Protocol #: 16-0391
Project Title: STAT (STatins and Aspirin in Trauma) Trial: A Phase II, pragmatic, prospective, randomized, double-blind, adaptive clinical trial examining the efficacy of statins and aspirin in the reduction of acute lung injury and venous thromboembolism in patients with fibrinolysis shutdown.

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I. Hypothesis and Specific Aims:

Patients sustaining life-threatening injuries are at high risk for thrombotic events both at the macro level such as venous thromboembolic (VTE) events as well as at the microvascular level leading to pulmonary dysfunction (acute lung injury or ALI and adult respiratory distress syndrome or ARDS).[1, 2] These complications have been traditionally attributed to an unbalance in injury induced inflammatory processes but recent investigations have suggested that hypercoagulation is a contributing mechanism. [3] Emerging evidence has indicated that, in contrast to rapid clot formation, early post-injury resistance to clot degradation (resistance to fibrinolysis or fibrinolysis shutdown) may play a significant mechanistic role in trauma-induced coagulopathy (TIC) and its sequelae.[4, 5]

A recent pilot study in cardiac surgery patients suggested that while coagulation activity was increased and more fibrin was formed immediately postoperatively in patients who subsequently developed ALI, there was insufficient fibrinolytic activity to remove the increased amount of fibrin.[6] Fibrin degradation is modulated by plasminogen and plasminogen-activator-inhibitor-1 (PAI-1). [3] PAI-1 is the major inhibitor of two plasminogen activators, namely, urokinase-type plasminogen activator (u-PA), which activates fibrinolysis at the tissue level, and tissue-type plasminogen activator (t-PA), which activates intravascular fibrinolysis. [7]

Trauma and surgery patients do present increased levels of PAI-1, which correlated with the subsequent development of organ failure, such as ALI and ARDS. [2] Platelets play a role in both VTE and lung injury, thus anti-platelet agents (such as aspirin) have been increasingly used in populations at-risk for thrombotic complications, including trauma patients. [1, 3, 8, 9]

Statins and aspirin have been proposed and studied as potential therapeutic agents that can block the above described prothrombotic mechanisms.[3, 10, 11] Current guidelines for cardiac surgery patients recommend the use of aspirin and statins as safe and effective agents to reduce thrombotic complications. [12]

The comparison between cardiac surgery patients and trauma patients is pertinent, as major surgical procedures are often labeled as “schedule trauma” or “controlled injury” (see NIGMS PAR at http://grants.nih.gov/grants/guide/pa-files/PAR-13-291.html and Silliman et al. [13]). Specifically in healthy individuals (as the majority of trauma patients), the large JUPITER trial has convincingly demonstrated a reduction in the incidence of VTEs with the use of statins (rosuvastatin). [14]

Currently, there are no pharmacologic interventions in use in the intensive care unit (ICU) setting to treat resistance to fibrinolysis (shutdown). Moreover, current interventions have not been successful in further reducing thrombotic complications such as VTE and lung injury in trauma and surgical patients. [1, 15]

Therefore, we hypothesize that rosuvastatin and aspirin (ASA), two FDA-approved medications, will reduce the development of venous thromboembolism by modifying both the platelet- and endothelial-derived fibrinolytic regulators contributing to fibrinolysis shutdown.

The primary objective of this study is to evaluate the efficacy of combination of rosuvastatin and aspirin in the reduction of the incidence of VTE (i.e. pulmonary embolism and deep venous thrombosis) in critically ill trauma patients.

As a secondary objective, we propose to evaluate the efficacy of rosuvastatin and aspirin in the incidence of ALI (as measured by the Berlin criteria[16]) and on ventilator days (as measured by ventilator-free days) in critically ill trauma patients. Another secondary objective is to evaluate the efficacy of rosuvastatin and aspirin on reducing other adverse outcomes in critically ill trauma patients (30-day mortality, ICU days, and arterial thrombosis such as myocardial infarction, MI and stroke). In addition, we will further explore the mechanisms through which these agents influence the coagulation cascade in patients at-risk for thrombotic complications (fibrinolysis phenotypes incidence, systemic PAI-1 and tPA levels).

This protocol describes a Phase II, pragmatic, prospective, randomized, double-blind, adaptive clinical trial comparing rosuvastatin and aspirin (in combination) to placebo.

II. Background and Significance:

Trauma is the leading cause of death for individuals in the first four decades of life in the United States.[17] The majority of these deaths occur within just a few hours following injury.[18] Patients surviving the initial insult remain at risk of death due to either macro-vascular (VTE) or micro-vascular (ALI, ARDS) thrombotic complications.[18] Recent investigations into post-trauma care have focused on the reduction of the hypercoagulability component of trauma-induced coagulopathy.[19] This work has concentrated on understanding the mechanisms behind large vein thrombosis in an effort to reduce the risk of VTE events. Geerts et al. studied 350 critically injured patients and found that greater than 50% had evidence of DVT based upon venography screening.[20] Based upon these background data, they conducted a randomized study comparing unfractionated versus fractionated heparin as prophylaxis against DVT. They found that heparin decreased the incidence by 19%, while low molecular weight heparin (LMWH) therapy decreased the incidence by 43%.[21] Other groups, including ours, have shown that less rigorous screening and use of LMWH can reduce the incidence of venous thrombi to rates ranging from 2.5-9%.[22-24] Despite this and other extensive work, pulmonary embolism (PE) remains one of the most common causes of post-injury mortality and has been associated with up to 12% of late trauma-related deaths.[25] Furthermore, these deaths continue to occur in the setting of pharmacological and mechanical prophylactic therapies, the current standard of VTE-prevention care.[26]

Postinjury micro-thrombosis may manifest as distant organ dysfunction or postinjury multiple organ failure, including ALI and ARDS.[27, 28] The pulmonary system is nearly always the first to exhibit dysfunction.[29] Overall, these patients spend an average of 20 days on the ventilator with an average hospital-stay greater than 30 days.[30] The mechanism of post-trauma ALI remains unclear and is most likely multifactorial. However, trauma-induced coagulopathy (TIC) has been suggested to play a significant role in the development of secondary pulmonary pathology.[31]

The first investigations linking coagulation dysfunction and ALI were performed by Hardaway, et al. in the 1960s and 1970s.[32-34] This group found that hypercoagulability and resistance to clot breakdown (fibrinolysis shutdown) developed in trauma patients following hemorrhagic shock and resuscitation. They proposed that these factors predispose the trauma patient to microvascular thrombosis of the pulmonary system, resulting in ALI.[33, 34] Based upon this hypothesis, they conducted a phase II clinical trial evaluating the efficacy of tissue plasminogen activator (tPA; a blood protein that activates fibrinolysis, commonly used in MI and stroke patients to dissolve life-threatening occlusive clots) on pulmonary function in trauma patients when administered within 48 hours of injury.[35] Though this trial had promising results, administration of systemic tPA remains high-risk following trauma and major surgery and is contraindicated in the setting of neurosurgery. Furthermore, tPA has been shown to act on neuronal receptors and has been linked to excitotoxicity and subsequent cell death.[36] As such, the use of tPA in trauma patients, particularly those having sustained head injury, remains controversial and has seen little clinical use given the appreciable risks of ongoing bleeding, secondary bleeding and potential neurologic sequelae.

While VTE is related to venous macrovascular insult and ALI to arteriolar microvascular thrombosis, review of the existing literature engenders the concept that post-trauma ALI and VTE are manifestations of the same pathologic process, both a hyper-coagulable and fibrinolysis resistant state.[31, 37] Currently, there are no clinical methods to measure and quantify microvascular occlusion. However, animal models have been employed to demonstrate microthrombi within the pulmonary vascular beds resulting in foci for leukocyte-induced lung injury[38] which can be attenuated with pharmacologic thrombolytic therapy.[39] However, as previously discussed, the use of thrombolytics, such as tPA, is high-risk and controversial in a post-trauma patient already predisposed to bleeding.

We propose that there is a missing component in the understanding of the mechanisms driving these pathologies, specifically resistance to fibrinolysis (shutdown).[40] Further, we hypothesize that both VTE and ALI remain significant clinical problems due to a failure to treat this pathology.

Leading to this hypothesis, we have observed that trauma patients treated with anti-platelet agents prior to injury have a reduced incidence of organ failure and improved overall survival.[41] Subsequently, Chen et al. conducted a retrospective study of 575 trauma patients and showed an independently associated decreased risk of acute respiratory distress syndrome (ARDS) in the cohort taking aspirin prior to

hospitalization.[42] Further, these effects appear to be even more robust when anti-platelet agents, such as aspirin, are used in conjunction with a statin.[43] On their own, statins have been reported to have protective effects following trauma, although no prospective data currently exists.[11] In each of these studies, the improvement in outcomes has been attributed to the anti-inflammatory effects of these drugs. However, our lab, as well as others, contends that they may be acting through a novel mechanism that acts to reduce resistance to fibrinolysis, microvascular and macrovascular thrombosis and their morbid sequelae.[10, 44-46]

In an evaluation of nearly 200 critically injured patients, we have identified a spectrum of fibrinolysis.[40] This spectrum consists of three distinct groups (termed phenotypes): normal (or as we termed "physiologic") fibrinolysis, hyper-fibrinolysis and a cohort the exhibits resistance to fibrinolysis (i.e. shutdown). As would be expected, the small cohort of patients exhibiting excessive clot breakdown (hyper-fibrinolysis) had an increased mortality attributable to hemorrhage. Interestingly, nearly 65% of this patient population had measured coagulation parameters consistent with fibrinolysis shutdown. In turn, this group had a higher mortality rate attributable to organ failure (Figure 1).

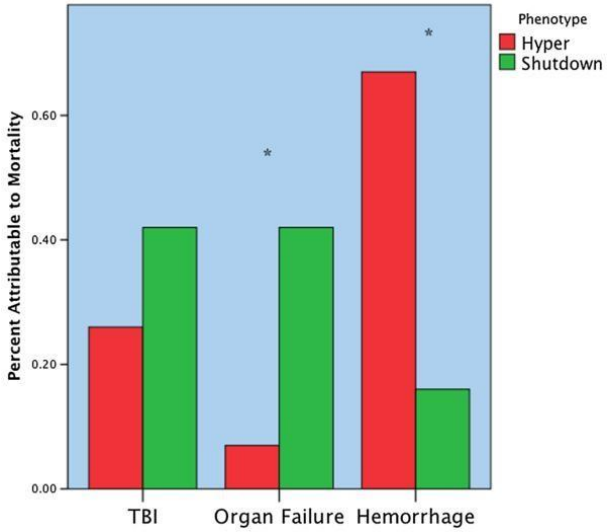


*Figure 1. Distribution of mortality according to fibrinolytic phenotype. The y axis represents the percentage of total mortality per phenotype. The hyperfibrinolytic phenotype had a high frequency of mortality associated with hemorrhage. The shutdown phenotype has a high frequency of organ failure-related death. TBI did not reach statistical difference between phenotypes but was more common in the shutdown cohort. *p < 0.05. Hyper, hyperfibrinolysis; Shutdown, fibrinolysis shutdown; TBI, traumatic brain injury.[20]*

Patients with physiologic fibrinolysis ultimately had the best outcomes (Figure 2). As such, correction of the pathologic fibrinolytic state to achieve a physiologic fibrinolysis profile appears to offer a window to a therapeutic intervention for coagulopathic patients following severe injury.

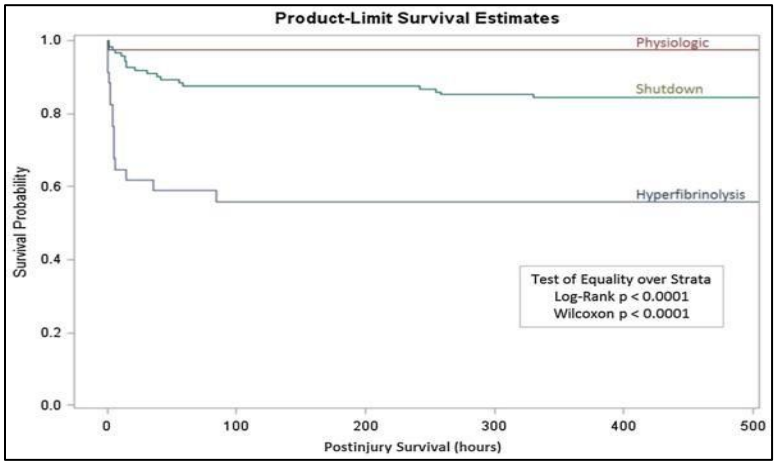


Figure 2. Survival curves of different phenotypes of fibrinolysis. Curve demonstrates the time from injury to survival patterns between the fibrinolysis phenotypes. The y axis represents the percentage of survival, and the x axis represents hours from injury. Survival, hours after injury.[20]

Furthermore, it is well known that circulating platelets contain multiple granules, with each granule containing prothrombotic and antifibrinolytic proteins.[31] Recently, we have shown that platelets are potent inhibitors of fibrinolysis[47], leading to the hypothesis that reduction in platelet activity will abrogate fibrinolysis shutdown and ultimately reduce the development of ALI and VTE events. In fact, the American College of Chest Physicians currently advocates for the use of antiplatelet therapy (in the form of aspirin) in orthopedic surgery patients at high risk for VTE. [48]

As previously mentioned, the addition of rosuvastatin, an HMG-CoA reductase inhibitor with a number of pleiotropic effects,[45, 49] has the potential to further reduce resistance to fibrinolysis. Indeed, statins have been shown to reduce the circulating level of plasminogen activator inhibitor 1 (PAI-1) in healthy volunteers with metabolic syndrome.[50] PAI-1 is a protein that prevents tPA from initiating the breakdown of a blood clot and is thought to be the primary regulator of tPA in trauma patients. *In vitro* studies suggest that statins reduce PAI-1 synthesis by blocking transcription (i.e. at the genetic level) in the endothelial cells that line the blood vessels.[51] In non-trauma patients with septic shock, PAI-1 levels are attributable to endothelial dysfunction and are highly predictive of mortality.[52] With respect to critically injured patients, we have preliminary data showing a nearly 300-fold increase in PAI-1 in this cohort when compared to healthy controls. Animal studies have shown that PAI-1 levels begin to rise several hours following hemorrhagic shock and resuscitation[53] and can arise from endothelial cells.[54]

Although ARDS directed trials using statins (simvastatin and rosuvastatin) [55, 56] have failed to demonstrate a benefit, there are several important differences in the severity of disease between septic patients with established ARDS and trauma populations. Moreover, these trials focused on treatment, while our objective is directed to prevention. Specifically, the differences between these trials and ours are:

a) Difference in strategies: in both abovementioned ARDS trials, statins were given as <u>treatment</u> for these patients, i.e., when the lung injury was already established, while we are proposing statins as a <u>prevention</u> strategy;

b) Differences in populations: there were only 6 trauma patients in the "Rosuvastatin for Sepsis-Associated Acute Respiratory Distress Syndrome study" [55], which included mostly patients with pneumonia, a complication that occurs in approximately 25% of ICU trauma patients. [2] Similarly, in the HARP-2 trial [56], which tested simvastatin to treat ARDS, of the 540 patients only 44 (8%) were victims of trauma.

c) Differences in mechanisms: in the case of statin therapy used to treat sepsis, the hypothesis has been that statins inhibit the HMG-CoA reductase thus reducing systemic inflammatory mediators (TNF-α, IL-1ß, IL-6, and IL-8). However, statins' inhibition of HMG-CoA results in a number of pleiotropic effects, as illustrated in Figure 3 below.[11, 45, 49, 57, 58] Statins have been shown to downregulate endothelial transcription of plasminogen activator inhibitor-1 (PAI-1), which is massively up-regulated post injury, resulting in suppressed fibrinolysis, which in turn is associated with macro- and micro-thrombotic complications. [59] Therefore, statins' inhibition of PAI-1 in the critically-injured represents a promising, novel therapeutic strategy for the prevention of micro-thrombotic complications such as acute lung injury and ARDS as well as macrothrombotic complications as VTE.

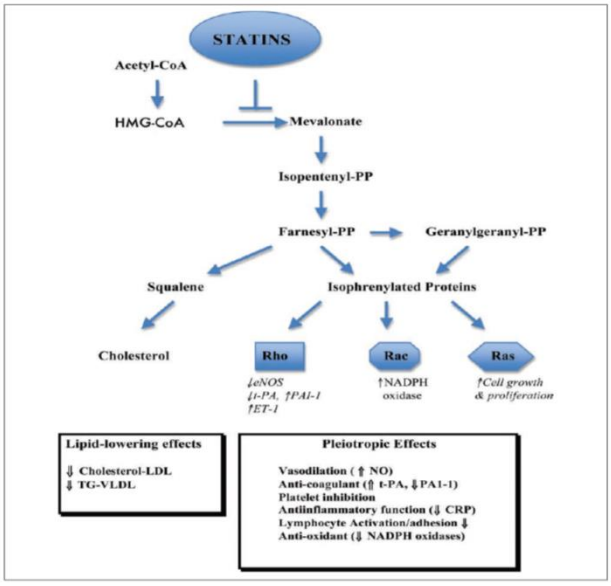


Figure 3: The mechanism of statins: statins inhibit the conversion of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) to mevalonate and thus prevent cholesterol synthesis. They further prevent the downstream production of the isoprenoid intermediates and give rise to the pleiotropic effects (Note: PP=pyrophosphate)[58]

Regarding the safety profile of statins, the SAILS [55] and HARP-2 trials [56] provided good evidence that statins were not associated with higher risk of adverse events among critically ill patients, although there was some concern about a potential contribution of rosuvastatin to renal and liver failure. It should be noted, however, that both organ dysfunctions are much more common in sepsis patients than in trauma patients. [2] Incidentally, the SAILS trials required FDA IND regulation because the study sponsor, the manufacturer of rosuvastatin, planned on submitting a new indication for the drug (a clear IND requirement).

Although most of current evidence on statins comes from the cardiovascular field, it has been suggested that statins may also have beneficial effects in non-cardiac surgical procedures such as vascular and general surgery. [45, 46, 57, 58] Moreover, the beneficial effects of statins were observed in normolipidemic patients, suggesting they are independent of lipid lowering effects. [60] Finally, there is some evidence that statins may improve recovery from DVT. In mice with DVT, rosuvastatin and atorvastatin induced significant reductions in platelet aggregation, clot stability, thrombus PAI-1, tissue factor, neutrophils, myeloperoxidase, neutrophil extracellular traps (NETs), and macrophages, most notably in the earlier DVT stages. [61] In patients with DVT, administration of 40 mg/day atorvastatin for three days increased plasma fibrin clot permeability (by 23%) and lysability (by 15–20%), independent of changes in lipid profile.[62] These effects may facilitate restoration of the patency of a thrombosed vein. Finally, the JUPITER trial provided convincing evidence that rosuvastatin prevented VTE events in healthy, normolipidemic patients, with a very safe profile. [14]

All of the statins currently on the market have similarly excellent safety profiles. [10, 63-65] There is no evidence of difference between them. [65, 66] High doses of statin have been associated with some reports of bleeding, but current evidence is strong that no risk of bleeding is associated with low dose (as in this study) statins. [10] We will be using a low dose of rosuvastatin (20mg) that has been thoroughly validated in the cardiovascular surgery literature as a safe and efficacious regimen to improve postsurgical outcomes. [67, 68] More important, we will be using the only statin (rosuvastatin 20 mg) thus far shown in a RCT to reduce VTE incidence in non-hyperlipidemic patients. [14]

Mechanistically, rosuvastatin also seems a logic choice. The following table, Table 1, shows some of the mechanisms as well as evidence of VTE reduction in clinical trials and whether or not these statins are recommended for cardiac surgery patients.

Table 1.

	Anti-thrombotic mechanism	**Clinical evidence in VTE prophylaxis**	**Recommended in cardiac surgery**
Simvastatin	**↓ TF expression** **↓ Thrombin formation** **↓ PAI-1** **↑tPA** **↓ Thrombin formation**	**No**	**2nd option**
Rosuvastatin	**↓ TF expression** **Down-regulation of PAI-1 monocytes, smooth muscle** **cells and endothelial cells** **↓ PAI 1** **↑tPA**	**Yes**	**1st option**
Atorvastatin	**↓ Thrombin formation;** **Up-regulates PAI-1 synthesis during macrophage differentiation, no effect in mature macrophages** **↓ PAI 1** **↑tPA** **↑Thrombomodulin transcription in endothelial cells**	**No**	**1st option**
Pravastatin	**↓ Thrombin formation** **No effect on PAI-1** **No effect on tPA**	**No**	**2nd option**

Rosuvastatin in a low (20mg) dose, based on the above specific effects, its safety profile and being the only statin for which there is a RCT demonstrating VTE prevention effects, seems to be a logical choice for

our trial. In addition, it is a first choice in conjunction with aspirin for the postoperative period of cardiac surgery. [12]

It is important to note that the dose of aspirin chosen for this trial is in tune with current guidelines. The most recent American Heart Association guidelines recommend the use of a higher dose of aspirin based on studies that demonstrated that lower doses of aspirin (100–200mg) may be insufficient to effectively inhibit platelet function as a result of resistance to the antiplatelet effect of aspirin in the postoperative period ("aspirin resistance"), which adversely affected postoperative vein graft patency. [68]

The primary adverse effect of aspirin therapy in any dose regimen is an increased risk of bleeding. However, in examining the difference between low-dose (75 - 162mg) and high-dose (>162 - 325mg) therapies, a meta-analysis of 22 randomized trials incorporating approximately 75,000 patients found no difference in hemorrhage risk between low- and high-dose regimens in all incidences of bleeding (RR 1.62; 95% CI 1.3 - 2.03) and major bleeding (RR 1.71; 95% CI 1.41 - 2.08). [69]

It is important to emphasize that the comparisons between trauma and cardiac surgery populations regarding potential safety of the combination of aspirin and statin is based on solid evidence, as explained below.

Comparison between cardiac surgery and trauma patients: Whether the research population (trauma patients) is potentially at higher risk from the study medication interventions is relevant to determine FDA IND exemption for this trial. Below, we provide a comparison between the population for whom these medications are currently recommended (cardiac surgery patients) versus our trauma population.

We examined several studies to compare the two populations head to head. Data from the study by Berger et al. [70] are summarized below in table 2.

Table 2: Patient characteristics on admission with detail of the diagnostic categories (Berger et al. [55])

Variable	Complex cardiac surgery	Trauma	Subarachnoid hemorrhage
Number	**113**	**66**	**21**
Gender, male/female	**70/43[a]**	**52/14[a]**	**5/16[a]**
Age, years	**70 ± 10[a]**	**40 ± 19[a]**	**54 ± 9**
Weight, kg	**75 ± 16**	**75 ± 13**	**68 ± 12**
Body mass index, kg/m^2	**26.5 ± 5.0**	**24.9 ± 3.9**	**24.5 ± 3.4**
SAPS II	**39.3 ± 10.4[d]**	**35.7 ± 15.4[d]**	**33.9 ± 13.6[d]**
SOFA score at admission	**9.1 ± 1.7[a]**	**7.5 ± 3.1[a]**	**6.3 ± 2.9[a]**

In the above randomized study, compared to trauma patients, cardiac surgery patients were more likely to be older, to have a higher BMI, and to have both a higher Simplified Acute Physiologic Score II (SAPS II) and Sequential Organ Failure Assessment (SOFA). In addition, they were much more likely to have prior acute renal failure (43% vs. 15%) and to develop acute renal failure (44% vs. 19%) during hospital admission.

The 2015 study by Zbrozek and Magee [71] also compared these hospitalized populations head to head. They observed that, compared to trauma patients, cardiac patients were more likely to require blood transfusions (18% vs. 14%), were older and had more comorbidities (as measured by the Charlson Comorbidity Index). ICU mortality of cardiac surgery patients was higher than trauma patients, while hospital mortality was similar between the two groups.

Specifically regarding VTEs, trauma patients have an incidence varying from 1.8% to 6.0% depending on type of injuries and whether there is systematic surveillance. [72, 73] Mortality associated with VTE can also vary depending on the type of VTE but has been estimated to be associated with an adjusted odds ratio of

hospital mortality of 2.08 (95%CI: 1.15-3.78;p=0.016).[72] The most recent guidelines by the American College of Chest Physicians reported the risk of symptomatic VTE among trauma patients to range from 3% to 5%.[26] Relative contraindications to pharmacologic VTE prophylaxis in trauma include severe head injuries, non-operatively managed liver or spleen injuries, renal failure, spinal column fracture with epidural hematoma, severe thrombocytopenia, and coagulopathy, all of which would be excluded in our study. [26] After exclusion of these high-risk patients, the risk of bleeding in trauma patients receiving VTE prophylaxis has been estimated to be at 0.7% (95% CI, 0.2%-1.7%). Comparatively, the same 2012 CHEST systematic review [26] estimated cardiac patients to have a VTE risk of 0.5% to 1.1% (thus lower than trauma patients) while the risk of bleeding due to VTE prophylaxis for cardiac patients was estimated at 4.7% (range, 3.1%-5.9%), which is higher than trauma patients.

In conclusion, we believe that there is strong evidence that, compared to cardiac surgery patients, trauma patients are higher risk of VTE but (assuming appropriate exclusion of the trauma patients at high risk defined above) at lower risk of bleeding complications from VTE pharmacological prophylaxis. In light of this evidence, we believe there are no reasons to consider the proposed research population at higher risk than cardiac surgery patients for whom the proposed medications are considered standard of care.

In summary, critically injured patients represent a distinct patient population that is at a high risk for macro- and micro-thrombotic complications associated with high morbidity and mortality. The current pharmacologic therapies used to treat these patients in the intensive care unit are not targeted at reducing fibrinolysis shutdown. Medicating patients at high risk for fibrinolysis shutdown with rosuvastatin and aspirin early after injury has the potential to reduce the incidence and severity of post-trauma VTE events.

III. Other Preliminary Studies

Fibrinolysis shutdown measurements: Our lab has developed a novel assay to measure fibrinolysis shutdown. [74] This assay employs the technique of thrombelastography (TEG), a physical clot-shearing method employed to measure the rate that whole blood forms a clot, the strength of the clot, and the rate of clot degradation (i.e., fibrinolysis). The TEG LY30 is the primary parameter used to quantify fibrinolysis. However, a limitation to this assay is that it does not measure the magnitude of fibrinolysis shutdown. When patients at risk for organ failure from fibrinolysis shutdown are studied, it is necessary to ensure that they have a marked resistance to fibrinolysis, not just a low LY30.

To address this issue, we sought to modify the traditional TEG assay, adjusting LY30 sensitivity to better evaluate the resistance to fibrinolysis. By adding tPA to whole blood after it has been removed from the body, there is an increase in fibrinolytic activity that can be readily measured and quantified by conventional thrombelastography. We have previously described this assay as the "tPA-Challenged™ TEG." [59]

We have validated the tPA-Challenged™ TEG in 134 healthy volunteers, resulting in normal distributions of fibrinolytic activity using multiple distinct doses of tPA in a dose/response manner. When the level of fibrinolysis in the tPA-Challenged™ TEG of a healthy volunteer is compared to that of bluntly-injured trauma patients in the ICU, marked fibrinolysis shutdown is observed in the post-injury patients. Trauma patients with persistent fibrinolysis resistance (i.e. lower than expected fibrinolysis for 5 days post-injury) were found to have a trend towards increased VTE; however, our study population was too small to make a definitive conclusion based upon these results.

More recently, we evaluated serial blood samples drawn from trauma patients from the time of injury through hospital day 7. In the first several hours post-injury, patients had diverse levels of fibrinolytic activity. By 24 hours post-injury, however, more than 60% showed fibrinolysis resistance greater than the 95th percentile of the healthy volunteer population at the highest tested dose of tPA.

IV. Research Methods

A. Outcome Measure(s):

Primary Outcomes:

Incidence of VTE

- Based on screening duplex ultrasound (US) of legs and central line on day 3.

Secondary Outcomes:

Fibrinolysis phenotypes as measured by traditional and tPA-Challenged™ TEG LY30

PAI-1 and tPA levels

Incidence of ALI within 2 weeks post-injury based on the Berlin Criteria[16]
Ventilator days (as measured by ventilator-free days)
Incidence of arterial thrombotic complications: myocardial infarction (MI) and cerebrovascular accident (CVA)
All-cause 30-day mortality
ICU free days
Organ failure based off of Denver MOF score[75]

B. Description of Population to be Enrolled:

The setting of the study is the Denver Health Medical Center, a high volume, level 1 urban trauma center. The patient population to be enrolled consists of all adult trauma patients (ages 18-65 years) requiring admission to the surgical intensive care unit (SICU) and expected hospital stay for at least 3 days. Outside hospital transfer patients that require SICU admission less than 24 hours after their injury are also eligible for enrollment.

Exclusion criteria for prophylactic anti-coagulation and for the study are:

Known inherited bleeding disorder or coagulopathy
Known contraindication to pharmacologic anticoagulation
Spinal column fracture with epidural hematoma
Head trauma/central nervous system injury
- Severe TBI; defined as AIS Head >3
- Intracranial hemorrhage; subdural or epidural hematoma
- Neurosurgery service objection; neurosurgical contra-indications will be documented

Ongoing hemorrhage requiring blood product transfusion
Thrombocytopenia (platelet count < 50,000)
Non-operatively managed liver or spleen injuries Grade III or above
Known chronic kidney disease (GFR < 15ml/min)
Rising creatinine (Cr > 1.5x baseline) at the time of enrollment
Inclusion in any other clinical trial
Documented previous ischemic strokes

In addition, the following exclusion criteria apply:

Receiving statin or aspirin therapy pre-injury, as potentially being assigned for Control would increase patient's risks
Known allergy or other contraindication to statins or aspirin
Pregnant patients
Prisoners, as their ability to freely consent is impaired
Inability to obtain consent from patient or proxy prior to 48 hours post-injury
VTE event (DVT or PE) diagnosed during current hospitalization prior to obtaining informed consent

C. Study Design and Research Methods

This protocol describes a Phase II, pragmatic, prospective, randomized, double-blind, adaptive clinical trial comparing combination rosuvastatin and aspirin therapy to placebo. Currently, there is no treatment for fibrinolysis shutdown or effective measures to prevent macro- and micro-thrombosis post-injury, thus, placebos are acceptable as the control group. Treatment for both groups would otherwise be by standard of care, which includes pharmacologic thromboembolism prophylaxis. The safety of concomitant use of VTE pharmacological prophylaxis with statins and aspirin is well documented in cardiac surgery patients. [12]

Patients admitted to the SICU will be screened for eligibility in the STAT trial (see above inclusion/exclusion criteria) based on history, physical exam and clinical data obtained during their initial treatment. If deemed eligible for enrollment in the STAT trial, the patient, their legally authorized representative (LAR), or their proxy decision-maker will be contacted by our on-site professional research assistants (PRAs) for consent to enroll in the study. Only patients for whom consent is obtained within 48 hours post-injury will be eligible. If consent is not obtained until 48 hours post-injury subjects will not receive the first three blood draws that are to be performed at 6, 12, and 24 hours, respectively.

Upon consent, blood samples will be obtained at 6, 12, 24, 48, 72, 120 and 168 hours after injury. Traditional and tPA-Challenged™ TEG as well as ROTEM Platelet will be performed on these samples to evaluate the development of the three fibrinolysis phenotypes. Once the adminstration of prophylactic anti-coagulation to the study subject has begun, the DHMC pharmacy will randomize the patient to the control or intervention arms of the study. Randomization will occur using a computer-generated block (groups of 20 patients) random number sequence in sealed, opaque envelopes maintained at the DHMC pharmacy. Patients assigned to the intervention arm will receive the standard of care anti-coagulation plus the combination experimental drugs (20mg of rosuvastatin and 325mg of aspirin) daily either orally or via feeding tube. These doses are consistent with those currently recommended in the postoperative period following cardiac surgery. {Members, 2011 #61} Patients assigned to the control group will receive standard of care anti-coagulation plus identical-looking placebos either orally or via feeding tube. Healthcare providers, PRAs and patients will be blinded to study randomization allocation (double blind design). The DHMC pharmacist will be aware of the patient's treatment arm so that rapid un-blinding will be possible in the event of an adverse event possibly related to a study medication.

Trombo Therapeutics, Inc. is not involved in this study. tPA-challenged TEG assay is used for research purposes only. The data from this test is not accessible for the clinical care providers and does not affect the patient care. We are using this assay to study the clot sensitivity and as a marker for massive blood transfusion. The results of tPA-challenged TEG assay will not be used for any clinical decision or research subject assignment. At the moment there is no plan to submit tPA-challenged TEG data to the FDA.

The blood samples will be used to monitor for type of fibrinolysis, function of the biochemical mediators of coagulation (see table 3 below) and potential side effects of these medications, as explained in more detail below.

Table 3: Biochemical Mediators of Coagulation and Fibrinolysis

Coagulation Factors	**Fibrinolysis Factors**
PAI-1	Plasmin/Antiplasmin Complex (PAP)
tPA	TAFI
D-dimer	α2 macroglobulin
Thrombin/Anti-thrombin complex (TaT)	α2 anti-plasmin
Free Anti-thrombin	C1 esterase
Factor VII	Angiostatin
Protein C	α-enolase
Protein S	Polyphosphates
Platelet aggregation	Myosin
Functional fibrinogen	Histones

Multiple methods of laboratorial analysis will be employed to evaluate these parameters. The methods and techniques include: the aforementioned TEG analyses to evaluate the mechanical properties of the blood clots; ROTEM aggregometry to assess platelet function; enzyme-linked immunosorbent assays and quantitative proteomics to compare and contrast coagulation protein levels during statin and aspirin treatment; and quantitative mass-spectrometry metabolomics to investigate the links between the metabolic derangements that occur during trauma and shock with the function of the coagulation proteins (specific focus on the development of fibrinolysis phenotypes). Ultimately, sampling at each time-point will require approximately 21 ml of blood to perform the necessary assays (please, see table 4 below with the volume of blood required to perform the coagulation assays). Two additional samples may be collected for safety monitoring after consent is obtained and after the third dose of the study drug. To reduce redundancy, safety labs will not be collected if there has been a result within the past 12 hours. Over the course of the study, a maximum of 9 blood samples will be obtained with a maximum total of approximately 163 ml of blood drawn.

Table 4: Coagulation Assays & Safety Monitoring Labs with Amount of Blood Required

Assay Method	Blood (ml)
Rapid TEG	0.35
Kaolin and tPA-Challenged Kaolin	1.0
Native citrated TEG	0.35
Platelet mapping panel (ADP, TRAP, AA)	0.5
Platelet Aggregometry	0.3
Proteomics	2.0
AP/MS	1.5
Thrombin generation	1.5
ELISAS[tPA(total, bound, free); PAI-1 (total, bound, free); Plasminogen(total + PAP); Anti-plasmin(total); TAT; TAFO; Factor VIII; Protein C; Protein S; Anti-thrombin]	5.5
Total (Per Time Point)	21.0
Safety Monitoring Clinical Laboratory Tests	
AST, CK, Creatinine	4.0
Hemoglobin	4.0
Total (Potentially Collected Twice)	8.0
Total Maximum Amount of Blood Collected	**163**

Study medications or placebo will be administered during hospital stay while patients are receiving the standard of care dosing of prophylactic anticoagulation (i.e. heparin or heparin-derivatives) and will be interrupted concomitantly with interruption of pharmacological VTE prophylaxis. Patients diagnosed with VTE will be withdrawn from the study drugs and will receive the appropriate VTE treatment per current ICU protocols.

A lower extremity ultrasonography will be conducted on the third day the patient receives the study drug. If the subject is discharged sooner than the third day the patient receives the study drug, lower extremity ultrasonography will not be done. The test will be used to evaluate the central line sites and veins in the legs for blood clots. The results of the ultrasound test will not be available for patient clinical care as routine ultrasound surveillance for thrombotic events as this is not recommended for trauma patients according to the latest guidelines. [84]

We will monitor function/damage of liver, renal and muscle before initiation of therapy (if the patient already has a recent test of these functions within 12 hours of enrollment, the result of these tests will be used to avoid unnecessary sampling), and upon the end of the therapy or as clinically necessary (i.e., any clinical indications of organ dysfunction). Stopping rules are:

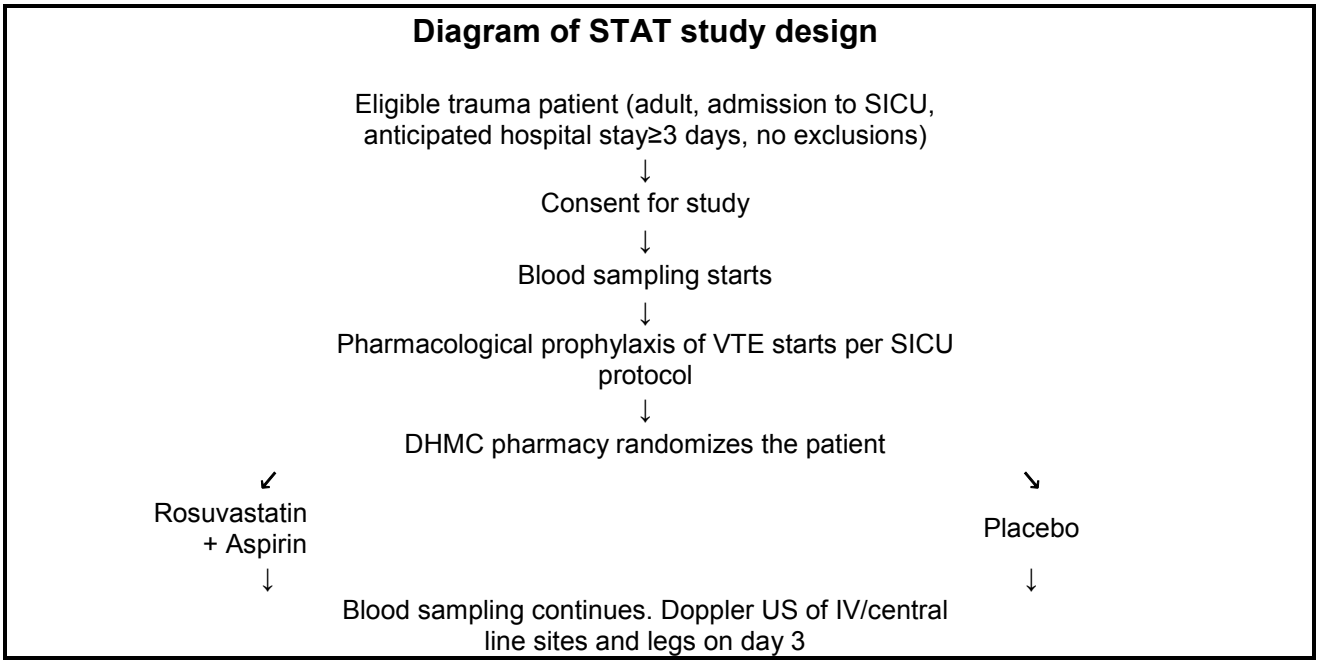
1. Bleeding: Any ongoing bleeding requiring blood transfusion or operative procedure to stop bleeding. Bleeding will be monitored via monitoring of the daily measurements of hemoglobin levels, which are an integral part of the ICU protocols for trauma patients. Study medications will be unblinded and stopped if there is an unexplained drop in hemoglobin level greater than 2g/dl within 24 hours after enrollment leading to discontinuation of VTE Prophylaxis OR a drop in hemoglobin level greater than 1g/dl daily for three consective days leading to discontinuation of VTE Prophylaxis.
2. Liver dysfunction: Alteration in liver chemistry is a rare sequela of statin therapy. A meta-analysis of 13 placebo-controlled trials incorporating nearly 50,000 patients examined the incidence of liver toxicity (defined as elevation of hepatic transaminases greater than 3 times the upper limit of normal) in patients receiving statin therapy. [77] This study reported the incidence of elevated AST or ALT in statin-treated patients vs placebo controls as 1.14% and 1.05%, respectively (OR 1.25; 95% CI 0.99 - 1.62). Further, the only individual drug associated with a statistically significant elevation in transaminases over a follow-up interval exceeding 3 years was fluvastatin. These and more recent data have informed expert opinion, [78] which ultimately prompted the FDA in 2012 to revoke the recommendation that liver function tests be monitored in patients taking statins. That said, given our at-risk patient population, we will use the accepted definition of 3 times the baseline of the AST test as the criterion for interruption of therapy;
3. Renal dysfunction: While transient proteinuria has been observed in patients undergoing statin therapy, acute kidney injury (AKI) is a rare complication. The JUPITER randomized controlled trial

incorporating approximately 17,000 patients and comparing rosuvastatin to placebo found no difference in AKI (6.0% v 5.4%; p = 0.08) between the groups. [79] In a randomized trial comparing rosuvastatin to atorvastatin, simvastatin and pravastatin, acute renal failure was observed in 2 of 420 patients receiving high-dose rosuvastatin. [80] We will define AKI as serum creatinine increase greater than 2x of baseline (>Grade 1, based on AKIN criteria) as the criterion to interrupt therapy.

4. Muscle injury: Myositis and rhabdomyolysis are rare sequela of statin therapy. A meta-analysis of 26 studies incorporating nearly 130,000 patients identified an incidence of between 1 and 4 per 10,000 patients developing rhabdomyolysis following statin therapy. [81] This meta-analysis includes a study of 12,000 patients comparing the efficacy of simvastatin 80mg with 20mg doses. In this study, the overall incidence of myolysis and rhabdomyolysis were 0.5 and 0.1 per 1000 person years, respectively, wherein all cases of rhabdomyolysis occurred in the high dose cohort. [82] The accepted definition of myositis is muscle pain in the setting of a serum creatine kinase concentration greater than 10 times the patient's baseline.[46] We will use this threshold to clinically quantify myositis, preceding potential development of rhabdomyolysis, as well as the criterion to interrupt therapy.
5. If the study patient receives aspirin, as prescribed by their SICU attending, administration of the study drug will be stopped.
6. Thrombocytopenia: If the study patient's platelet count decreases below 50,000 uL requiring transfusion of platelets while receiving the study drug, administration of the study drug will be stopped. This will be monitored and recorded in our twice daily safety monitoring log.

Any of the above mentioned stopping rules will be reported as a SAE and will trigger an immediate review by the DSMB.

Data collection/storage/maintenance: Trained PRAs will collect demographic, clinical, laboratory and outcome data, coordinate collection of blood samples with appropriate hospital personnel and perform laboratorial analyses. Data will be archived using Microsoft Excel. Samples will be stored for future research in our Sample Bank (if permission is obtained from the patient or the LAR). PRAs will use the patient's medical record for data collection. Data from medical records will be collected while the patient is being given the study intervention, or up to 30 days post-injury whichever is longer. If the study intervention is discontinued before 30 days, data collection will still continue to evaluate 30 day mortality.



D. Description, Risks and Justification of Procedures and Data Collection Tools:

Initial screening:

- History, physical exam and clinical data (laboratory and radiographic reports) obtained during the patient's trauma work-up and stabilization will be reviewed for enrollment eligibility
- These data will not subject the patient to any additional study-related risk, as no study intervention will take place

Blood collection/phlebotomy:

- Standard hypodermic venipuncture will be employed to obtain blood samples for evaluation only if no IV access already exists. Trained hospital personnel will draw blood samples.
- Approximately 21.5cc per collection (~150.5cc total over 7 days) will be required for analysis
- Risks are minimal and include pain, bruising, bleeding, infection and damage to surrounding structures
- These procedures are necessary to conduct laboratory analyses and its results will not interfere with patient care

Medications:

- Aspirin: aspirin is a non-steroidal anti-inflammatory drug that blocks the chemical messengers responsible for platelet activation; pertinent side-effects of aspirin are uncommon and include systemic bleeding, gastrointestinal bleeding and allergic reaction (see above for risks)
- Rosuvastatin: statins are HMG-CoA reductase inhibitors that have been shown to have multiple effects beyond cholesterol-synthesis regulation; pertinent risks of statin therapies are rare and include liver and kidney dysfunction, and myopathy (see above for risks)
- Both aspirin and rosuvastatin are FDA approved medications and standard of care in populations at lower risk for adverse events than the proposed trauma population
- As there is no current clinical treatment for fibrinolysis shutdown, a sequelae of trauma with both high morbidity and mortality, the proposed treatment strategy has acceptable risks that do outweigh the potential risks of treatment

Lower extremity ultrasonography:

- This is a non-invasive radiographic test that uses external sound waves to produce images of internal anatomic structures
- This test will be used to evaluate the central line sites and veins in the legs for blood clots
- The risks of ultrasonography are minimal
- This procedure is necessary to survey for and/or diagnose DVT ·
- The results of research ultrasound test will not be available for patient clinical care as routine ultrasound surveillance for thrombotic events is not recommended for trauma patients according to latest guidelines due to high number of false positives. [84]
- Ultrasound screening images will be stored in a secure location on Denver Health server and interpreted by an independent radiology specialist prior to interim analyses.

E. Potential Scientific Problems:

There are some potential problems with the significant variation in the current use of aspirin, which some surgeons use for VTE prophylaxis in trauma patients while others do not. As this is a pragmatic trial, the investigators will not interfere with the clinically indicated use of aspirin. If the study patient receives aspirin, as prescribed by their SICU attending, the patient will be withdrawn from the study and all blood draws will stop. The DHMC pharmacy will be aware of the group assignment; therefore, if the patient is placed on aspirin per his/her attending's prescription, no additional dose of aspirin will be given. If the patient is assigned to the experimental group, there is no contamination; however, if the patient is assigned to the control group, there is the potential for contamination of the study. We will adjust for this in the as–treated analysis (see below).

F. Data Analysis Plan:

Our sample size is defined and limited by our trauma center volume. Based on the analysis of our screening data in the past 16 months of study enrollment our annual SICU trauma admission volume was about 700 patients. The exclusion rate was over 90% in addition to 60% refusal rate. We were able to enroll roughly 1/3 of what we expected originally. This Phase II study may be underpowered to detect VTE reduction, depending on the observed incidence among patients with fibrinolysis shutdown. Trauma ICU series have reported variable VTE incidence under surveillance from 3-6%, in which case we could only detect reductions to 0-1% with 80% power, 95% confidence, and 2 equally spaced interim looks at the data. However, certain at-risk patients (possibly including shutdown patients) have higher VTE rates of 11-28%, in which case, the study could detect 39-64% relative reductions. Thus, we designed STAT as adaptive with pre-planned reassessment of sample size to detect a meaningful >50% relative reduction in VTE, in/exclusions, and other parameters during blinded interim analyses.

During the blinded interim analyses, the DSMB will reassess:

- Sample size, with a target to reach >50% relative reduction in VTE
- Inclusion and exclusion criteria
- Recruitment procedures
- Additional adverse events
- Addition of secondary outcomes
- Expansion to a multi-center trial

The analysis will contrast the outcome of the patients randomized to statin and aspirin therapy versus those randomized to placebo. In addition, we will test an interaction between group allocation and the presence of fibrinolysis shutdown on the development of thrombotic complications. All analyses will be conducted as intent-to-treat initially and as-treated subsequently. Primary and secondary outcomes as well as adverse events will be compared between groups. Subgroup analyses by VTE risk measured by the Greenfield score [83] are planned.

Continuous variables (i.e. PAI-1, LY30, ventilator free days, and intensive care unit free days) will be contrasted using a non-parametric test. Survival analysis will be performed using Cox proportional regression and Kaplan-Meier curves. Dichotomous outcomes (mortality, adverse events, thromboembolic events) will be contrasted between treatment arms with a chi-squared test or Fisher Exact test if expected cell values are <5.

We request permission to conduct the first interim analysis to be performed at the enrollment of 40 patients. In this adaptive trial, changes in sample size requirements were to be assessed at each interim analysis. The first interim analysis was originally planned upon enrollment of 147 subjects (two interim and one final analyses were planned to be performed in equally spaced intervals). However, due to unexpected slow enrollment (different than assumed during sample size calculations, see details above) it seems prudent to anticipate it in order to re-assess sample size requirements. The results of this interim analysis will allow us to re-assess our assumptions for a more precise sample size calculation for continuation of the trial.

If adjustment for confounders is necessary, Poisson regression with robust standard errors will be used for dichotomous outcomes and mixed models for continuous outcomes with appropriate transformations to approximate normality as needed.

Missing data will be minimized by careful, onsite, concomitant data collection through 24/7 coverage by our PRAs. For variables with more than 10% missing data, we will conduct missing data diagnosis (MAR, MNAR, MCAR), and if pertinent, missing data treatment with MICE.

Data Safety Monitoring Board: All analyses will be monitored by an independent Data Safety Monitoring Board (DSMB). All serious adverse events (SAE) and protocol violations (UAP) will be communicated to the DSMB. The DSMB will convene every 12 months for the 5 years of the study or more often if there are concerns about SAE or UAP as determined by the DSMB Chair. Each meeting will be approximately 1 ½ to 2 hours in duration and attended in-person or via conference call. We expect that each meeting will require a maximum of 2-hour preparation time (to read and review pertinent materials). The Chair of the DSMB may call *ad hoc* meetings as needed should unexpected or adverse events be identified and require review. These *ad hoc* reviews will consist of short conference calls or exchange of email. The first meeting will convene before enrollment starts. If necessary, the DSMB can require amendments to be submitted to the IRB to ensure safety of the subjects. Confirmed members of the DSMB are as follows:

- M. Margaret Knudson, MD (chair)
  - Professor of Surgery
  - University of California, San Francisco
  - Division of General Surgery
  - Medical Director for the Military Health System (MHS) Strategic-ACS Partnership
- Alden Harken, MD
  - Professor of Cardiothoracic Surgery
  - University of California, San Francisco
  - Chief, UCSF-East Bay Surgery Program
  - Chair, Department of Surgery, Alameda County Medical Center
  - Division of UCSF-East Bay Surgery
- Sung-Joon Min, PhD
  - Associate Professor of Healthcare Policy and Research
  - University of Colorado AMC
- Michael Wang, MD
  - Associate Professor of Pediatrics-Hematology/Oncology
  - Clinical Director, Hemophilia and Thrombosis Center
  - University of Colorado AMC

G. Summarize Knowledge to be gained:

On the basis of this randomized, controlled Phase II study, we hope to better understand the following:

- the efficacy of aspirin and rosuvastatin in the reduction of the incidence of VTE in critically injured trauma patients at risk for these complications
- the mechanisms involved in the coagulation and inflammatory cascades linking fibrinolysis to VTE and other thrombotic complications by examining the effect of aspirin and statin therapy on PAI-1, tPA levels and fibrinolysis phenotypes

The results of this Phase II study will assist in determining the efficacy and safety of combination statin and aspirin therapy in severely-injured patients, will assist in determining the pathway through which trauma-induced coagulopathy determines late post-injury thrombotic complications and will enable the design of a multicenter, randomized, controlled study adequately powered to determine the level of reduction in the incidence of VTE in this population. Furthermore, we will develop a systematic algorithm to treat the diverse fibrinolysis phenotypes. In sum, this and future studies will help broaden our understanding of the complex mechanisms that underlie trauma-induced coagulopathy.

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