other Populations
The following safety information is based on a total of 1,127 individuals who were treated with Argatroban in clinical pharmacology studies (n=211) or for various clinical indications (n=916).

Intracranial Bleeding. Intracranial bleeding only occurred in patients with acute myocardial infarction who were started on both Argatroban and thrombolytic therapy with streptokinase. The overall frequency of this potentially life-threatening complication among patients receiving both Argatroban and thrombolytic therapy (streptokinase or tissue plasminogen activator) was 1% (8 out of 810 patients). Intracranial bleeding was not observed in 317 subjects or patients who did not receive concomitant thrombolysis (see **WARNINGS**).

Allergic Reactions. 156 allergic reactions or suspected allergic reactions were observed in 1,127 individuals who were treated with Argatroban in clinical pharmacology studies or for various clinical indications. About 95% (148/156) of these reactions occurred in patients who concomitantly received thrombolytic therapy (e.g., streptokinase) for acute myocardial infarction and/or contrast media for coronary angiography.

Allergic reactions or suspected allergic reactions in populations other than HIT/HITTS patients include (in descending order of frequency*):

- Airway reactions (coughing, dyspnea): 10% or more
- Skin reactions (rash, bullous eruption): 1 to <10%
- General reactions (vasodilation): 1 to 10%

*The CIOMS (Council for International Organization of Medical Sciences) III standard categories are used for classification of frequencies.

OVERDOSAGE

Symptoms/Treatment

Excessive anticoagulation, with or without bleeding, may be controlled by discontinuing Argatroban or by decreasing the Argatroban infusion dosage (see **WARNINGS**). In clinical studies at therapeutic levels, anticoagulation parameters generally return to baseline within 2 to 4 hours after discontinuation of the drug. Reversal of anticoagulant effect may take longer in patients with hepatic impairment.

No specific antidote to Argatroban is available; if life-threatening bleeding occurs and excessive plasma levels of Argatroban are suspected, Argatroban should be discontinued immediately, aPTT and other coagulation tests should be determined. Symptomatic and supportive therapy should be provided to the patient (see **WARNINGS**).

Single intravenous doses of Argatroban at 200, 124, 150, and 200 mg/kg were lethal to mice, rats, rabbits, and dogs, respectively. The symptoms of acute toxicity were loss of righting reflex, tremors, clonic convulsions, paralysis of hind limbs, and coma.

DOSAGE AND ADMINISTRATION

Argatroban, as supplied, is a concentrated drug (100 mg/mL), which must be diluted 100-fold prior to infusion. Argatroban should not be mixed with other drugs prior to dilution in a suitable intravenous fluid.

Preparation for Intravenous Administration

Argatroban should be diluted in 0.9% Sodium Chloride Injection, 5% Dextrose Injection, or Lactated Ringer's Injection to a final concentration of 1 mg/mL. The contents of each 2.5 mL vial should be diluted 100-fold by mixing with 250 mL of diluent. Use 250 mg (2.5 mL) per 250 mL of diluent or 500 mg (5 mL) per 500 mL of diluent. The constituted solution must be mixed by repeated inversion of the diluent bag for one minute. Upon preparation, the solution may show slight but brief haziness due to the formation of microprecipitates that rapidly dissolve upon mixing. The pH of the intravenous solution prepared as recommended is 3.2-7.5.

Heparin-Induced Thrombocytopenia (HIT/HITTS)

Initial Dosage: Before administering Argatroban, discontinue heparin therapy and obtain a baseline aPTT. The recommended initial dose of Argatroban for adult patients without hepatic impairment is 2 µg/kg/min, administered as a continuous infusion (see Table 8).

Table 8. Standard Infusion Rates for 2 μg/kg/min Dose
(1 mg/ml final concentration)

Body Weight (kg)	Infusion Rate (ml/hr)
50	6
60	7
70	8
80	10
90	11
100	12
110	13
120	14
130	16
140	17

Monitoring therapy: In general, therapy with Argatroban is monitored using the aPTT. Tests of anticoagulant effects (including the aPTT) typically attain steady-state levels within 1-3 hours following initiation of Argatroban. Dose adjustment may be required to attain the target aPTT. Check the aPTT 2 hours after initiation of therapy to confirm that the patient has attained the desired therapeutic range.

Dosage adjustment: After the initial dose of Argatroban, the dose can be adjusted as clinically indicated (not to exceed 10 μg/kg/min), until the steady-state aPTT is 1.5 to 3 times the initial baseline value (not to exceed 100 seconds)(see **CLINICAL STUDIES** for mean values of aPTT obtained after initial doses of Argatroban).

Percutaneous Coronary Interventions (PCI) in HIT/HITTS Patients
Initial Dosage: An infusion of Argatroban should be started at 25 μg/kg/min and a bolus of 350 μg/kg administered via a large bore intravenous (IV) line over 3 to 5 minutes. Activated clotting time (ACT) should be checked 5 to 10 minutes after the bolus dose is completed. The procedure may proceed if the ACT is greater than 300 seconds.

Dosage Adjustment: If the ACT is less than 300 seconds, an additional IV bolus dose of 150 μg/kg should be administered, the infusion dose increased to 30 μg/kg/min, and the ACT checked 5 to 10 minutes later. If the ACT is greater than 450 seconds, the infusion rate should be decreased to 15 μg/kg/min, and the ACT checked 5 to 10 minutes later. Once a therapeutic ACT (between 300 and 450 seconds) has been achieved, this infusion dose should be continued for the duration of the procedure.

In case of dissection, impending abrupt closure, thrombus formation during the procedure, or inability to achieve or maintain an ACT over 300 seconds, additional bolus doses of 150 μg/kg may be administered and the infusion dose increased to 40 μg/kg/min. The ACT should be checked after each additional bolus or change in the rate of infusion.

Monitoring therapy: Therapy with Argatroban is monitored using ACT. ACTs should be obtained before dosing, 5 to 10 minutes after bolus dosing and after change in the infusion rate, and at the end of the PCI procedure. Additional ACTs should be drawn about every 20 to 30 minutes during a prolonged procedure.

Continued Anticoagulation after PCI: If a patient requires anticoagulation after the procedure, Argatroban may be continued, but at a lower infusion dose [see **DOSAGE AND ADMINISTRATION, Heparin-Induced Thrombocytopenia (HIT/HITTS)**].

Dosing in Special Populations

Hepatic Impairment:

For patients with heparin-induced thrombocytopenia with hepatic impairment, the initial dose of Argatroban should be reduced. For patients with moderate hepatic impairment, an initial dose of 0.5 μg/kg/min is recommended, based on the approximate 4-fold decrease in Argatroban clearance relative to those with normal hepatic function. The aPTT should be monitored closely and the dosage should be adjusted as clinically indicated (see **PRECAUTIONS**).

Hepatic Impairment in HIT/HITTS Patients Undergoing PCI:

For hepatically impaired HIT/HITTS patients undergoing PCI, refer to **PRECAUTIONS, Hepatic Impairment**.

Renal Impairment:

No dosage adjustment is necessary in patients with renal impairment (see **PRECAUTIONS**).

CONVERSION TO ORAL ANTICOAGULANT THERAPY

Initiating Oral Anticoagulant Therapy

Once the decision is made to initiate oral anticoagulant therapy, recognize the potential for combined effects on INR with co-administration of Argatroban and warfarin. A loading dose of warfarin should not be used. Initiate therapy using the expected daily dose of warfarin.

Co-Administration of Warfarin and Argatroban at Doses Up to 2 μg/kg/min

Use of Argatroban with warfarin results in prolongation of INR beyond that produced by warfarin alone. The previously established relationship between INR and bleeding risk is altered. The combination of Argatroban and warfarin does not cause further reduction in the vitamin K dependent factor Xa activity than that which is seen with warfarin alone. The relationship between INR obtained on combined therapy and INR obtained on warfarin alone is dependent on both the dose of Argatroban and the thromboplastin reagent used. The INR value on warfarin alone (INRW) can be calculated from the INR value on combination Argatroban and warfarin therapy (see **CLINICAL PHARMACOLOGY**, Figure 3 explanation).

INR should be measured daily while Argatroban and warfarin are co-administered. In general, with doses of Argatroban up to 2 μg/kg/min, Argatroban can be discontinued when the INR is >4 on combined therapy. After Argatroban is discontinued, repeat the INR measurement in 4 to 6 hours. If the repeat INR is below the desired therapeutic range, resume the infusion of Argatroban and repeat the procedure daily until the desired therapeutic range on warfarin alone is reached.

Co-Administration of Warfarin and Argatroban at Doses Greater than 2 μg/kg/min
For doses greater than 2 μg/kg/min, the relationship of INR between warfarin alone to the INR on warfarin plus Argatroban is less predictable. In this case, in order to predict the INR on warfarin alone, temporarily reduce the dose of Argatroban to a dose of 2 μg/kg/min. Repeat the INR on Argatroban and warfarin 4 to 6 hours after reduction of the Argatroban dose and follow the process outlined above for administering Argatroban at doses up to 2 μg/kg/min.

STABILITY/COMPATIBILITY
Argatroban is a clear, colorless to pale yellow, slightly viscous solution. If the solution is cloudy, or if an insoluble precipitate is noted, the vial should be discarded.

Solutions prepared as recommended are stable at 25°C (77°F) with excursions permitted to 15°-30°C (59°-86°F) in ambient indoor light for 24 hours; therefore, light resistant measures such as foil protection for intravenous lines are unnecessary. Solutions are physically and chemically stable for up to 48 hours when stored at 2° to 8°C in the dark. Prepared solutions should not be exposed to direct sunlight. No significant potency losses have been noted following simulated delivery of the solution through intravenous tubing.

HOW SUPPLIED
Argatroban Injection is supplied in 2.5 mL solution in single-use vials at the concentration of 100 mg/mL. Each vial contains 250 mg of Argatroban.

NDC 0007-4407-01 (Package of 1)

Storage
Store the vials in original cartons at room temperature [25°C (77°F) excursion permitted to 15°-30°C (59°-86°F)]. Do not freeze. Retain in the original carton to protect from light.

DATE OF ISSUANCE APR. 2002

Manufactured by **Abbott Laboratories**, North Chicago, IL 60064
Distributed by **GlaxoSmithKline**, Research Triangle Park, NC 27709 for **Texas Biotechnology Corporation**, Houston, TX 77030

Appendix 2.0 DOSING ALGORITHM

Suggested Titration Algorithm

The algorithm below is a guideline for assisting investigators in making dosage adjustment to attain the protocol target aPTTs of 1.75 and 2.25 times patient baseline value. Please note that due to inter-patient variability these adjustments may not definitely result in the achievement of the specified aPTT ranges and that variations in the dosage amounts may be required.

Protocol Target aPTT Values:

- Low dose target = 1.75 times baseline
- High dose target = 2.25 times baseline

A +/- 5% range is permitted when attempting to attain the target aPTT values.

1.75 range = 1.66 – 1.84 (5% of 1.75 = 0.0875)
2.25 range = 2.14 – 2.36 (5% of 2.25 = 0.1125)

Suggested algorithm:

If the aPTT value is greater than or equal to +/- 10% of the target value (± 10% of 1.75 = 1.58 – 1.93 and ± 10% of 2.25 = 2.03 – 2.48), begin dosage adjustments at 0.5 μg/kg/min increments. Once the aPTT is within 10% of the target value, decrease dosage adjustments to 0.25 μg/kg/min increments. As the target range is approached, the dosage increments may need to be decreased by 0.125 μg/kg/min amount depending on the patient's sensitivity to the drug. See Figures below for a graphical example of dose titration as well as an example patient titration schedule.

A secure website will allow entering of the patient's weight and baseline PTT. After entering the data, the website will generate a printable (custom to only that individual patient) titration schedule (according to high vs. low dose schedule).

ADDITIONAL SAFETY NOTE:
Low-Dose Target: Instructions for aPTT values from 80 seconds up to greater than 100 seconds:

If the aPTT elevates to **between 80 and 100** seconds, decrease the infusion by 50% (i.e. if 2 μg/kg/min decrease to 1 μg/kg/min), check the aPTT 1 hour following the adjustment. If the follow-up aPTT remains between 80-100, decrease the rate again by 50% and check the PTT 1 hour following the second rate decrease. Continue this process until the aPTT is <80 seconds, then follow the titration protocol generated by the website to maintain the target aPTT.

If the aPTT **exceeds 100** seconds, immediately discontinue the infusion and check the aPTT every hour following the discontinuation until the aPTT is **less than 80** seconds. Once the aPTT is below 80 seconds, re-initiate the infusion (without the bolus dose) at the lowest previous dose for that patient that achieved an acceptable aPTT value. In the event an acceptable previous dose was never reached (i.e., all previous aPTTs were greater than target), restart the infusion at 50% of the previous rate.

High-Dose Target: Instructions for aPTT values from 110 seconds up to greater than 130 seconds:

If the aPTT elevates to **between 110 and 130** seconds, decrease the infusion by 50% (i.e. if 2 μg/kg/min decrease to 1 μg/kg/min), check the aPTT 1 hour following the adjustment. If the follow-up aPTT remains between 110-130, decrease the rate again by 50% and check the PTT 1 hour following the second rate decrease. Continue this process until the aPTT is <110 seconds, then follow the titration protocol generated by the website to maintain the target aPTT.

If the aPTT exceeds **130** seconds, immediately discontinue the infusion and check the aPTT every hour following the discontinuation until the aPTT is **less than 110** seconds. Once the aPTT is below 110 seconds, re-initiate the infusion (without the bolus dose) at the lowest previous dose for that patient that achieved an acceptable aPTT value. In the event an acceptable previous dose was never reached (i.e., all previous aPTTs were greater than target), restart the infusion at 50% of the previous rate.

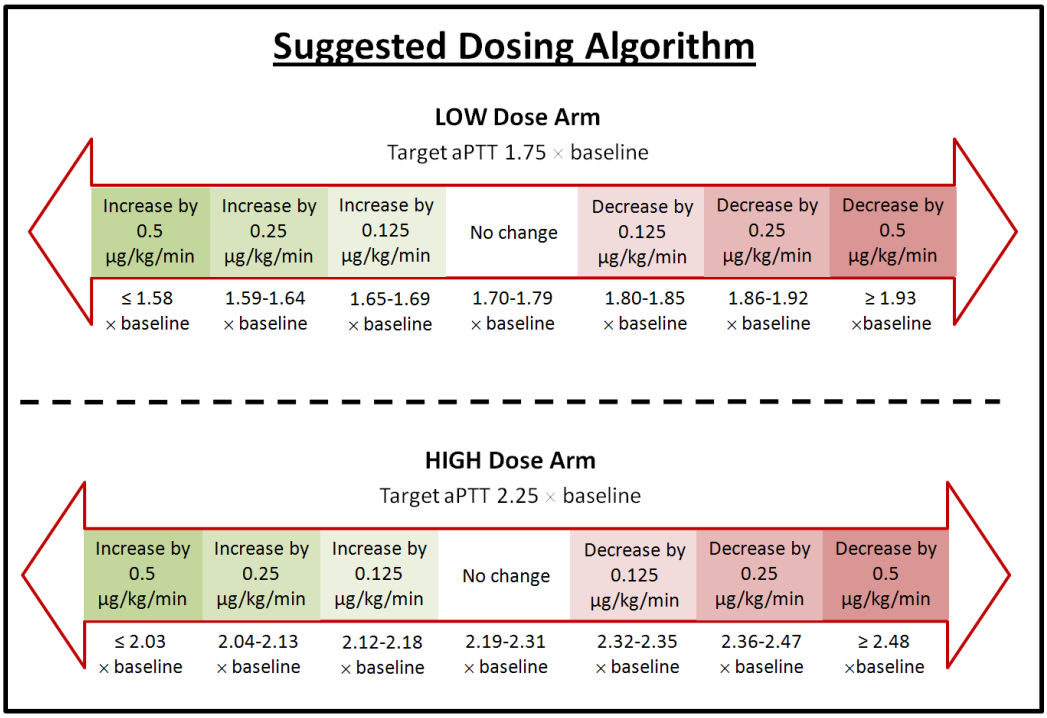


Figure 1. Example titration schedule for the low-dose arm: 80kg patient with a baseline aPTT of 25.

Patient Initials: ade Weight: 80 Kg Baseline aPTT: 25 sec. Calculate

Calculated Bolus: **8.0 mg = 8.0 cc = 8000 µg:** [100µg/kg] Current As of: 10/20/2010 08:47
Initial infusion: Low Dose 1µg/kg/min: **4.8 cc/hr**

Use this table for approx. dosage adjustment based on latest aPTT result vs. target of 1.75 x baseline minor adjustments may be needed to avoid over-shooting target

aPTT multiplier Range	if latest aPTT Level is:	Adjust Rate (µg/kg/min)
≤ 1.58 x Baseline	≤ 39.7	Increase by 0.5
1.59 - 1.64 x Baseline	39.8 - 41.2	Increase by 0.25
1.65 - 1.69 x Baseline	41.3 - 42.4	Increase by 0.125
1.70 - 1.79 x Baseline	42.5 - 44.8	No Change
1.80 - 1.85 x Baseline	44.9 - 46.3	Decrease by 0.125
1.86 - 1.92 x Baseline	46.4 - 48.0	Decrease by 0.25
≥1.93 x Baseline	≥ 48.1	Decrease by 0.50

Appendix 3.0 SAFETY REPORTING PROCEDURES

3.1 Definitions:

The investigator is responsible for the detection and documentation of events meeting the criteria and definition of an Adverse Event (AE) or Serious Adverse Event (SAE) as provided in this protocol. During the study, when there is a safety evaluation, the investigator or site staff will be responsible for detecting AEs and SAEs, as detailed within this Appendix.

The definition of an Adverse Event (AE) includes: Any untoward medical occurrence in a patient or clinical investigation subject, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product.
For marketed medicinal products, this also includes failure to produce expected benefits (i.e. lack of efficacy), abuse or misuse. This also includes an exacerbation of pre-existing conditions or events, intercurrent illness, drug interaction or the significant worsening of the disease under study that do not represent a clinically significant exacerbation or worsening need not be considered an adverse event. An adverse experience includes significant failure of expected pharmacological or biological action.

A serious adverse event is any untoward medical occurrence that, at any dose:
a) results in death.
b) is life-threatening.
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.
c) requires hospitalization or prolongation of existing hospitalization.
NOTE: In general, hospitalization signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or out-patient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether hospitalization occurred or was necessary, the AE should be considered serious. Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.
d) results in disability/incapacity, or
NOTE: The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g. sprained ankle) which may interfere or prevent everyday life functions but do not constitute a substantial disruption.
e) is a congenital anomaly/birth defect.

f) other Serious (Important Medical Events). Medical or scientific judgment should be exercised in deciding whether 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 medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious. Examples of such events are invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

Lack of efficacy per se will not be reported as an AE. The signs and symptoms or clinical sequelae resulting from lack of efficacy will be reported if they fulfill the AE or SAE definition (including clarifications).

Abnormal laboratory findings (e.g., clinical chemistry, hematology, urinalysis) or other abnormal assessments (e.g., abnormal liver function, renal clearance, ECGs, X-rays, vital signs, etc.,) that are judged by the investigator as clinically significant will be recorded as AEs or SAEs if they meet the definition of an AE, or SAE, as defined above. Clinically significant abnormal laboratory findings or other abnormal assessments that are detected during the study or are present at baseline and significantly worsen following the start of the study will be reported as AEs or SAEs. However, clinically significant abnormal laboratory findings or other abnormal assessments that are associated with the disease being studied, unless judged by the investigator as more severe than expected for the subjects condition, or that are present or detected at the start of the study and do not worsen, will not be reported as AEs or SAEs. The investigator will exercise his or her medical and scientific judgment in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant.

An SAE considered related to study participation (e.g., procedures, invasive tests, a change in existing therapy), even if it occurs during the pre- or post-treatment period, will be reported promptly to the DSMB and IRB.

3.2 Reporting Safety Data

The standard time period for collecting and recording AEs and SAEs will begin at the receipt of investigational product and will end after the Day 90 assessment. Additionally, in order to fulfill international reporting obligations, SAEs that are related to study participation (e.g., procedures, invasive tests, a change from existing therapy) or are related to a concurrent medication will be collected and recorded from the time the subject consents to participate in the study until Day 90 assessment is complete.

When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory, and diagnostics reports) relative to the event. The investigator will then record all relevant information regarding an AE/SAE on the SAE forms. The investigator will attempt to establish a diagnosis of the event based on signs, symptoms,

and/or other clinical information. In such cases, the diagnosis should be documented as the AE/SAE and not the individual signs/symptoms.
The investigator will make an assessment of intensity for each AE and SAE reported during the study. The assessment will be based on the investigators clinical judgment. The intensity of each AE and SAE recorded in the SAE form should be assigned to one of the following categories:

Mild: An event that is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.

Moderate: An event that is sufficiently discomforting to interfere with normal everyday activities.

Severe: An event that prevents normal everyday activities. An AE that is assessed as severe should not be confused with a SAE. Severity is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe. An event is defined as ,serious when it meets one of the pre-defined outcomes.

The investigator is obligated to assess the relationship between the study drug (Argatroban) and the occurrence of each AE/SAE. The investigator will use clinical judgment to determine the relationship. Alternative causes, such as natural history of the underlying diseases, concomitant therapy, other risk factors, and the temporal relationship of the event to the investigational product will be considered and investigated. The investigator will also consult the Investigators Brochure and/or Product Information, for marketed products, in the determination of his/her assessment.

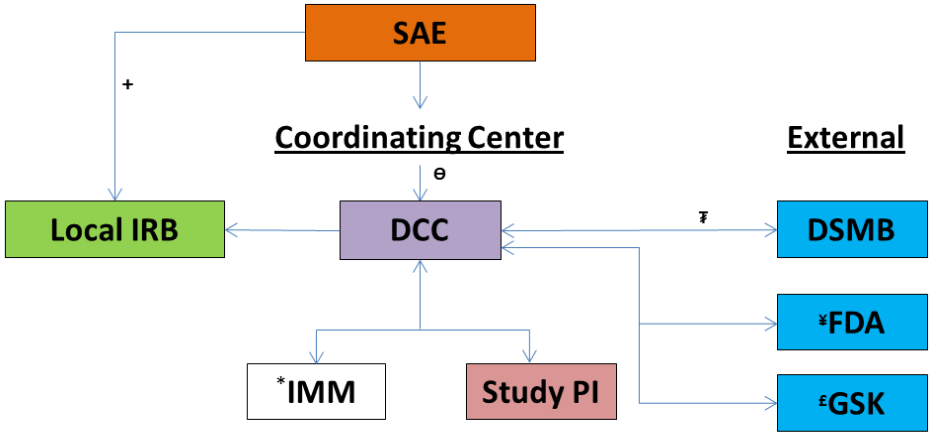
3.3 Follow-up AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each subject on the subject's condition. All AEs and SAEs documented at a previous visit/contact and are designated as ongoing, will be reviewed at subsequent visits/contacts. All AEs and SAEs will be followed until resolution, until the condition stabilizes, until the event is otherwise explained, or until the subject is lost to follow-up. Once resolved, the appropriate AE/SAE forms page(s) will be updated. The investigator will ensure that follow-up includes any supplemental investigations as may be indicated to elucidate the nature and/or causality of the AE or SAE. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.

3.4 Regulatory Requirements for SAE Reporting

Any SAE will be reported to the Data Coordinating Center within 24 hours of learning of the event. The investigator, or responsible person according to local requirements, will comply with the applicable local regulatory requirements related to the reporting of SAEs to regulatory authorities and the Institutional Review Board (IRB). SAEs will be reviewed by the independent safety medical monitor. Depending on the severity, cause and circumstances of the SAE, a

report to the FDA might be warranted. This report will be generated by the coordinating PI. Further, all SAEs will be reported to both Glaxo-Smith-Kline, Inc (provider of study drug) and the Data Safety Monitoring Board. See Figure 1 for more detail.



Abbreviations: **SAE** – Serious Adverse Events; **DCC** – Data Coordinating Center; **STUDY PI** – overall study PI at UTH – University of Texas-Houston; **IMM** – Independent Medical Monitor; **GSK** – Glaxo-Smith-Kline; **FDA** – Food & Drug Administration; **DSMB** – Data Safety Monitoring Board; **IRB** – Internal Review Board; **PH2** – Parenchymal Hemorrhage Type 2; **sICH** – Symptomatic Intracerebral Hemorrhage

+ All local sites will report occurrences of SAEs to their local IRB (per their local guidelines). In many cases, this will happen before final study adjudication.
ɵ DCC will receive the SAE within 24 hours of the event.
*Preliminary review occurs by both Study PI and IMM. The IMM will have 14 calendar days to make the final decision regarding SAE adjudication and send back to DCC.
Ŧ DCC will send the SAEs report to DSMB with the following ways:
- Report all un-adjudicated sICHs, PH2s, serious systematic bleedings, deaths and SAEs that are unexpected and related to the study drug within 7 calendar days by telephone or fax of the determination. Follow-up adjudicated written report will be submitted no later than 15 calendar days of the determination.
- Report all other SAEs after every 10 patients that completed the day 90 visit.

¥ The FDA will receive all SAEs that are unexpected fatal or life-threatening adverse experience associated with use of the drug:
- Preliminary report will be provided by telephone or fax within 7 calendar days of the determination. Final adjudicated written reports will be submitted no later then 15 calendar days of the determination. An annual report will be sent to the FDA including all SAEs

£ GSK will receive SAEs for every 10 patients that completed the day 90 visit.

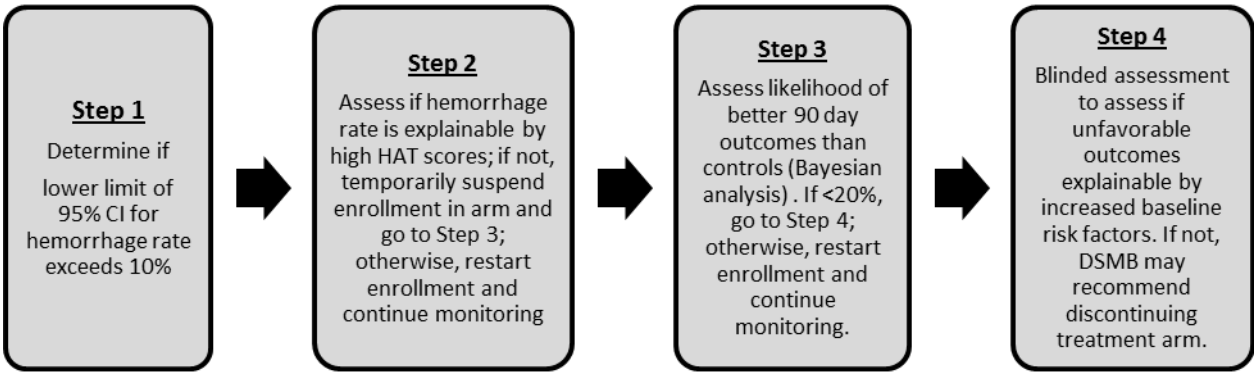
Figure 1. Procedures for reporting Serious Adverse Events (SAEs).

Example: a local site has an SAE:

1. All clinical (Local) sites report any incidents of SAEs to both their local IRB and the DCC. An automatic notification system alerts the overall principle investigator (PI) at UT-Houston (UTH) or a designee (e.g., project coordinator).
2. Preliminary review occurs by both UTH principle investigator and IMM. If an SAE is confirmed not to be study related, then the SAE will be reported to DCC, GSK and the DSMB (by the PI or his designee at UTH). Conversely, if the SAE is confirmed to be unexpected and related to study drug, then the PI (or his designee) at UTH also reports to the FDA.
3. The DCC will assist the PI or his designee at UTH to assemble regularly scheduled DSMB reports.
4. If the FDA and DSMB request changes in the status of the SAEs then this information will be sent back to DCC/UTH (now an adjudicated SAE).
5. These updated report(s) will be distributed to all local IRBs (including the UTH coordinating center IRB).

* For timing of each report (i.e., within 24-hours of knowledge of SAE occurrence), see the study manual of procedures.

Appendix 4.0 SAFETY STOPPING RULES



Explanation:

Relatively complex stopping rules are necessary to minimize the possibility that a truly effective medication that would benefit very large numbers of patients would be abandoned due to misleading findings for a small number of study patients. To this end, stopping rules will be based on 1) net clinical benefit (i.e., better long term outcomes could occur despite higher than expected early hemorrhage rates; 2) a four step safety stopping rule for low and high dose Argatroban arms that involves:

1. **identification of a high symptomatic or PH-2 hemorrhage rate (lower limit of 95% CI exceeds 10%) whenever it occurs in either Argatroban arm;**

2. **assessment of whether the high hemorrhage rate is explainable by a high-base line risk of patients in that arm (assessed by the HAT score);**

3. **If not, suspension of enrollment in that arm and comparison of 90 day outcomes with the controls pending a Bayesian analysis of the likelihood of better 90 day outcome than in controls;**

4. **If likelihood of treatment benefit is ≥20%, restart enrollment with continued monitoring. If <20%, conduct a blinded assessment of each treatment arm to determine if unfavorable outcomes are explained by increased baseline risks. The DSMB may recommend discontinuing the corresponding Argatroban arm.**

Step 1

Intracerebral hemorrhage (raw) rate calculation

Using group sequential method (calculated using exact binomial probabilities), halting rules are calculated: 1) after the first group of 10, 17 and 25 patients that mandate halting of a study arm if the lower limit of 95% confidence interval for the true hemorrhage rate exceeded 10% (see table 1 on next page).

Using table 1 below, if 4 of the first 10 patients experience a significant ICH (60 percent), we would be 95% confident that the true hemorrhage rate is > 10% (in this example, the lower limit is 12.2%).

In the event that Step 1 is triggered, two things will occur:

1) The treatment arm will be suspended until 90 day outcome is assessed for all patients enrolled at that treatment arm.
2) Step 2 will be instituted

Step 2

Compare HAT (hemorrhage after thrombolysis) scores between the treatment and control arms.

The HAT score is a validated five-point scale based on NIH Stroke Scale score, extent of hypodensity on CT scan, serum glucose >200 at baseline or history of diabetes that has been shown to predict the risk of hemorrhage after thrombolysis[1,2] in the absence of Argatroban administration. In the original manuscript, the rate of symptomatic ICH was greater in patients with more HAT points: 2% (0 point), 5% (1 point), 10% (2 points), 15% (3 points), and 44% (>3 points).

If the percentage of patients with a HAT score ≥3 in the treatment group is the same or higher than control, this might account for the higher hemorrhage rates and allow continued enrollment.

Using this step will avoid early termination due to play of chance that the treatment arm included patients with a higher risk of bleeding at enrollment.

In the event that the % of HAT score 3 (or greater) is the same or less than the control group (i.e., the baseline risk of ICH in the treatment group is the same or less than control patients), step 3 will be instituted.

Score Assignment in the HAT score

Characteristic	Points
History of diabetes mellitus or baseline blood glucose >200 mg/dL upon admission	
No	0
Yes	1
Pretreatment NIHSS score	
<15	0
15-20	1
≥20	2
Presence of easily visible hypodensity on initial head CT scan	
No	0
<1/3 of MCA territory	1
≥1/3 of MCA territory	2

Step 3

Compare clinical outcomes at 90 days between the treatment arm and control patients

The net clinical benefit of an experimental treatment includes the benefits minus the harm. It is possible that due to the known neuroprotective effects of thrombin-inhibition, patients might experience somewhat higher early bleeding risk, but better long-term stroke recovery.

Therefore, the % of mRS 0-1 (good clinical outcome) at 90 days will be compared between the treatment and control patients. Using a neutral prior, Bayesian probabilities have been established- see table 2 on next page. The study arm would potentially be terminated in the event that the posterior probability of treatment benefit (vs. control patients) is ***less than 20%.***

For example, at 17 patients if 5 ICHs have occurred, then there would need to be 4 fewer good outcomes in the treatment arm. At this point the estimated probability of treatment benefit would be 8%.

If the rate of good outcome in any Argatroban arm indeed is <20%, then Step 4 will be instituted.

Step 4

Blinded assessment of baseline patient characteristics

In the event that rates of hemorrhage are high, HAT scores are no worse than control patients and clinical outcomes are also worse, two stroke neurologists (Co-PIs: Andrew Barreto and James Grotta) will assess the clinical characteristics of each arm of the study enrolled to date. This blinded assessment will include baseline NIH stroke scale, age, mRS, vessel of occlusion, and other medical comorbidities. The two stroke neurologists will determine if the groups appear equal in these characteristics (i.e., baseline risk). If there does not appear to be any difference among the baseline risk factors between study arms (and the high rate of hemorrhage is in an Argatroban arm), the DSMB may then decide to terminate the arm.

Table 1. Halting rules for 95% Confidence Interval (CI) using Group Sequential Interval Estimation Technique

Sample Size (N)	Number of ICH (X)	Percent P= X/N	Lower limit of 95% CI	Upper limit of 95% CI
10	4	0.40	0.122	0.738
17	5	0.29	0.103	0.559
25	7	0.28	0.121	0.494

Table 2. Bayesian probabilities of a treatment effect at 90 days. Treatment arm can be terminated in the event the probability of **any** benefit is < 20%.

Assumptions: neutral prior, centered at a relative risk of 1 with 95% credible interval (0.33, 3) and assumes a 3% absolute increase in excellent outcome (mRS 0 or 1): treatment (32%) and control group (29%).

Number of excellent outcomes at 90 days (mRS 0 or 1)	Probability of incorrectly stopping the treatment arm (**Type I error -** Chance of calling an effective treatment ineffective**)**
Treatment: x Control: x+3	**8%**
Treatment: x Control: x+4	**8%**
Treatment: x Control: x+5	**9.4%**

Models and recommendations for rules for suspending or stopping enrollment were based on:

1) The model used assumes that the treatment and control arm are independent and the primary outcome of clinical outcome at 90 days follows a Binomial distribution. Prior distributions for the probability of a good clinical outcome are assumed to follow Beta distributions (Beta[5,12]) in both arms which gives a neutral prior on the relative risk centered at 1 with 95% credible intervals of 0.3 and 3.0. The posterior distribution for the proportion of good outcomes will also be a Beta distribution. This Beta distribution provides the posterior point estimates for proportion of good outcome and the corresponding 95% credible interval as well as posterior probability of benefit (i.e., Pr(RR>1)).
2) At least a 3% absolute true increase in excellent outcomes in treatment vs. control patients (32% versus 29%, assumed true event rates). This would correspond to a number needed to treat for one patient to benefit of 33 which would be a lower number to treat (that is, a stronger treatment effect) for a more important outcome than for many widely accepted therapies commonly used in clinical practice.
3) It is important to study sufficient patients to assess treatment effects. Stopping rules used previously by DSMBs have been criticized for terminating trials too prematurely for negative

results.[3] With the large number of future stroke patients who would be harmed if a truly beneficial therapy were abandoned because of chance findings in a small sample of patients, it is important not to abandon a promising therapy unless the findings indicate a high likelihood it is not beneficial. A 20% threshold is the highest that seems reasonable; a lower threshold could also be considered.

As long as there is no evidence that the hemorrhage rate in either argatroban arm exceeds 10%, the full 105 patients will be enrolled. If the lower limit for the 95% confidence interval for hemorrhage exceeds 10% at the sample sizes shown below, enrollment in that arm will be suspended until clinical outcome has been determined for all patients. If the probability of benefit is at least 20%, enrollment in that arm will resume (as argatroban might improve outcome despite some increase in hemorrhage). Otherwise, enrollment in that arm will be resumed until enrollment reaches the point at which stopping would be reconsidered.

After 10,000 trial simulations the probability of incorrectly stopping a treatment arm for a truly beneficial therapy is displayed in table 2 below. The stopping rule would be incorrect 8% of the time (92% probability that there is no treatment effect) after 10 treatment patients (and 10 controls) have been enrolled. For example, if 4 of 10 treatments experience a significant ICH and at 90 days there are 3 less excellent outcomes in the experimental group, then the trial would be stopped. The same scenario is also listed for the halfway point of the study (17 patients per arm) and at approximately 2/3^{rds} of enrollment (25 patients per arm).

References

1. Lou, M., et al., *The HAT Score: a simple grading scale for predicting hemorrhage after thrombolysis.* Neurology, 2008. **71**(18): p. 1417-23.
2. Tsivgoulis G, Saqqur M, **Barreto A**, Demchuk AM, Ribo M, Rubiera M, Sharma VK, Stamboulis E, Schellinger PD, Molina CA, Alexandrov AV. Validity of HAT score for predicting symptomatic intracranial hemorrhage in acute stroke patients with proximal occlusions: data from randomized trials of sonothrombolysis. *Cerebrovascular Diseases* 2011;31:471-476.
3. Fernandes, R. M., van der Lee, J. H. and Offringa, M. (2011), Data monitoring committees, interim analysis and early termination in paediatric trials. Acta Paediatrica, 100: no. doi: 10.1111/j.1651-2227.2011.02282.x

Appendix 5.0 HEALTH RELATED QUALITY OF LIFE PROTOCOL AND FORMS

Health Quality and Economics Sub-Study

Argatroban rt-PA Stroke Study-2/Health Related Quality of Life after Ischemic Stroke Study

Introduction

The goal of this study is to estimate the health economic impact of the Argatroban TPA Stroke Study-2 (ARTSS-2) a Phase IIb trial for the combined use of tPA (Tissue Plasminogen Activator) and Argatroban in the treatment of acute ischemic stroke patients. The ARTSS-2 trial will include a sample of patients located in several collaborating medical centers in the US and England. The economic component of ARTSS-2 involves a cost-effectiveness analysis (CEA) which allows for an objective comparison of alternative interventions based on parameters such as quality of life gains for patients. The results from the economic study will complement the clinical findings in reaching a conclusion on the effectiveness and safety of the intervention.

Methods

The CEA will measure marginal differences in costs (i.e., resources consumed) and quality of life (quality adjusted life years, or QALY) for three treatments: a control (tPA only applied according to pre-determined standards of care); and two interventions (tPA in combination with a low dosage of Argatroban, and tPA in combination with a high dosage of Argatroban). The CEA will be done from the provider perspective and will involve patient data from each collaborating medical center, starting at the moment of initial treatment and continuing through a 90-day post-treatment follow-up period.

Primary Economic Outcomes or Endpoints

The health outcome measure in the model will be incremental quality-adjusted-life years (QALYs) while the economic outcome measure is incremental healthcare costs between each treatment. QALY is a health status index, which incorporates the impact of quantity and quality of life for various treatments, and has become a standard for health economics studies. The following ratios will be conducted to measure pre- and post- cost-effectiveness of the interventions:

- Cost per intervention
- Cost per QALY gained in trial
- Incremental cost-utility per intervention

Cost-Effectiveness Analysis

The CEA will involve three general steps:

1. Measuring **patient utility** and estimating **quality-adjusted life years**. Utility measures in combination clinical assessments of long-term survival will be used to estimate QALYs by patient.
2. Estimating ***medical costs*** associated with implementing each treatment. These are patient-level costs and include medical, hospital, and broad estimates of indirect costs (e.g., opportunity cost of lost work days).
3. Estimating **incremental cost-effectiveness ratios** (ICERs) using the cost and QALY data obtained in the previous steps. In this study we will use patient QALY as a measure of effectiveness and we consider a cost-utility analysis a variation of a cost-effectiveness analysis. CERs are stochastic measures allowing for the statistical comparison of cost and health outcome differences between the three treatments.

Patient Utility Measurement and QALYs Estimation

We consider reported patient utility as an approximation of quality of life and as such can be used to make ordinal comparisons between individuals of varying degree of health. In order to ensure quality patient-level utility data, we will use two self-administered questionnaires: 1) the Paper Standard Gamble (PSG, shown in Appendix A), which is used to measure patient utility by means of a standard gamble approach; and, 2) the EuroQoL (EQ-5D,

shown in Appendix B), which is used to determine general health conditions and patient utility and is based on time trade-off values. The PSG is suitable for postal surveys and has proven to provide highly reliable patient utility estimates in a relatively inexpensive manner [1][2]. We have modified the original instrument described by Ross (2003) to include a graphical component alongside line descriptions of different "gamble" situations. The EQ-5D is an instrument recommended by the Agency for Health Research and Quality [3]and used extensively in health economic studies. Also, the EQ-5D has been well validated in hundreds of studies, and incorporates utility into the quality of life ratings

We will estimates QALYs with two-step approach. First, with the assistance of the medical team at the UT Med Center and using patient disability measures (e.g., modified Rankin Scale) we will obtain estimates of life expectancy for each patient. A complementary analysis will include constructing a decision tree based on the rates obtained in the trial combined with a Markov model. Second, we will calculate QALYs for each patient with the product of life expectancy estimates and reported utility measures.

The PSG and the EQ-5D will be implemented for two observational periods; one day prior to discharge, and 90 days after discharge. Patient responses obtained from the EQ-5D survey will be matched to US-and UK-specific utility preference weights (EQ-5D value sets) in order to obtain country-specific estimates of utility.

Medical Costs Estimation

For these analyses, we will focus primarily on estimating direct and indirect total intervention costs. These include

- Direct costs of cardiology medical care at each participating site, including labor costs (physician, nursing, technician), laboratory, pharmaceuticals, hospital care, and other identifiable costs.
- Indirect costs of care, including the time the patient spends receiving the intervention and lost wages due to illness.

We will use three main approaches for collecting cost data. First, we will use an instrument called the Resource Utilization Form (shown in Appendix C), a self-administered questionnaire developed by the Texas School of Public Health, which captures patient demographic and social information as well as use of healthcare services. Second, we will maintain direct contact with the finance areas of each participating medical center in order to secure physician and hospital cost data by patient. The required data includes unit costs for physician and medical resources used by each patient such as procedures, hospital stay, and medication. Appendix D presents the Patient Case Report which outlines a proposed format to be used by each participating medical center for reporting cost data. Third, we will apply a Micro Costing Analysis which is a modified time-study method used to identify intervention-specific costs for in-hospital and post-discharge provider-patient encounters. The Micro Costing Study will be applied randomly throughout the duration of the study, but only for a sub-sample of patients in the UT Med Center.

Total hospital and provider cost data will be collected throughout the duration of the trial. The Resource Utilization Form will be implemented once, 90 days after discharge. We will use the provider perspective in calculating cost using payment rates to value (RVU) for physician services and procedures, cost-adjusted charges for hospital care, and wholesale prices for drugs. In order to quantify costs of resource consumption rates reported in the Resource Utilization Form we will use nationally representative payment rates whenever possible, including the national Medicare payment rate for physician visits, procedures, and laboratory tests. Health care cost for each person throughout will be related to their demographic characteristics, risk factors, and socioeconomic characteristics. In this manner we will be able to estimate health care costs for persons with missing data and to extrapolate costs beyond the trial period. Future treatment costs from end of follow-up until death will be estimated from a simulation model that projects trends established for each arm of the study. Health care use will be documented in terms of frequency of and lengths of stay of hospitalizations, number of diagnostic procedures, surgical procedures, physician visits, and type and daily dose of any pharmaceuticals. We will also try to estimate future health care costs by age sex adjusted health care spending rates for the general population from the Medical Expenditure Panel Survey.

Before receiving any questionnaire, every patient will have a brief explanation of the instruments and the participants' understanding will be discussed prior to self-completion by enrolled individuals. Honesty and completeness will be encouraged at this time and a quiet area in proximity to the clinician will be provided to complete the questionnaire. Approximate time for questionnaire completion will be 30 minutes. Appendix E presents a protocol logistics outline illustrating the proposed mechanism for integrating QALY and cost data from all participating medical centers. The general protocol data flow is illustrated in figure 1 while a summary of these instruments and primary domains is shown in table 1.

Incremental Cost-effectiveness Ratio Estimates

We will obtain incremental cost-effectiveness ratios (ICERs) by comparing cost and QALY changes for all three treatments (tPA only, tPA + Arg/n low, and tPA+Arg/n high):

$$ICER_i = (Cost_i - Cost_j) \div (QALY_i - QALY_j)$$

WHERE *COST* INVOLVES TOTAL DIRECT AND INDIRECT COSTS, AND $I \neq J$.

We will incorporate in our analyses a variety of statistical or economic methods to analyze the data. These methods include:

- ICER scatter plots
- Weighted means analysis, to analyze means of intervention strategies
- Discounted cash flow (DCF) analysis, to adjust future years relative to the base years
- Analysis of variance, and other statistical methods to describe data and explore statistical significance in differences of cost-effectiveness of intervention groups
- Sensitivity analysis, to determine the effects of alternative parameter estimates on ICEA ratios

As mentioned before various approaches will be used (e.g., Markov model) to project costs, and outcomes beyond the 90-day duration of the trial. An average 30-year time horizon will be considered in each model, but this horizon span will be adjusted according to age-specific groups of patients. As reported in the literature, future costs will be discounted at 3% per year and effects will be discounted at a 5% annual rate [4][5]. Costs estimates and projections for sites outside the US will be calculated in local currency and final ICER will be used to compare results within and between countries. Appendix F includes the Montreal Cognitive Assessment (MoCA) form and instructions.

Investigators

Dr. James Langabeer II will serve as the primary analytical investigator for the cost-effectiveness analyses. Dr. Langabeer is an associate professor of healthcare management and policy, and is the Director of the Center for Emergency Research (a decision science/cost effectiveness center focused on acute emergency healthcare). He is the five books and over 50 research articles.
Dr. Rigoberto Delgado will serve as the co-investigator and will focus on data integrity and analytical methods. Dr. Delgado is a research fellow in Health Economics at the University Of Texas School of Public Health, and has conducted numerous economic evaluations in health care.

Definition of Roles

All roles in the Scope of Work will be performed by Drs. Langabeer and Delgado, with the following exceptions:

Role & Responsibility	Langabeer/ Delgado	PI/Providers
Use instruments to interview patients at prescribed time intervals		X
Provide training to clinical staff/PI on instruments and data collection process	X	
Perform primary statistical significance on clinical outcomes		X
Define intervention costs and outcomes for cost protocol		X
Enter data from patient questionnaires	X	X
Collect hospital cost and charge data by patient	X	
Collect micro cost data only for patients in the UT Med Center	X	
Obtain cost-effectiveness estimates for each treatment	X	

Figure 1: Research Instruments - Health Quality and Economics

Argatroban TPA Stroke Study-2/ Health Related Quality of Life after Ischemic Stroke Study

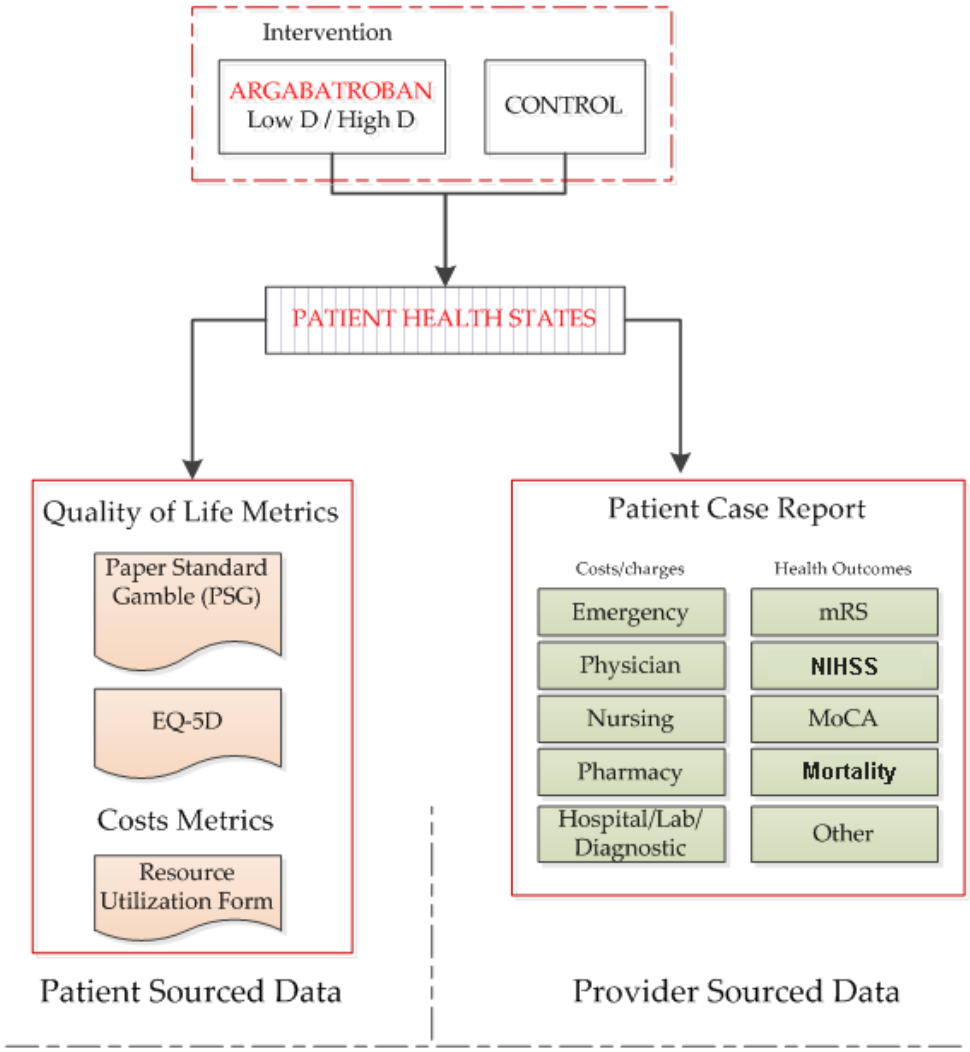


Table 1: Summary of Analytical Instruments and Data Sources Used in this Protocol

Instrument/ Data Source	***Type of Measure***	***Domains***	***Assessment (****days)*	
			Discharge	***90 days post-discharge***
PSG	Quality adjusted life years	Utility of current health status	X	X
EQ-5D	Quality adjusted life years	Mobility, self-care, usual activity, pain/discomfort, anxiety/depression	X	X
Resource Utilization Form	Patient Resource Costs	Economic		X
Hospital and Physician Costs	Hospital, provider, pharmacy costs	Economic	Duration of trial	
Micro Costing Study	Clinical procedure costs	Economic	Duration of trial	

References

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2. Phillip L. Ross, e. a. (2003). A Paper-based Measure of Standard Gamble Utility for Current Health. *International Journal of Technology Assessment in Health Care, 19*(1), 135-147.

3. AHRQ. (2005, December). *U.S. Valuation of the EuroQol EQ-5D Health States.* Retrieved October 20, 2010, from Agency for Healthcare Research and Quality: http://www.ahrq.gov/rice/EQ5Dproj.htm

4. S.C. Fagan, e. (1998, April). Cost-effectiveness of tissue plasminogen activator for acute ischemic strok. *Neurology, 50*, 883-890.

5. Shanon E. Sinclair, e. a. (2001). Cost-Utility Analysis of Tissue Plasminogen Activator Therapy for Acute Ischaemic Stroke, A Canadian Healthcare Perspective. *Pharmaeconomics, 19*(9), 927-936.

Appendix A. Paper Standard Gamble

PSG Form **Page 1 of 1**

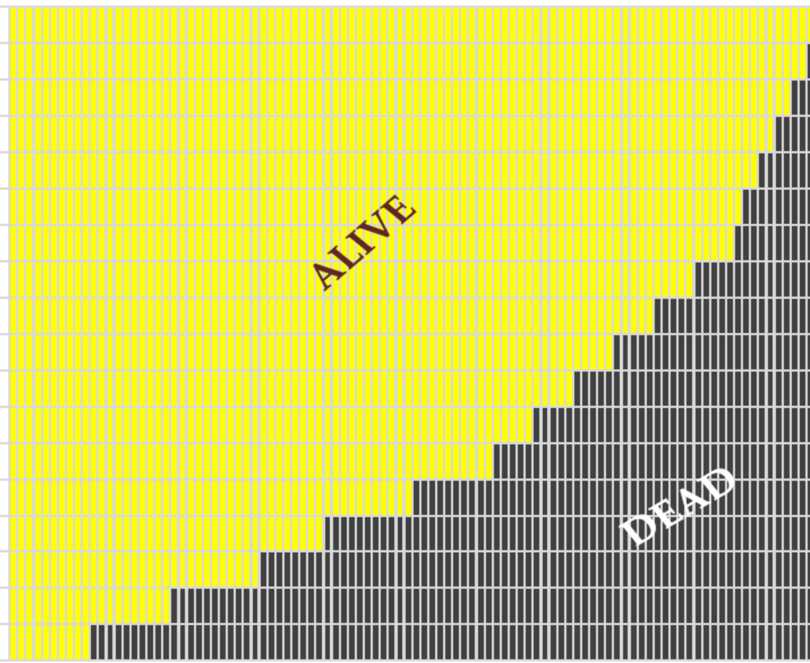
Health State Selection: Self-Completion Questionnaire

Patient ID#: ________________ **Date:** ________________

Imagine a new (make-believe) treatment is now available for ***all*** your ***current*** health problems. Your doctor advises you that if you undergo the treatment today and it works, it cures every health problem you ***currently*** have for the rest of your life. However, if you have the treatment today and it ***does not*** work, it causes a sudden and painless death in your sleep tonight. Your doctor has no way of predicting which patients will be cured by this new (make-believe) treatment, and will support whatever decision you make. Given everything you know about your current health, how it may change in the future, and your treatment options, we want to know what you think about this treatment.

→ ***Would you undergo the treatment right now if you knew that…*** *(Please circle "Yes" or "No" for every question.)*

out of 100 patients, 100 are cured and none die in their sleep?	Yes	No
out of 100 patients, 99 are cured and 1 dies in his sleep?	Yes	No
out of 100 patients, 97 are cured and 3 die in their sleep?	Yes	No
out of 100 patients, 95 are cured and 5 die in their sleep?	Yes	No
out of 100 patients, 93 are cured and 7 die in their sleep?	Yes	No
out of 100 patients, 91 are cured and 9 die in their sleep?	Yes	No
out of 100 patients, 90 are cured and 10 die in their sleep?	Yes	No
out of 100 patients, 85 are cured and 15 die in their sleep?	Yes	No
out of 100 patients, 80 are cured and 20 die in their sleep?	Yes	No
out of 100 patients, 75 are cured and 25 die in their sleep?	Yes	No
out of 100 patients, 70 are cured and 30 die in their sleep?	Yes	No
out of 100 patients, 65 are cured and 35 die in their sleep?	Yes	No
out of 100 patients, 60 are cured and 40 die in their sleep?	Yes	No
out of 100 patients, 50 are cured and 50 die in their sleep?	Yes	No
out of 100 patients, 40 are cured and 60 die in their sleep?	Yes	No
out of 100 patients, 30 are cured and 70 die in their sleep?	Yes	No
out of 100 patients, 20 are cured and 80 die in their sleep?	Yes	No
out of 100 patients, 10 are cured and 90 die in their sleep?	Yes	No



Form

Appendix B. EQ-5D Forms

EQ-5D Form **Page # 1**

Health Assessment Self-Completion Questionnaire

Patient ID#: _____ **Date:** _____

Place a checkmark in ONE box for each group below, indicating which statements best describes your own health state today.

Mobility:

- □ I have no problems in walking about
- □ I have some problems in walking about
- □ I am confined to bed

Self-Care:

- □ I have no problems with self-care
- □ I have some problems washing or dressing myself
- □ I am unable to wash or dress myself

Usual Activities (e.g., work, study, housework, family, or leisure activities):

- □ I have no problems with performing my usual activities
- □ I have some problems with performing my usual activities
- □ I am unable to perform my usual activities

Pain/Discomfort:

- □ I have no pain or discomfort
- □ I have moderate pain or discomfort
- □ I have extreme pain or discomfort

Anxiety/Depression:

- □ I am not anxious or depressed
- □ I am moderately anxious or depressed
- □ I am extremely anxious or depressed

Own Health Scale

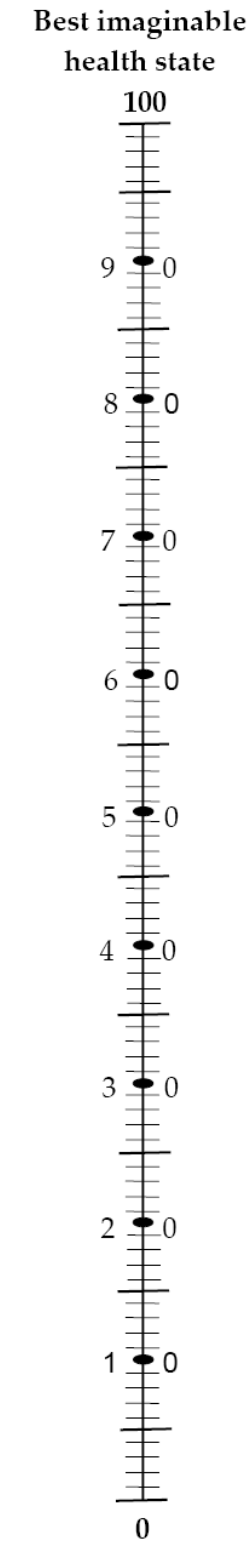
Patient ID#: _____

Date: _____

To help people say how good or bad a health state is, we have drawn a scale (rather like a thermometer) on which the best state you can imagine is marked 100 and the worst state you can imagine is marked 0.

We would like you to indicate on this scale how good or bad your own health is today, in your opinion. Please do this by drawing a line from the box below to whichever point on the scale indicates how good or bad your health state is today.

Your own health state today



Appendix C. Resource Utilization Forms

Patient # _ _ - _ _ _ Patient Initials _ _ _ **Date**: (mm) __ __/ (dd) __ __/ (yyyy) __ __ __ __

DEMOGRAPHIC INFORMATION

Sex (M/F): ___ DOB (mm) __ __/ (dd) __ __/ (yyyy) __ __ __ __

Race: ☐ White ☐ African American ☐ Asian ☐ Hispanic/Latino
☐ Other (specify) _____

Marital Status: ☐ Single ☐ Married ☐ Separated ☐ Divorced ☐ Widowed

Zip Code: _ _ _ _ _

Education: Highest degree (select one):
☐ GED/High School diploma ☐ Some College
☐ College Degree ☐ Graduate School

Employment Status (select one):

☐ Full time ☐ Part time ☐ Unemployed ☐ Disabled
☐ Housewife ☐ Retired ☐ Student

Your current job title: _____

Your estimated annual income:
☐ less than $10,000 ☐ $10-25,000 ☐ $25-50,000 ☐ $50-75,000 ☐ $75-100,000
☐ over $100,000

Medical Insurance:

☐ State/local government program
☐ Commercial HMO
☐ Commercial PPO
☐ Medicare
☐ Medicare managed care
☐ Medicaid
☐ Medicaid managed care
☐ Self pay
☐ Other (specify) _____

☐ None

HEALTH CARE USE

1. Over the past **90 days**, have you received any of the following therapies or were admitted to a special care facility **because of your stroke condition?** If yes, please indicate the number of days.

a. Received **inpatient** rehabilitation?

☐ No ☐ Yes If yes, how many days? _____

Name of hospital? _____

b. Received **in-home** physical therapy or occupational therapy?

☐ No ☐ Yes If yes, how many days? _____

c. Received **outpatient** physical therapy or occupational therapy?

☐ No ☐ Yes If yes, how many days? _____

Name of clinic or Hospital? _____

d. Were admitted to a **Skilled Nursing Facility**?

☐ No ☐ Yes If yes, how many days? _____

Name of Facility? _____

e. Were admitted to a **Nursing Home**?

☐ No ☐ Yes If yes, how many days? _____

Name of Facility? _____

f. Were admitted to a **Long-term Acute Care Hospital**?

☐ No ☐ Yes If yes, how many days? _____

Name of Facility? _____

2. Over the past **90 days and after discharge**, have you done any of the following **because of your stroke condition**? If yes, please indicate the number of times.

a. Been to the hospital **emergency department**?

☐ No ☐ Yes If yes, how many times? _____

Name(s) of hospital (s)? _____

b. Been seen by a **specialist doctor** (cardiologist, neurologist, etc.)?

☐ No ☐ Yes If yes, how many times? _____

Name of Doctor(s)? _____

c. Been seen by your **family doctor**?

☐ No ☐ Yes If yes, how many times? _____

d. Been admitted to the **hospital overnight** as an inpatient?

☐ No ☐ Yes If yes, how many times? _____

1st time: Hospital Name _____ Start date: _____

Estimated # of Days as an Inpatient? _____ days

2nd time: Hospital Name _____ Start date: _____

Estimated # of Days as an Inpatient? _____ days

e. Used **ambulance services** or **EMS** on any of the occasions you have told us about above?

☐ No ☐ Yes If yes, how many times? _____

3. Over the past **90 days and after discharge**, have you had any of the tests or investigations listed below **because of your stroke condition**? If yes, please tell us the number of times.

a. Blood work (cholesterol, INR adjusted, etc.) ☐ No ☐ Yes If yes, how many times? _____

b. Cardiac Catheterization ☐ No ☐ Yes If yes, how many times? _____

b.1. Angioplasty and Stent Insertion ☐ No ☐ Yes If yes, how many times? _____

c. Ultrasound of heart (echocardiogram) ☐ No ☐ Yes If yes, how many times? _____

d. CAT scan of the brain ☐ No ☐ Yes If yes, how many times? _____

e. CT Angiogram (injection of dye during CT) ☐ No ☐ Yes If yes, how many times? _____

f. Ultrasound of the neck (carotid ultrasound) ☐ No ☐ Yes If yes, how many times? _____

g. Magnetic Resonance Imaging of head (MRI) ☐ No ☐ Yes If yes, how many times? _____

h. Electrocardiogram (EKG) ☐ No ☐ Yes If yes, how many times? _____

i. Electroencephalogram (EEG) ☐ No ☐ Yes If yes, how many times? _____

4. Over the past **90 days and after discharge**, have you seen any of the following **people because of your stroke condition**? If yes, please tell us the number of times.

a. A home healthcare nurse? ☐ No ☐ Yes If yes, how many times? _____

b. A social worker? ☐ No ☐ Yes If yes, how many times? _____

c. A psychologist? ☐ No ☐ Yes If yes, how many times? _____

5. Over the past **90 days and after discharge**, did you have any other extra expenses for equipment that you needed **because of your stroke condition**? If yes, please tell us the amount you spent.

a. Wheel chair	☐ No	☐ Yes	If yes, how much did you spend?	_____
b. Shower chair	☐ No	☐ Yes	If yes, how much did you spend?	_____
c. Walking aids	☐ No	☐ Yes	If yes, how much did you spend?	_____
d. Hospital bed	☐ No	☐ Yes	If yes, how much did you spend?	_____
e. Pumps	☐ No	☐ Yes	If yes, how much did you spend?	_____
f. Other	☐ No	☐ Yes	If yes, how much did you spend?	_____

6. Over the past **90 days and after discharge**, did you have any other additional expenses on your house **because of your stroke condition** (add a ramp, widened a door, etc.)?

☐ No ☐ Yes

If yes, please tell us the reason and amount spent on each item:
Reason_____
Amount spent:_____

7. Over the past **90 days and after discharge**, were you prescribed any medicines in addition to those you were prescribed at discharge?

☐ No ☐ Yes

If yes, please list all the names of additional drugs and doses you were prescribed after discharge:

_____
_____
_____

8. Over the past **90 days and after discharge**, what kind of transportation have you been using to make your medical appointments?

☐ Family member takes me ☐ Metro
☐ Ambulance ☐ I drive myself
☐ Other (specify) _____

9. How many days over the last **90 days have you missed work** due to physician appointments, hospitalizations, or anything else **related to your stroke condition**? _____ days

Appendix D. Patient Case Report

Patient Case Report – Health Quality and Economics

Argatroban TPA Stroke Study-2/ Health Related Quality of Life after Ischemic Stroke Study

Patient Study ID: _____

Study Group/ ID: _____

Site Location/ ID: _____

Is the patient insured? Yes ☐ No ☐ Insurer: _____

Patient Length of Stay in days: _____

Total Gross/Billed Charges - Initial Stay _____ (Reported in $USD ☐ or £ ☐)

Total Costs – Initial Stay

Principal Diagnosis Code: _____

Costs: Reported in $USD ☐ or £ ☐

Emergency _____
Physician _____
Nursing _____
Pharmacy _____
Hospital/Lab/Diagnostic (CT, MRI) _____
Total _____

Follow-Up Visits

Total # of visits in 90-day period _____

Costs: Reported in $USD ☐ or £ ☐

Emergency _____
Physician _____
Nursing _____
Pharmacy _____
Hospital/Lab/Diagnostic (CT, MRI) _____
Total _____

Readmission

Patient readmitted for neurology related cases? Yes ☐ No ☐

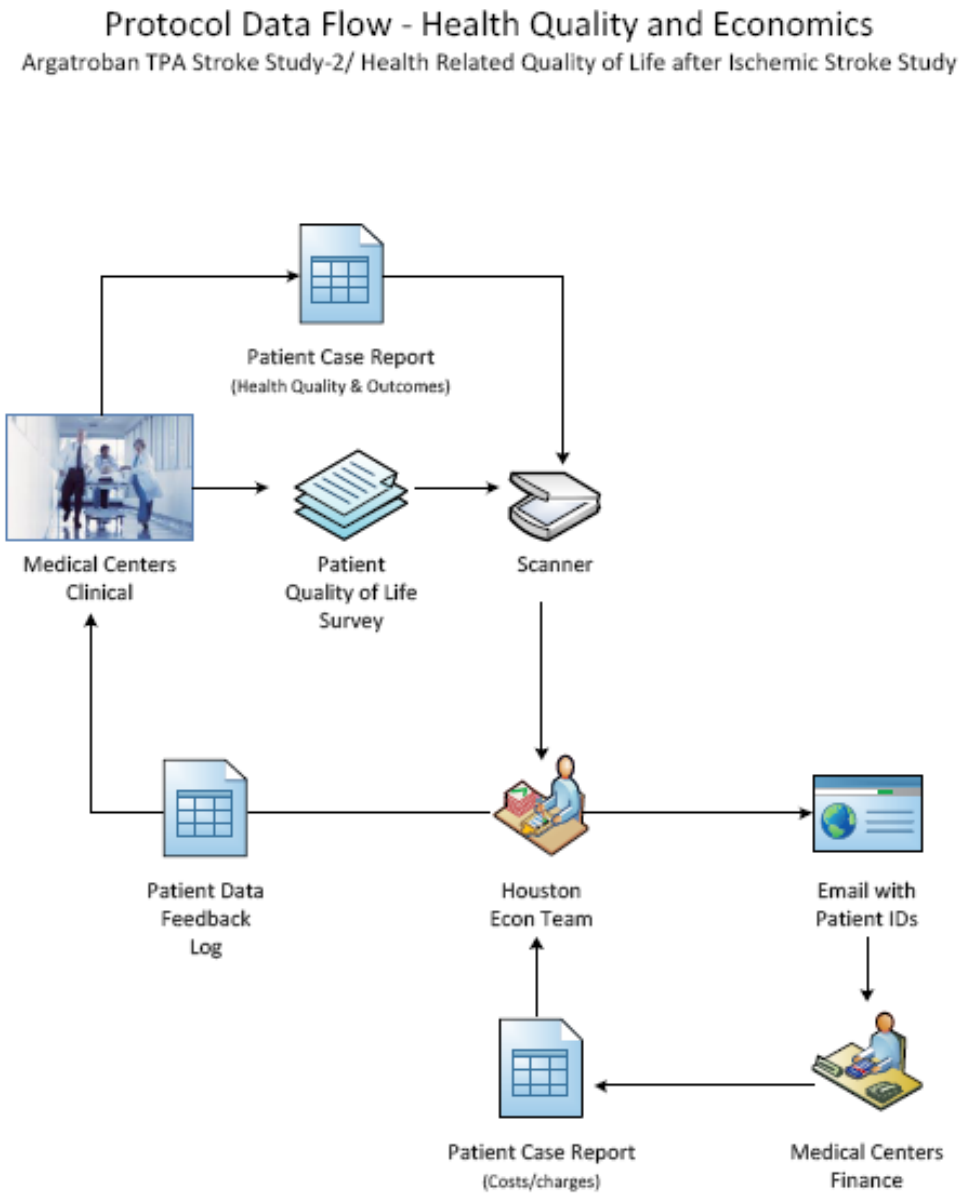
# of times readmitted _____

Readmission	1st	2nd	3rd	4th
Principal Diagnosis Code:				
Total costs of readmissions				

Health Quality & Outcomes (To be obtained from Coordinating Center Data-core database)

Modified Rankin Scale
NIH Stroke Score
Death, if it happens before study ends: Before discharge ☐ or Days post-discharge _____
Other Health Outcomes (e.g., MoCA)
Patient ultimate Disposition post-admission
Other

Appendix E. Mechanism for integrating QALY and cost data from all clinical sites.

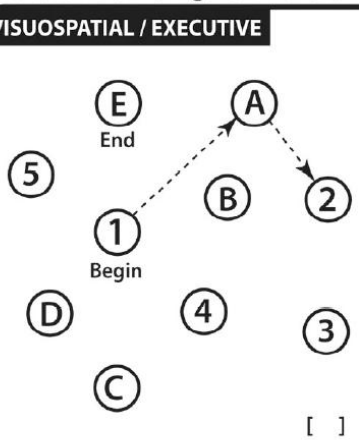


Appendix F. Montreal Cognitive Assessment form and instructions.

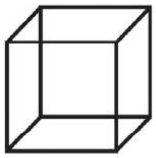
MONTREAL COGNITIVE ASSESSMENT (MOCA)
Version 7.1 Original Version

NAME :
Education : Date of birth :
Sex : DATE :

VISUOSPATIAL / EXECUTIVE — POINTS



Copy cube

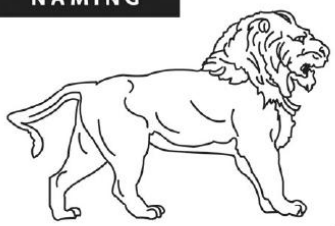


[ ] [ ]

Draw CLOCK (Ten past eleven) (3 points)

[ ] Contour [ ] Numbers [ ] Hands __/5

NAMING



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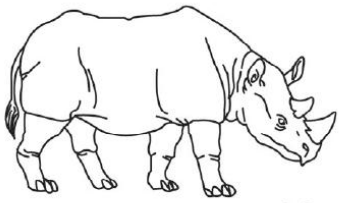

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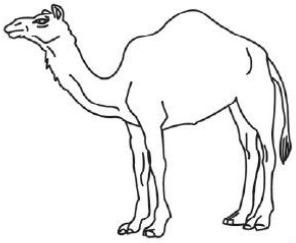


[ ] __/3

MEMORY Read list of words, subject must repeat them. Do 2 trials, even if 1st trial is successful. Do a recall after 5 minutes.

	FACE	VELVET	CHURCH	DAISY	RED
1st trial					
2nd trial					

No points

ATTENTION Read list of digits (1 digit/ sec.).
Subject has to repeat them in the forward order [ ] 2 1 8 5 4
Subject has to repeat them in the backward order [ ] 7 4 2 __/2

Read list of letters. The subject must tap with his hand at each letter A. No points if ≥ 2 errors
[ ] F B A C M N A A J K L B A F A K D E A A A J A M O F A A B __/1

Serial 7 subtraction starting at 100 [ ] 93 [ ] 86 [ ] 79 [ ] 72 [ ] 65
4 or 5 correct subtractions: **3 pts**, 2 or 3 correct: **2 pts**, 1 correct: **1 pt**, 0 correct: **0 pt** __/3

LANGUAGE Repeat : I only know that John is the one to help today. [ ]
The cat always hid under the couch when dogs were in the room. [ ] __/2

Fluency / Name maximum number of words in one minute that begin with the letter F [ ] _____ (N ≥ 11 words) __/1

ABSTRACTION Similarity between e.g. banana - orange = fruit [ ] train – bicycle [ ] watch - ruler __/2

DELAYED RECALL — **Optional**

	FACE	VELVET	CHURCH	DAISY	RED	
Has to recall words WITH NO CUE	[ ]	[ ]	[ ]	[ ]	[ ]	Points for UNCUED recall only
Category cue						
Multiple choice cue						

__/5

ORIENTATION [ ] Date [ ] Month [ ] Year [ ] Day [ ] Place [ ] City __/6

© Z.Nasreddine MD **www.mocatest.org** Normal ≥ 26 / 30

TOTAL __/30
Add 1 point if ≤ 12 yr edu

Administered by: _____

Montreal Cognitive Assessment (MoCA)

Administration and Scoring Instructions

The Montreal Cognitive Assessment (MoCA) was designed as a rapid screening instrument for mild cognitive dysfunction. It assesses different cognitive domains: attention and concentration, executive functions, memory, language, visuoconstructional skills, conceptual thinking, calculations, and orientation. Time to administer the MoCA is approximately 10 minutes. The total possible score is 30 points; a score of 26 or above is considered normal.

1. Alternating Trail Making:

Administration: The examiner instructs the subject: *"Please draw a line, going from a number to a letter in ascending order. Begin here* [point to (1)] *and draw a line from 1 then to A then to 2 and so on. End here* [point to (E)]."

Scoring: Allocate one point if the subject successfully draws the following pattern: 1 –A- 2- B- 3- C- 4- D- 5- E, without drawing any lines that cross. Any error that is not immediately self-corrected earns a score of 0.

2. Visuoconstructional Skills (Cube):

Administration: The examiner gives the following instructions, pointing to the **cube**: "*Copy this drawing as accurately as you can, in the space below"*.

Scoring: One point is allocated for a correctly executed drawing.
- Drawing must be three-dimensional
- All lines are drawn
- No line is added
- Lines are relatively parallel and their length is similar (rectangular prisms are accepted)

A point is not assigned if any of the above-criteria are not met.

3. Visuoconstructional Skills (Clock):

Administration: Indicate the right third of the space and give the following instructions: *"Draw a **clock**. Put in all the numbers and set the time to 10 past 11".*

Scoring: One point is allocated for each of the following three criteria:
- Contour (1 pt.): the clock face must be a circle with only minor distortion acceptable (e.g., slight imperfection on closing the circle);
- Numbers (1 pt.): all clock numbers must be present with no additional numbers; numbers must be in the correct order and placed in the approximate quadrants on the clock face; Roman numerals are acceptable; numbers can be placed outside the circle contour;

• Hands (1 pt.): there must be two hands jointly indicating the correct time; the hour hand must be clearly shorter than the minute hand; hands must be centred within the clock face with their junction close to the clock centre.

A point is not assigned for a given element if any of the above-criteria are not met.

4. Naming:

Administration: Beginning on the left, point to each figure and say: *"Tell me the name of this animal".*

Scoring: One point each is given for the following responses: (1) lion (2) rhinoceros or rhino (3) camel or dromedary.

5. Memory:

Administration: The examiner reads a list of 5 words at a rate of one per second, giving the following instructions*: "This is a memory test. I am going to read a list of words that you will have to remember now and later on. Listen carefully. When I am through, tell me as many words as you can remember. It doesn't matter in what order you say them"*.
Mark a check in the allocated space for each word the subject produces on this first trial. When the subject indicates that (s)he has finished (has recalled all words), or can recall no more words, read the list a second time with the following instructions: *"I am going to read the same list for a second time. Try to remember and tell me as many words as you can, including words you said the first time."* Put a check in the allocated space for each word the subject recalls after the second trial.
At the end of the second trial, inform the subject that (s)he will be asked to recall these words again by saying, *"I will ask you to recall those words again at the end of the test."*

Scoring: No points are given for Trials One and Two.

6. Attention:

Forward Digit Span: Administration: Give the following instruction: "*I am going to say some numbers and when I am through, repeat them to me exactly as I said them*". Read the five number sequence at a rate of one digit per second.

Backward Digit Span: Administration: Give the following instruction: "*Now I am going to say some more numbers, but when I am through you must repeat them to me in the backwards order.*" Read the three number sequence at a rate of one digit per second.

Scoring: Allocate one point for each sequence correctly repeated, (*N.B.*: the correct response for the backwards trial is 2-4-7).

Vigilance: Administration: The examiner reads the list of letters at a rate of one per second, after giving the following instruction: "*I am going to read a sequence of letters. Every time I say the letter A, tap your hand once. If I say a different letter, do not tap your hand*".

Scoring: Give one point if there is zero to one errors (an error is a tap on a wrong letter or a failure to tap on letter A).

Serial 7s: Administration: The examiner gives the following instruction: *"Now, I will ask you to count by subtracting seven from 100, and then, keep subtracting seven from your answer until I tell you to stop."* Give this instruction twice if necessary.

Scoring: This item is scored out of 3 points. Give no (0) points for no correct subtractions, 1 point for one correction subtraction, 2 points for two-to-three correct subtractions, and 3 points if the participant successfully makes four or five correct subtractions. Count each correct subtraction of 7 beginning at 100. Each subtraction is evaluated independently; that is, if the participant responds with an incorrect number but continues to correctly subtract 7 from it, give a point for each correct subtraction. For example, a participant may respond "92 – 85 – 78 – 71 – 64" where the "92" is incorrect, but all subsequent numbers are subtracted correctly. This is one error and the item would be given a score of 3.

7. Sentence repetition:

Administration: The examiner gives the following instructions: *"I am going to read you a sentence. Repeat it after me, exactly as I say it* [pause]: ***I only know that John is the one to help today."*** Following the response, say: *"Now I am going to read you another sentence. Repeat it after me, exactly as I say it* [pause]: ***The cat always hid under the couch when dogs were in the room."***

Scoring: Allocate 1 point for each sentence correctly repeated. Repetition must be exact. Be alert for errors that are omissions (e.g., omitting "only", "always") and substitutions/additions (e.g., "John is the one who helped today;" substituting "hides" for "hid", altering plurals, etc.).

8. Verbal fluency:

Administration: The examiner gives the following instruction: *"Tell me as many words as you can think of that begin with a certain letter of the alphabet that I will tell you in a moment. You can say any kind of word you want, except for proper nouns (like Bob or Boston), numbers, or words that begin with the same sound but have a different suffix, for example, love, lover, loving. I will tell you to stop after one minute. Are you ready?* [Pause] *Now, tell me as many words as you can think of that begin with the letter F.* [time for 60 sec]. *Stop."*

Scoring: Allocate one point if the subject generates 11 words or more in 60 sec. Record the subject's response in the bottom or side margins.

9. Abstraction:

Administration: The examiner asks the subject to explain what each pair of words has in common, starting with the example: *"Tell me how an orange and a banana are alike".* If the subject answers in a concrete manner, then say only one additional time: *"Tell me another way*

in which those items are alike". If the subject does not give the appropriate response *(fruit)*, say, *"Yes, and they are also both fruit."* Do not give any additional instructions or clarification. After the practice trial, say: *"Now, tell me how a train and a bicycle are alike".* Following the response, administer the second trial, saying: *"Now tell me how a ruler and a watch are alike"*. Do not give any additional instructions or prompts.

Scoring: Only the last two item pairs are scored. Give 1 point to each item pair correctly answered. The following responses are acceptable:
Train-bicycle = means of transportation, means of travelling, you take trips in both;
Ruler-watch = measuring instruments, used to measure.
The following responses are **not** acceptable: Train-bicycle = they have wheels; Rulerwatch = they have numbers.

10. Delayed recall:

Administration: The examiner gives the following instruction: *"I read some words to you earlier, which I asked you to remember. Tell me as many of those words as you can remember."* Make a check mark (√) for each of the words correctly recalled spontaneously without any cues, in the allocated space.

Scoring: **Allocate 1 point for each word recalled freely without any cues.**

Optional:
Following the delayed free recall trial, prompt the subject with the semantic category cue provided below for any word not recalled. Make a check mark (√) in the allocated space if the subject remembered the word with the help of a category or multiple-choice cue. Prompt all non-recalled words in this manner. If the subject does not recall the word after the category cue, give him/her a multiple choice trial, using the following example instruction, *"Which of the following words do you think it was, NOSE, FACE, or HAND?"*
Use the following category and/or multiple-choice cues for each word, when appropriate:

FACE:	category cue: part of the body	multiple choice: nose, face, hand
VELVET:	category cue: type of fabric	multiple choice: denim, cotton, velvet
CHURCH:	category cue: type of building	multiple choice: church, school, hospital
DAISY:	category cue: type of flower	multiple choice: rose, daisy, tulip
RED:	category cue: a colour	multiple choice: red, blue, green

Scoring: **No points are allocated for words recalled with a cue.** A cue is used for clinical information purposes only and can give the test interpreter additional information about the type of memory disorder. For memory deficits due to retrieval failures, performance can be improved with a cue. For memory deficits due to encoding failures, performance does not improve with a cue.

11. Orientation:

Administration: The examiner gives the following instructions: “Tell me the date today”. If the subject does not give a complete answer, then prompt accordingly by saying: *“Tell me the [year, month, exact date, and day of the week].”* Then say: *“Now, tell me the name of this place, and which city it is in.”*

Scoring: Give one point for each item correctly answered. The subject must tell the exact date and the exact place (name of hospital, clinic, office). No points are allocated if subject makes an error of one day for the day and date.

TOTAL SCORE: Sum all subscores listed on the right-hand side. Add one point for an individual who has 12 years or fewer of formal education, for a possible maximum of 30 points. A final total score of 26 and above is considered normal.

Appendix G. Assessment of Capacity Evaluation (ACE) Questionnaire

1. Medical Condition:

QUESTION	RESPONSE	SCORE
What medical condition led to your admission to the hospital?	I had a stroke. I had a blood clot in the head. I'm weak/lost sensation/etc (stroke symptoms) on the right/left (body location) side of my body.	**YES**
What medical condition led to your admission to the hospital? Q:What is/was your most serious medical problem during your hospital stay?	I don't know. I had a stroke. I had a blood clot in the head. I'm weak/lost sensation/etc (stroke symptoms) on the right/left (body location) side of my body.	**UNSURE**
What medical condition led to your admission to the hospital? Q: Did you have stroke? Why are/were you in the hospital?	I don't know. No, I don't know.	**NO**

***If the patient scores a No to question #1, stop this exam and proceed with remainder of evaluation using the patient's proxy.**

2. Proposed Treatment:
Say this: "For the next few questions, please answer using the scenario we are going to tell you. Here is the scenario…"

Imagine you have suffered a second stroke that is disabling [you are paralyzed] and you are in the emergency room. The doctors offer you an optional new clotbuster medicine that gives you a good chance of being cured, but with a small risk of bleeding in the brain that could make you worse. If you don't want the clotbuster, you could receive standard treatment – aspirin, but your disability would probably stay the same. Of course, you have the right to refuse any treatment.

QUESTION	RESPONSE	SCORE
What is the treatment that is offered?	Clotbuster for my stroke.	**YES**
What is the treatment that is offered? Can you have a new clotbuster medicine?	I don't know. For my stroke. You tell me. Yes	**UNSURE**
What is the treatment? Can you have a new clotbuster medicine?	I don't know. I don't know/no.	**NO**

3. Alternatives:

QUESTION	RESPONSE	SCORE
Are there any other treatments? [from the scenario]	Yes, aspirin/standard treatment/care.	**YES**
Are there any other treatments? Can you take aspirin?	I don't know. Yes.	**UNSURE**
Are there any other treatments? Can you take aspirin?	I don't know. I don't know/no.	**NO**

4. Option of Refusing Proposed Treatment (including withholding or withdrawing proposed treatment):

QUESTION	RESPONSE	SCORE
Who decides your treatment?	I do.	**YES**
Who decides your treatment? Can you refuse the new clotbuster?	I don't know. Yes	**UNSURE**
Can you refuse the new clotbuster?	I don't know/No.	**NO**

5. Consequences of Accepting Proposed Treatment:

QUESTION	RESPONSE	SCORE
What could happen to you if you **take** the new clotbuster medicine?	I could get better. I could be cured. I could bleed. I could get worse.	**YES**
What could happen to you if you **take** the new clotbuster medicine? Could the clotbuster make you better? Could it also cause some bleeding?	I don't know. Yes.	**UNSURE**
What could happen to you if you **take** the new clotbuster medicine? Could the clotbuster medicine make you better? Could it also cause some bleeding?	I don't know. I don't know/no	**NO**

6. Consequences of Refusing Proposed Treatment:

QUESTION	RESPONSE	SCORE
What could happen to you if you **don't take** the new clotbuster medicine?	My stroke/symptoms/disability would stay the same.	**YES**
What could happen to you if you **don't take** the new clotbuster medicine? Would your stroke symptoms stay the same?	I could take aspirin. I don't know. Yes / probably.	**UNSURE**
What if you **don't take** the new clotbuster medicine? Would your stroke symptoms stay the same?	I don't know / nothing. I don't know. I could get worse. [*Try rediscussing consequences and repeat the questions. If no better answer, score no.] For example: *"Remember, if you don't want the clotbuster medicine, you could take aspirin and your stroke symptoms would probably stay the same."*	**NO**

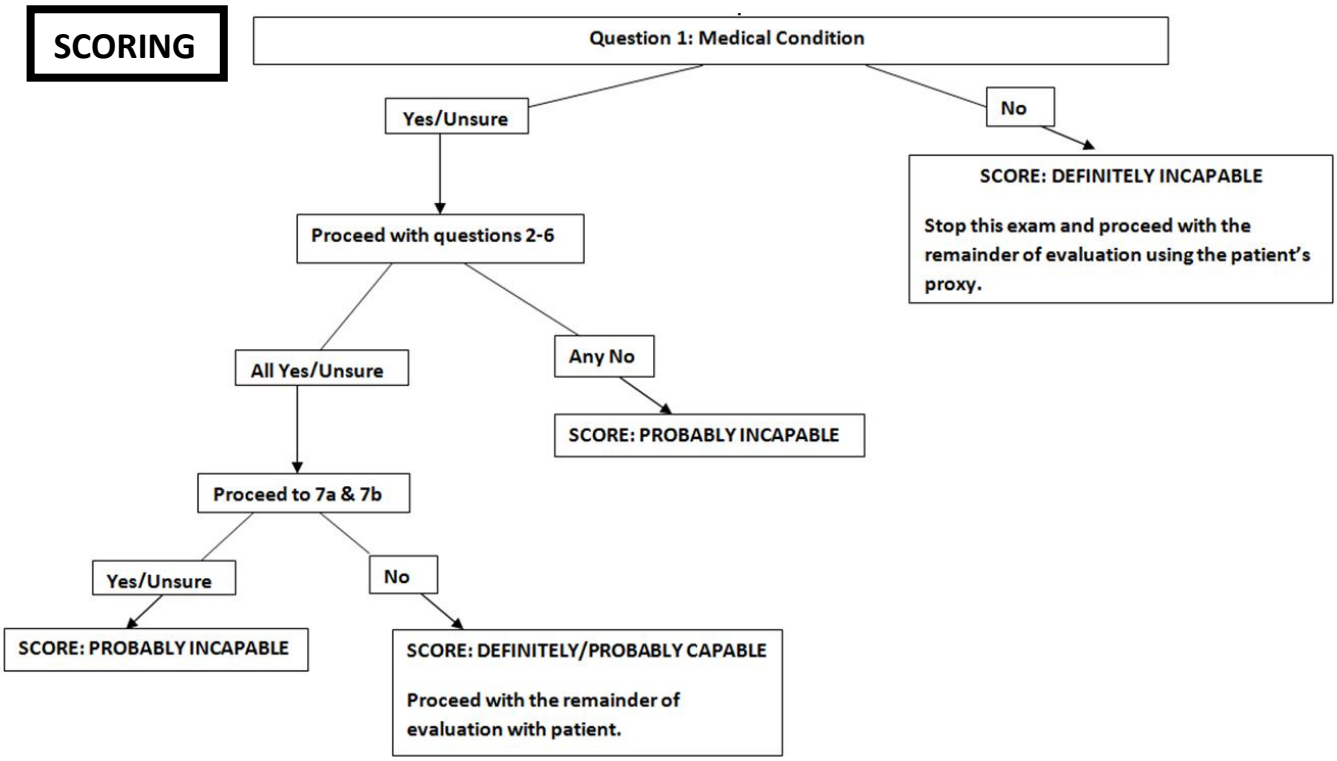
7a. The Person's decision is affected by Depression:

- Why would you **not want to have** the new clotbuster medicine?

I'm a bad person. I've had a bad life. I deserve to die. I'm being punished. I'm not worth it.	**YES** [definitely depressed]
Nothing seems to work. I have no hope. I'm very sad. I'm all alone. I've suffered too much.	**UNSURE** [possibly depressed]
I've lived a full and complete long life. I don't want to have any bleeding in my brain. I'm not sure it would work. [*Needs further discussion of the **good** chance at a cure versus a **small** risk of brain bleeding].	**NO** [not depressed]

7b. The Person's Decision is affected by Delusions / Psychosis:

QUESTION	RESPONSE	SCORE
Do you think that the doctors offering the new clotbuster medicine are trying to hurt or harm you?	You are vampire or any bizarre response.	**YES** [definitely delusional]
Do you think that the doctors offering the new clotbuster medicine are trying to hurt or harm you?	Yes. You are trying to kill me. You want me to bleed and die.	**UNSURE** [possibly delusional]
Do you think that the doctors offering the new clotbuster medicine are trying to hurt or harm you?	No.	**NO** [not delusional]



Impression:

Definitely Capable	☐	**PASS: Proceed with *Patient***
Probably Capable	☐	
Probably Incapable	☐	**FAIL: Proceed with *Proxy***
Definitely Incapable	☐	