Tranexamic Acid Mechanisms and Pharmacokinetics In Traumatic Injury (TAMPITI Trial)

Background:

Trauma is the leading cause of death in persons younger than 40 years. Hemorrhage is the etiology in 30% of these deaths, and remains the leading cause of potentially preventable mortality (66-80%) [1]. As a result, the prevention of death from hemorrhagic shock has been the subject of intensive research and effort. Death secondary to hemorrhagic shock occurs from both surgical bleeding and coagulopathy. Acute traumatic coagulopathy is characterized by a hypocoagulable state, where the net balance of coagulation is such that there is low clot forming capacity and strength, which does not allow for adequate hemostasis. Acute traumatic coagulopathy occurs early in patients who are in shock from hypoperfusion and is not due to coagulation factor consumption or dysfunction because of acidosis, moderate hypothermia, or dilution[2]. However shock (oxygen debt) itself is associated with a coagulopathy that is due to the systemic activation of anticoagulant and fibrinolytic pathways[1, 3]. The protein C pathway is implicated in this process, in addition to fibrinolysis which is mediated by de-inhibition of tPA through PAI-1 consumption[2]. Low levels of PAI-1, with increased plasminogen activator release from the vessel wall contributes to hyperfibrinolysis. It has been suggested that TAFI is the main driver of fibrinolysis inhibition, and that reduction in TAFI activation by the competitive binding of protein C to T-TM is the mechanism for increased fibrinolysis with activation of protein C [1].

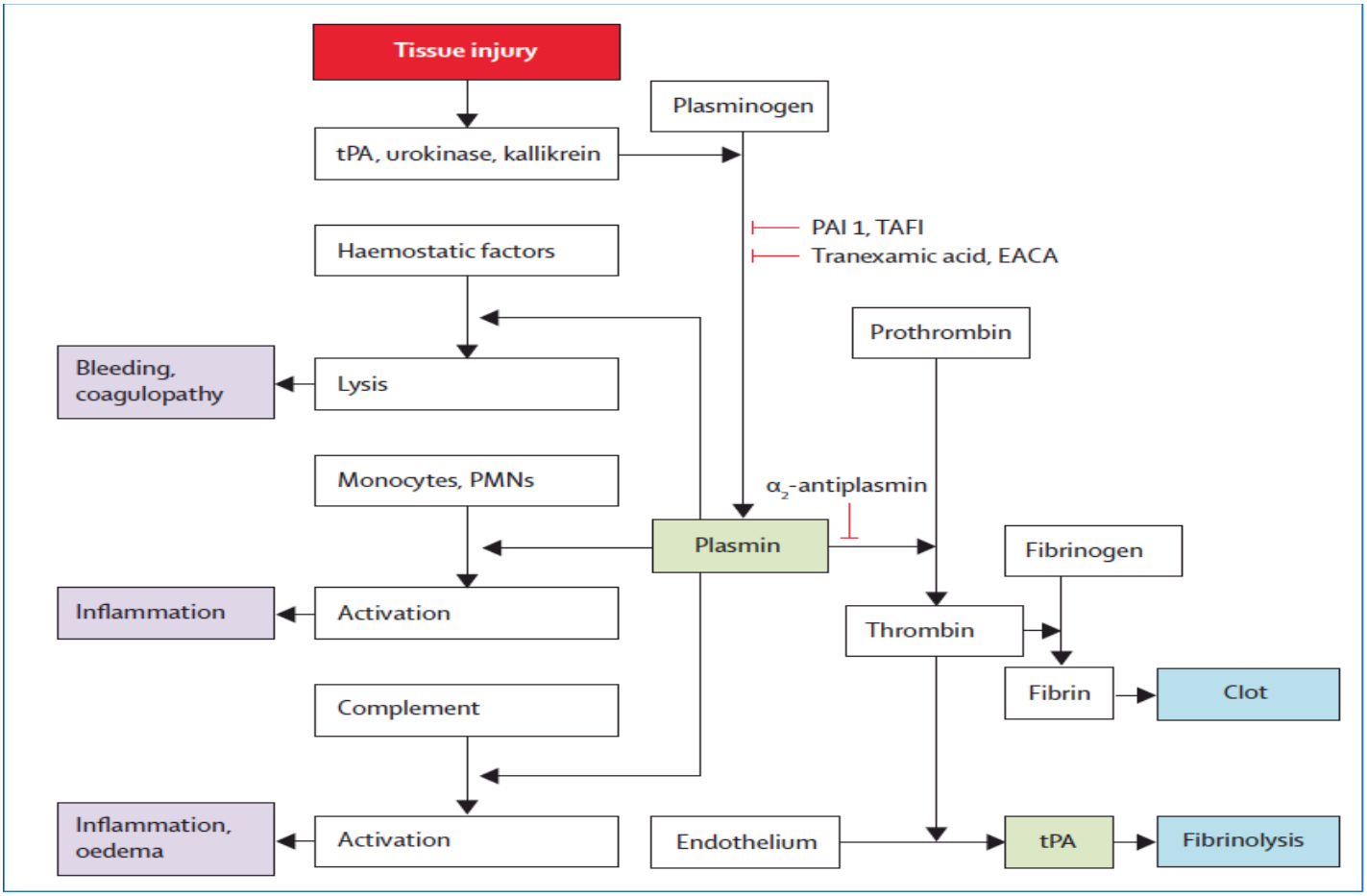
Due to the knowledge of increased fibrinolysis promoting a hypocoagulable state in severe trauma, trials have been performed to determine if antifibrinolytics such as tranexamic acid (TXA) could reduce morbidity and mortality by reducing death from hemorrhage[4]. TXA is an antifibrinolytic that inhibits both plasminogen activation and plasmin activity, thus preventing clot break-down rather than promoting new clot formation. TXA occupies the lysine-binding sites on plasminogen, therefore preventing its binding to lysine residues on fibrin. This reduces plasminogen activation to plasmin. Similarly, blockade of lysine-binding sites on circulating plasmin prevents binding to fibrin and thus prevents clot break-down. TXA is excreted largely unchanged in urine and has a half-life of approximately 2 hours in circulation when studied in patients without traumatic injury. Intravenous administration of TXA was approved by the FDA in 1986 for the prevention or reduction of bleeding in patients with hemophilia undergoing dental procedures. The FDA approved use of the oral form of TXA to control heavy menstrual cyclic bleeding in 2009. Despite the extensive and routine use of TXA in many surgical populations and an increasing use in severe trauma patients, TXA does not have an FDA approved indication for patients with traumatic injuries[1].

In 2010, the results of the landmark CRASH-2 (Clinical Randomization of an Antifibrinolytic in Significant Hemorrhage) trial were published, creating widespread international interest [5]. This multicenter, multinational study randomized 20,211 adult trauma patients to either one gram of TXA infused over ten minutes followed by one gram of TXA infused over eight hours versus an equivalent volume of normal saline placebo administered within eight hours of injury. Inclusion criteria consisted of systolic blood pressure less than 90 mmHg and/or with a heart rate greater than 110 beats per minute, or patients deemed to be at risk of significant hemorrhage. The primary outcome measure was in-hospital death within four weeks of injury. Secondary outcomes included thromboembolic events (myocardial infarctions, cerebrovascular accidents, pulmonary emboli, and deep vein thromboses), surgical interventions, blood transfusions, and the total units of blood transfused. The study found a significant decrease in all-cause mortality (14.5 vs. 16.0%, p=0.0035) and deaths from bleeding (4.9 vs. 5.7%, p=0.0077) in patients receiving TXA compared to placebo. More recently, data from the Military Application of Tranexamic Acid in Trauma Emergency Resuscitation Study (MATTERs) has been published, providing perspective from a different and likely more severely injured patient population[6]. MATTERs is a retrospective observational study that evaluated data from the Camp Bastion Surgical Hospital in Afghanistan. Patients were included in the study if they sustained combat-related injury and subsequently received a minimum of one unit of packed red blood cells. The primary outcome measures were 24 and 48-hour as well as in-hospital mortality (which was inclusive of any mortality occurring within 30 days of injury). Secondary endpoints included transfusion requirement and correction of coagulopathy based on resolution of extended prothrombin and thromboplastin times between arrival in the emergency department and arrival in the intensive care unit postoperatively. Overall, 896 consecutive patients were analyzed, with 293 receiving TXA. Patients receiving TXA were more severely injured than those not receiving the drug (ISS 25.2 vs. 22.5, p<0.001), and a greater proportion presented with severe TBI and admission systolic blood pressure ≤ 90 mmHg. Patients receiving TXA were given more packed red blood cells, fresh frozen plasma, platelet, and cryoprecipitate transfusions than patients not receiving the drug. In spite of these admission characteristics, patients receiving

TXA had lower overall 48-hour (11.3 vs. 18.9%, p=0.004) as well as in-hospital (17.4 vs. 23.9%, p=0.03) mortality. Additionally, although more patients who received TXA were hypocoagulable on admission, fewer TXA patients were hypocoagulable on arrival to the ICU, and there was a significant reduction in the proportion of hypocoagulable patients in the TXA group between admission and the ICU. While these data add to the beneficial profile of TXA, MATTERs also reported a greater number of pulmonary emboli (8 vs. 2, p=0.001) and deep vein thromboses (7 vs. 1, p=0.001) in TXA-treated patients.

Despite both the CRASH-2 trial and MATTERS studies indicating TXA use reduced the risk of mortality, questions regarding mechanisms of action still remain. Interestingly, in CRASH-2, while improved survival was due to decreased death from hemorrhage, half of the patients did not require blood transfusions or surgical intervention. This would suggest that correction of coagulopathy was not the only mechanism contributing to improved outcomes in this trial. Figure 1 describes plasmin's multiple effects to include leukocyte activation and chemokinesis. Given that TXA's primary effect is purported to be inhibition of plasminogen activation preventing plasmin generation, evaluating monocyte and neutrophil activity and downstream effects in the adaptive immune response in the setting of TXA administration are required. The immune suppressive effects of TXA on immune function have not been thoroughly examined, especially in patients with severe traumatic injury. It is possible that the improved outcomes that have been reported with early administration of TXA in trauma patients are related to its immune suppressive effects. The study of the effects of TXA use on endothelial activation and injury is also important due to the inter-relationship between coagulation and endothelial function. Endothelial injury secondary to local hypoperfusion causes acute traumatic coagulopathy with fibrinolysis. Therefore a thorough and comprehensive evaluation of the effects of TXA on immune, coagulation, and endothelial parameters is important to allow for a better understanding of the mechanisms of action of this agent.

Figure 1: Trauma and fibrinolysis



Optimal dosing is also unknown for patients with traumatic bleeding. The lack of pharmacokinetic studies in this population contributes to the uncertainty regarding TXA dosing for patients with traumatic injury and

hemorrhage. While the CRASH-2 trial utilized a bolus dose followed by an 8-hour infusion, the MATTERS study used multiple IV bolus doses without an 8-hour infusion. The intravenous dosing of TXA for patients with hemophilia with dental bleeding, which is the only FDA approved indication for IV administration, is an IV bolus dose of 10mg/kg every 6 to 8 hours one day prior to surgery. The FDA approved indication for the intravenous form of TXA does not include a continuous infusion dose over 8 hours after a bolus dose. The common, but off label use of TXA in multiple surgical populations often utilizes an IV bolus dose followed by an 8-hour continuous infusion dose. However, in a trauma patient with potentially limited vascular access, a one-time IV bolus dose would be more practical and may facilitate increased plasma concentration in the immediate time period where hemostatic control is critical. The CRASH-2 trial utilized a total dose of 2mg/kg IV, and the MATTERS study utilized doses that ranged between 1-4 mg/kg IV (personal communication with Dr Joseph J. Debose). Therefore the analysis of multiple bolus doses (within range of current practice) on mechanisms and pharmacokinetics is important and especially relevant to the practical application of TXA. It is critical for formal and thorough pharmacokinetic analyses to be done in patients with traumatic injury with active bleeding since this population has different physiology than elective surgery patients. The pharmacokinetic analysis of multiple bolus doses in addition to mechanistic, outcomes and safety data will inform the medical community regarding the most appropriate dosing of TXA in the acutely injured, critically ill, trauma patient.

In addition to the potential risk of thrombosis from the use of TXA, seizures have also been recognized as a possible adverse effect. The proposed mechanism for seizures is the structural similarity of TXA to γ-aminobutyric acid, which has a potential to cause neurotoxicity. TXA use in cardiac surgery has been associated with an increased risk of postoperative convulsive seizures. [85] These findings are associated with TXA doses that are well above the 2 and 4-gram IV doses proposed in our trial. For a typical 70 kg patient, a 4-gram dose of TXA is 57 mg/kg. A recent study of over 1000 patients requiring cardiac surgery indicated that doses > 100mg/kg or 7 grams IV were associated with an increased risk of early seizure. [85]It is important to recognize that while the risk of early seizure was increased at doses > 100mg/kg the overall incidence of seizure was still very low at 1.3% of patients. For the typical 70 kg patient in this study that received andose of 4 grams of TXA IV, the probability of seizure was 0.01%, with a narrow 95% CI of (0.006-0.014. [85]

A currently unpublished survey of all US trauma centers by our research group that had a 132/187 (71%) response rate, indicated 52% of level one US trauma centers incorporate TXA into their massive transfusion protocols. This data demonstrates that equipoise exists regarding its routine use in this population. Therefore it is essential and appropriate that prospective randomized control trials are performed to provide evidence regarding its mechanism of action data, pharmacokinetic information, and efficacy and safety data.

Hypotheses:

1. We hypothesize that early TXA use in patients with severe traumatic injuries, reduces a pro-inflammatory state and monocyte activation. To test this hypothesis in 150 patients (50 in each study group), we will determine the effects on immune function measures, at multiple time points, in all three study groups. We expect reduced inflammation, and monocyte activation in TXA treated patients compared to placebo.
2. We hypothesize that the pharmacokinetics of TXA administration are affected by the degree of shock measured by admission lactate, StO2, presence of acute renal failure, and blood products administered in patients with severe traumatic injury. We expect that the degree of shock, renal function, and blood products transfused will affect TXA pharmacokinetics.
3. We hypothesize that early use of TXA is safe and associated with improved outcomes. We expect that the use of TXA at both 2-gram IV and 4-gram IV doses will be safe and will not be associated with an increased risk of thromboembolic events, ARDS, seizures or any adverse events compared to placebo.

Aim 1: To determine the effects of TXA on immune parameters.

To evaluate the effects of TXA on immune function parameters we will, in a RCT, analyze samples from 150 patients (50 in each study group), at multiple time points. Parameters are:

a. Cytokines: TNF-α, IL-6, IL-10, and IFN-γ measured at time 0, 6, 24 and 72 hours.

b. Flow cytometric analyses on leukocytes measured at time 0, 6, 24 and 72 hours:

   CD66+/ROS+ to identify activated polymorphonuclear cells

   CD4+/CD69+ and CD8+/CD69+ to identify activated lymphocytes

   CD14+/HLA-DR+ to identify activated monocytes

   CD4+/Foxp3+ to identify T regulatory cells

Aim 2: To determine the pharmacokinetics and pharmacodynamics of multiple TXA dosing regiments.

To determine if the pharmacokinetics of TXA administration are affected by the degree of shock measured by admission lactate, StO2, presence of acute renal failure, and blood products administered in patients with severe traumatic injury, we will perform the following analyses in a total of 100 patients within both TXA treated groups (50 patients per TXA treatment group).

a. Perform pharmacokinetic analyses

b. Compare pharmacokinetic data between patients with varying degrees of shock (lactate and StO2 measures) and according to total amount of blood products transfused in the first 12 hours of injury.

Aim 3: To collect safety and clinical outcomes data on TXA use in patients with traumatic injuries.

To measure safety and outcome data in this RCT of 150 patients with severe trauma we will:

a. Determine the incidence of thromboembolic events (DVT, MI, PE, Stroke) in all three study groups. Subjects will be assessed daily by the study team until hospital discharge or up to 30 days, (which ever comes first) after receiving study drug for any signs and symptoms of a thromboembolic event (i.e. shortness of breath, chest pain, extremity swelling, fever of unknown etiology, etc.) In addition, the study team will further ensure the safety of our subjects by screening all subjects at day 7 or hospital discharge (if prior to 7 days) for DVT using duplex ultrasonography. Stroke, MI, and PE will each be diagnosed clinically according to standard definitions (see appendix at end of document).

b. Determine the incidence of seizures at 24 hours in all three study groups.

c. Determine the incidence of all adverse events in all three study groups. Adverse events for up to 30 days after study drug administration will be characterized according to severity, relatedness, duration, and resolution.

d. Compare mortality, mechanical ventilation and ICU free days, incidence of multiple organ failure, Acute Kidney Injury (AKI), Acute Respiratory Distress Syndrome (ARDS) and sepsis in all three study arms.

Aim 4: To develop a repository of blood samples for future analysis.

These banked samples would be analyzed for measures of coagulation and endothelial function to include but not limited to:

1. Complement: CH50 measured at time 0, 6, 24 and 72 hours

2. Endothelial activation and injury: Soluble Flt-1, soluble E-Selectin, VCAM-1, and PAI-1, Ang1/Ang2, Syndecan 1, Syndecan 2 and vWF measured at time points 0, 1,6, 24 hours.

3. Coagulation and Fibrinolysis:

    a. Inhibition of plasmin activity by TXA: D-dimer, PAP, alpha2-antiplasmin, TAFI, clot lysis (ROTEM), measured at times 0, 1, 6, and 24 hours

    b. Activation and inhibition of fibrinolysis: tPA and PAI-1 measured at times 0, 1, 6, and 24 hours

    c. Activation of the coagulation system: thrombin generation assays, TAT complexes, antithrombin, fibrinogen, fibrin monomer, fibrinopeptide A and B, Factor XIII, Factor XI, measured at times 0, 1, 6, and 24 hours

Methods:

Research Design: A single center, double- blind, randomized, placebo-controlled study will be performed under an IRB approval for exception from informed written consent (with community consultation) and with IND approval from the FDA. Participants will be randomized into 1 of 3 treatment arms (1:1:1): TXA 2 gram IV bolus, TXA 4 gram IV bolus, or placebo. A transfusion guideline will be in place to standardize transfusion practice in this trial.

Study period: The study period is from time of enrollment to hospital discharge or transfer. The study intervention will occur only once upon enrollment in the trial.

Power Analysis: Recent data indicates that in severely injured trauma patients 6.2 ± 0.8% of monocytes express CD14 at 72 hours post injury [86]. We consider a 10% decrease in CD14+/HLA-DR+ monocytes from 6 ± 0.8% to 5.4 ± 0.8% to be potentially clinically relevant. The comparison between the group receiving 2 grams of TXA and placebo is independent of the comparison for the group receiving 4 grams of TXA and placebo. Therefore, using a a two-tailed α of 0.05 and a (1-β) of 0.90, and a very conservative 20% dropout rate, a total of 47 patients per arm or a total of at least 141 patients will be required. To account for patients that may have missing data we plan to enroll 50 patients in each study group or a total of 150 patients.

Inclusion criteria:

1.) Patients with traumatic injury that are ordered to receive at least 1 blood product

and/or

2.) Patients admitted to the Emergency Department with a traumatic injury and require immediate transfer to the operating room

3.) Able to receive the study drug within 2 hours from time of injury

Exclusion criteria:

1.) Patients known to be < 18 years of age

2.) Acute MI or stroke

3.) Known inherited coagulation disorders

4.) Known history of thromboembolic events (DVT, PE, MI, Stroke)

5.) Known history of seizures and/or seizure after injury/on admission related to this hospitalization

6.) Suspected or known pregnancy

7.) Known to be lactating

8.) Suspected or known prisoners

9.) Futile care

10.) Known current state of immunosuppression (i.e. on high dose steroids, chemotherapeutics, etc.)

11.) Unknown time of injury

12). Patients wearing an "Opt Out" TAMPITI Study bracelet

13). Known presence of subarachnoid hemorrhage.

Randomization: This study is a placebo controlled (1:1:1), double-blinded, randomized clinical trial of 150 severely injured trauma patients. Participants will be randomized (1:1:1) to TXA dose 1 (2 gram IV bolus), TXA dose 2 (4 gram IV bolus), and matching volume of Normal Saline placebo using an envelop randomization method. Study staff will contact the Investigational Drug Services Pharmacy (IDS Pharmacy) to inform them of a study participant and will request that they randomize the subject by opening a random envelope which will secretly assign the participant to one of the 3 randomization assignments. IDS Pharmacy will dispense the study drug with a label, which will contain a randomization code. The study team member responsible for overseeing the study drug administration will contact the IDS Pharmacy to verify that the study drug code matches the assigned code for the specific participant to ensure that the **right patient gets the right drug**. Once study drug is obtained by the pharmacy, it will be administered (blinded) by IV over 10 minutes.

Blinding: Since this is a double-blinded study, all study investigators and team members (with the exception of the study statistician) will be blinded to treatment assignment. In addition to the statistician, the only individual who will know the study group assignment will be the investigational pharmacist. The Investigational Pharmacist can reveal the subject's treatment assignment for safety or treatment concerns. Accidental and suspected un-blinding will be considered a protocol deviation and will be submitted to the IRB according to Institutional policies and procedures. Data from subjects who were unblinded will be included in the intent-to-treat analysis.

Treatment groups: The three treatment arms will be TXA dose 1 (2 gram IV bolus), TXA dose 2 (4 gram IV bolus), and matching volume of Normal Saline placebo.

Primary outcomes: Primary outcomes include differences in the proportion of activated monocytes among the 3 treatment arms (TXA dose 1, TXA dose, 2, and placebo) from time 0 to time 72 hours.

Secondary outcomes: Secondary outcomes measures include differences in cytokine profiles and leukocyte function parameters, clinical outcomes, and PK analyses between the three study groups (as detailed in Aims 1-4 below). These comparisons will be exploratory and because of the large number of such comparisons, analytic results will be interpreted cautiously.

Patient screening: During the patient recruitment phase of the trial there will be research nurse coordinators in house 24 hours a day, 7 days a week to facilitate constant patient screening and immediate determination of eligibility, initiate the randomization process, ensure blood sample acquisition and processing, and prospective data collection. All research coordinators will carry a trauma pager and will be present in the Emergency room to screen and enroll study patients. An on-call system for additional research laboratory staff will be funded to facilitate trial execution. In order to detect any potential biases, a screening log will be maintained to record the number of eligible patients, the number of patients eligible not randomized, and the reason for their exclusion. This should allow detection of any selection bias.

Anticipated cohort and recruitment rate: Trauma Registry data from 2011 report an incidence of 150 patients per year that met eligibility criteria for this trial. These patients have a mean age of 40, ISS of 16, and in hospital mortality of 18%.

Data Collection: The schedule of events details timing of study related procedures, including timing and volume of blood samples being collected (See Appendix C). The 16 ml of blood listed for PK studies is the total volume collected in 24 hours at multiple time points. Data to be collected on all patients will include epidemiologic data, diagnoses, surgical procedures, blood products (RBC, plasma, platelets, cryoprecipitate), coagulation factor concentrates, concomitant medications, vital signs, clinical labs, AIS/ISS, presence of acute renal failure according to RIFLE criteria [78], and clinical outcomes. The date and time of administration for all medications, blood products and coagulation factor concentrates will be recorded. Storage duration, additive solutions and processing methods used for blood products will also be recorded. Seizure, severe adverse

thrombotic events, and all adverse events will be captured from the time of study drug administration until hospital discharge or up to 30 days, (which ever comes first) although the primary analysis of the risk of seizure and adverse thrombotic events will most likely occur within the first 24 hours and 7 days of the trial respectively. The risk of seizure secondary to TXA has never been reported to occur after the first 24 hours post TXA administration [76].

Clinical outcomes measured will include mortality at 24 hours, 30 days, and hospital discharge/transfer, ventilator and ICU free days, AKI, incidence of multiple organ failure and sepsis/severe sepsis between TXA treated and placebo study groups. Multiple organ failure will be defined according to the Marshall Score (See Appendix A). Sepsis and ARDS will be defined according to standard definitions (See Appendix A). Clinical and safety data will be collected daily until day of hospital discharge or transfer.

Banking of patient samples: All samples collected for banking for future analysis will be frozen at -80 degrees Celsius. Samples will be cataloged and appropriately labeled.

Protocol Deviations: Patients will be considered adherent to the protocol only if they receive TXA at the appropriate dose or placebo as intended by the randomization process. Patients who receive the wrong TXA dose or TXA instead of placebo or placebo instead of TXA will not be adherent to the protocol and will be classified as protocol deviations. Patients not adherent to protocol will be included in the intent-to-treat analysis but will be excluded from the per-protocol analysis. The decision to withdraw care will not be considered an exclusion criterion, if made after patient entry. These cases will be kept in the ITT analysis.

Laboratory Methods:

Aim 1: To determine the effects of TXA on immune parameters. To evaluate the effects of TXA on immune function parameters we will, in an RCT, analyze samples from 150 patients (50 in each study group), at multiple time points. Parameters to be measured are:

a. Cytokines: TNF-α, IL-6, IL-10, and IFN-γ measured at time 0, 6, 24 and 72 hours.

b. Flow cytometric analyses on leukocytes measured at time 0, 6, 24 and 72 hours:

   CD66+/ROS+ to identify activated polymorphonuclear cells

   CD4+/CD69+ and CD8+/CD69+ to identify activated lymphocytes

   CD14+/HLA-DR+ to identify activated monocytes

   CD4+/Foxp3+ to identify T regulatory cells

Utilizing 5 mL whole blood samples collected in standard citrate tubes at time 0, 6, 24 and 72 hours later, blood will be centrifuged, and the plasma will be collected and stored at -80C. This plasma will subsequently be thawed and utilized to perform ELISA-based measurements of the concentration of plasma cytokines, TNF- α, IL-6, IL-10, and IFN- γ. After harvesting plasma, the Buffy Coat will be gently pipetted and contaminating erythrocytes will be lysed with an ammonium chloride lysis solution. After washing twice with buffer (1% BSA and 1 mM EDTA) and 0.1% sodium azide (NaN3) in HBSS without phenol red, calcium, and magnesium, cells will be resuspended in 4% Hanks' azide buffer (HBSS without calcium, magnesium, or phenol red, with 4% BSA, 0.1% sodium azide, 0.2% anti-CD16/32, and 1 mM EDTA), and then stained for analysis via flow cytometry utilizing commercially available flow cytometry antibodies as follows. Cells staining CD66+ and ROS+ will identify activated polymorphonuclear cells. Cells staining CD4+ or CD8+ concurrently with CD69+ will be identified as activated lymphocytes. Cells staining CD14+ and HLA-DR+ will identify activated monocytes. Finally, cells staining CD4+ and Foxp3+ will be identified as regulatory T cells. For intracellular staining of ROS, this will be performed following extracellular staining utilizing commercially available fixation and permeabilization solutions. Flow cytometric analysis will be completed on a FACScan flow cytometer (Becton Dickinson). Each cell population will be evaluated and reported as total positive counts, percentage of total leukocytes. Additionally, the mean fluorescence intensity for each cell population in each sample will be obtained and reported.

Aim 1 Statistical analyses: The outcome measures in this aim are continuous variables that will be measured at multiple longitudinal time points.

Aim 2: To determine the pharmacokinetics and pharmacodynamics of multiple TXA dosing regiments.

To determine if pharmacokinetics of TXA administration are affected by the degree of shock measured by admission lactate, StO2, presence of acute renal failure, and blood products administered in patients with severe traumatic injury, we will perform the following analyses in a total of 150 patients within both TXA treated groups (50 patients per TXA treatment group).

a. Perform pharmacokinetic analyses

b. Compare pharmacokinetic data between patients with varying degrees of shock (lactate and StO2 measures) and adjusting for acute renal failure and total amount of blood products transfused in the first 12 hours of injury.

To facilitate the frequent sampling of patients for this PK analysis, research staff will be present at the clinical site 24 hours a day, 7 days a week with an on-call system for additional research laboratory support when needed. The research staff will follow subjects from admission throughout their hospitalization. To reduce the volume of blood phlebotomized from patients, research nurses will only sample the even or odd number time points from individual patients. For example if a patient is sampled according to even number sampling times, blood will be drawn at time 0, 20 min, 1 hr, 2 hr, 4 hr, 6 hr, 8 hr, and12 hr. A patient sampled on odd number sampling times will have samples drawn at time 0, 10 min, 40 min, 1.5 hr, 3 hr, 6 hr, 10 hr and 24 hr.

Each blood sample will be collected into a standard citrate tube, centrifuged and the supernatant (plasma) will be stored at -80C before analysis. Samples (stripped of any identifiers and only containing the subject's study number) will later be shipped on dry ice to our collaborating site at Emory for full analysis. TXA will be extracted from plasma samples using solid phase microextraction, and the concentrations will be measured using tandem liquid chromatography- mass spectrometry [77]. Pharmacokinetic data will be analyzed with NONMEM, using both the first-order and conditional non-Laplacian (with centering) estimation techniques. We will consider two- and three-compartment models, parameterized in terms of both compartment volumes and clearances (distribution and elimination). We will compare a basic model (in which pharmacokinetic parameters were independent of weight) to a model in which the pharmacokinetic parameters will be assumed to be proportional to weight. The optimal model will be selected on the basis of the objective function logarithm of the likelihood of the results) using standard criteria (NONMEM guide). Blood is collected into 2.0 buffered sodium citrate tubes (0.109M-3.2 %), and tubes are inverted 5 times to ensure proper mixing with the anticoagulant. Plasma is separated by centrifugation at 4000x g for 10 minutes at 4^0C and stored at -80^0C until analysis.

Aim 2 Statistical Analysis: The pharmacokinetic and pharmacodynamic (henceforth, PK/PD) analysis will be performed using pooled data from the entire study population. This will be accomplished by nonlinear mixed effects modeling, using the software package NONMEM (version 7.2.0). The basic features of NONMEM may be best understood by considering a typical PK experiment. In the typical pharmacokinetic experiment, the drug is given to a subject, blood samples are drawn at defined intervals after dosing, the blood concentration is determined as a function of time, and a compartment model is then "fit" to the data to derive pharmacokinetic parameters for individual patients. To understand pharmacokinetic variability among patients, this same approach will be repeated in multiple subjects. One can then simply calculate the mean of the individual pharmacokinetic parameters and also their variances or standard deviations. This approach is called the two-stage method and it is laborious, time consuming, and logistically challenging since it requires enough samples from each patient to determine their individual PK parameters. An alternate approach to determining the pharmacokinetic parameters for multiple patients and then averaging them is to draw a small number of samples from a large number of patients (after drug administration) and then perform what is termed a "mixed-effects" analysis on the pooled data. The difference between the observed and the predicted concentration is attributed to 1) measurement error and 2) the individual variation of the patient's pharmacokinetic parameters from the mean value for the population. For any observation, we can define the "likelihood" of the difference of the observed concentration and that predicted by the model. The likelihood is essentially the probability that if the model were true given the present values of the model parameters, the present observations would have been observed. If the observations are quite unlikely for the present model, then the present model is not a very good description of the observations. If the observations are quite likely for the present model, then the present model is most likely a valid explanation of the data.

We will begin our analysis comparing one, two, and three compartment models. The minimization of the objective function (in other words, the maximization of the above expression for the likelihood) is mathematically and computationally complex. We will select as parameters for the PK model the compartment volumes and intra-compartmental and elimination clearances. The one compartment model would be characterized by a single compartment volume and the elimination clearance (two "structural" parameters), the two-compartment model by the central and peripheral compartment volumes, the intra-compartmental clearance, and the elimination clearance (four structural parameters), and comparably for the three-compartment model (which will have six structural parameters). We will compare the minimal objective function for the one, two, and three compartment models. Given that the objective function has a χ^2 distribution, one would select a model with an additional parameter only if the objective function improves, i.e., decreases, by more than 3.84 to achieve significance at the p=0.05 level. Thus, we would select as our basic model the two-compartment model only if the objective function improves by more than 7.68 and similarly for selecting a three-compartment model rather than a two-compartment model. In addition to this basic statistical test, we will also reject more complex models if the minimization does not converge with a full covariance matrix (in general we can anticipate that parameters will not only have a variance, but that there will be covariance between parameters). We will also graphically analyze the models by plotting residuals (difference between predicted and observed concentrations) vs. time and predicted concentration to look for systematic (nonrandom) variation. With mixed-effect modeling one can determine empiric (post-hoc) Bayes estimates of individual patient parameters. These are the most probable parameter estimates given the estimate of the mean parameter, the variance of the parameter, and the observed drug concentrations in individual patients. This allows us to compare observed drug concentrations to drug concentrations predicted for the individual patient, as well as the drug concentrations predicted with mean parameters. We will reject more complex models if they result in a marked increase in residuals or systematic variation in the residuals.

In addition to determining the optimal basic model, the first stage of analysis will also require consideration of weight-based pharmacokinetic parameters. It is intuitive that pharmacokinetic parameters will be proportional with body mass in some manner. However, it is well known in PK analysis that if there is insufficient heterogeneity in the body mass of patients in the study group, weight-adjusted parameters lead to no better a fit of the data than non weight-adjusted parameters. After establishing the optimal compartmental model (1, 2, or 3 compartments), we will compare models in which we assume that compartment volumes and clearances are proportional to weight to those in which weight is not a factor.

Following determination of the optimal compartment model and whether we need weight-based modeling, we will explore the role of covariates. We will evaluate as potential covariates age, degree of shock as assessed by admission lactate and StO2, presence of renal failure, and blood products administered using this approach of graphical analysis of the relationship between the covariate and the specific PK parameter and then formal modeling of the parameter as a linear function of the covariate. In summary our PK/PD analysis will entail the following methods and steps

1. Use of pooled data and nonlinear mixed effects modeling using NONMEM
2. Initial analysis using the first-order approximation and estimation algorithm
3. Determination of the optimal compartment model (1, 2, or 3 compartments parameterized in terms of compartment volumes and clearances).
4. Selection of the optimal model using the χ^2 distribution of the objective function and also graphical inspection of the residuals for detection of systematic variation.
5. Evaluation of weight-based analysis.
6. Analysis of covariates (age, lactate, StO2, and volume of blood products) by graphical analysis of the relationship between the relevant η and the covariate and then inclusion of the covariate in the formal model and rejection of the covariate as significant based on the χ^2 distribution of the objective function.
7. Analysis of pharmacodynamic models using a sigmoid-E_{max} model and nonlinear mixed effects modeling.

Aim 3: To collect safety and clinical outcomes data on TXA use in patients with traumatic injuries.

To measure safety and outcome data in this RCT of 150 patients with severe trauma we will:

a. Determine the incidence of thromboembolic events (DVT, MI, PE, Stroke) in all three study groups. Subjects will be assessed daily by the study team (while in the hospital) for up to 30 days after receiving study drug for any signs and symptoms of a thromboembolic event (i.e. shortness of breath, chest pain, extremity swelling, fever of unknown etiology, etc.) In addition, the study team will further ensure the safety of our subjects by screening all subjects at day 7 or hospital discharge (if prior to 7 days) for DVT using duplex ultrasonography. Stroke, MI, and PE will each be diagnosed clinically according to standard definitions (see appendix A).

b. Determine the incidence of seizures at 24 hours in all three study groups.

c. Determine the incidence of all adverse events in all three study groups. Adverse events will be captured daily while subject remains in the hospital or for up to 30 days, (whichever comes first) and will be characterized according to severity, relatedness, duration, and resolution.

d. Compare mortality, mechanical ventilation and ICU free days, incidence of multiple organ failure, AKI, ARDS and sepsis in all three study arms.

e. Correlate mechanistic data collected with clinical outcomes measured.

Seizure, thromboembolic events, and all adverse events will be captured from the time of study drug administration until discharge/transfer from the hospital or for up to 30 days (whichever comes first). ICU and ventilator free days will be calculated according to a 30-day model.

Aim 3 Statistical Analysis: The incidence of thromboembolic events will be determined at 7 days or on the day of hospital discharge if the hospital stay is less than 7 days. Incidence of seizures will be determined 24 hours from time of randomization. We expect that approximately 20% of the sample will being diagnosed with a thrombotic event based on previous work in a similar cohort of routinely screened transfused trauma patients by Spinella [28]. Because most of these events will be asymptomatic, we will not be able to determine the specific day on which the event occurred. Thus, we will be unable to use survival models for this outcome. Instead, chi square tests will provide tests of the null hypothesis that rates are identical across groups. These will be followed by logistic regression analyses that adjust for appropriate and a priori determined covariates. To be conservative, subjects who die before 7 days will be assumed to have had a thrombotic event in our primary analysis. While there is no published literature on the risk of seizure in a cohort of transfused trauma patients, we estimate that it will occur in approximately 2% of the study population. Because we will know the day on which this occurred, survival methods will be applied to this variable, with the subject being censored on day 7 or the day of death or hospital discharge when either occurs prior to 7 days. Log rank tests will be used to compare survival curves across groups and Cox regression analyses will provide an adjustment for covariates. Secondary outcomes in this aim include mortality, which will be evaluated using the log rank test and Cox regression and the incidence of multiple organ failure and sepsis, which will be assessed using chi square tests and logistic regression. We will most likely apply Poisson regression models to outcomes such as the number of severe and related adverse events. However, a final determination of the appropriate method will have to await an evaluation of the pattern and frequency of occurrence of these events.

Aim 4: To develop a repository of blood samples for future analysis.

Blood samples will be drawn at time 0, 1, 6, 24, and 72 hours, centrifuged and stored at -80 degrees Celsius.

These banked samples would be analyzed for measures of coagulation and endothelial function to include but not limited to: Complement, CH50 measured at time 0, 6, 24 and 72 hours; Endothelial activation and injury, Soluble Flt-1, soluble E-Selectin, VCAM-1, and PAI-1, Ang1/Ang2, Syndecan 1, Syndecan 2 and vWF measured at time points 0, 1,6, 24 hours. Bank samples for future analysis at times 0, 1, 6, and 24 hours; Inhibition of plasmin activity by TXA: D-dimer, PAP, alpha2-antiplasmin, TAFI, clot lysis (ROTEM), Activation and inhibition of fibrinolysis: tPA and PAI-1, Activation of the coagulation system: thrombin generation assays, TAT complexes, antithrombin, fibrinogen, fibrin monomer, fibrinopeptide A and B, Factor XIII, Factor XI.

Adverse Events

TXA has been used for decades and possesses a well-established safety profile. While there have been rare cases of thromboembolic events and seizures potentially associated with high dose (> 100mg/kg) intravenous TXA administration, there is no evidence that the TXA treatment regimens proposed in this trial (maximum 57mg/kg for a 70 kg patient) will be associated with an increased risk of thromboembolic events or seizures. We will collect data on these events and will report such occurrences to the DSMB according to the DSMP (see Appendix B). Emergency un-blinding will be immediately available if deemed necessary by the clinical care team, DSMB, or Investigators for the safety or treatment related decisions during the course of the study. The IDS pharmacist will be able to un-blind in such situations.

Adverse events will be collected daily on all subjects from study drug administration until hospital discharge or up to 30 days, (which ever comes first).

Adverse Event Definitions

- **Adverse event (AE):** is any untoward medical occurrence in a study subject. An AE can therefore be any unfavorable and unintended sign, symptom or disease temporally associated with the use of an investigational product, whether or not considered related to the investigational product.
- **Suspected Adverse Reaction:** is any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For IND safety reporting reasonable possibility means that there is evidence to suggest a causal relationship between the drug and the adverse event.
- **Adverse Reaction:** any adverse event caused by a drug.
- **Serious Adverse Event:** an adverse event is considered serious if it results in any of the following outcomes:
    - Results in death
    - Considered to be life-threatening
    - Requires hospitalization or prolongation of existing hospitalization
    - Results in persistent or significant disability or incapacity
    - Results in a congenital anomaly or birth defect
- **Life-threatening** refers to an event in which the subject was at risk of death at the time of the event; it does not include an event that might have caused death if it were more severe. All other events that are considered medically serious by the investigator should also be reported.

Adverse Event Assessment and Reporting

The relationship of the investigational product to the adverse event and severity of the event must be determined using the following classifications:

- **Relationship** to investigational product characterized as:
    - Unrelated – if there is not a reasonable possibility that the study drug caused the AE.
    - Unlikely – suggesting that only a remote connection exists between the study drug and the event.
    - Possible and Probable – suggesting that there exists a reasonable temporal sequence of the AE with the study drug.
    -
- **Severity:** The following definitions should be used to determine the severity rating of all AEs:
    - Mild: Awareness of signs or symptoms, but these are easily tolerated and are transient and mildly irritating only. There is no loss of time from normal activities, and symptoms do not require medication or a medical evaluation.
    - Moderate: Discomfort enough to cause interference with usual activities or require therapeutic intervention, such as concomitant medication.
    - Severe: Incapacity with inability to work or do usual activities.

All AEs occurring during the study are to be followed up in accordance with GCP guidelines and will be followed until resolved; or if a chronic condition, until fully characterized.

All Suspected Unexpected Serious Adverse Drug Reactions (SUSARs) will be subject to expedited reporting. Additionally, post-study SUSARs that occur after the subject has completed a clinical study and are reported by the Investigator to the Sponsor (or an authorized representative) qualify for expedited reporting.

Suspected, unexpected serious adverse drug reactions (SUSARs) will be reported to the FDA, and IRBs within the required timeframes of seven calendar days for SUSARs which are fatal or life-threatening, and fifteen calendar days for all other SUSARs.

Study Drug Discontinuation Rules: Any thromboembolic event (MI, PE, Ischemic stroke, or symptomatic DVT) or obvious seizure activity while the study drug is being infused will warrant immediate cessation of the study drug administration and a medical safety and DSMB review.

Written IND Safety Reports : A written IND Safety Report (i.e., completed FDA Form 3500 A) will be reported to the responsible new drug review division of the FDA for any observed or volunteered adverse event that is determined to be a *serious and unexpected, suspected adverse reaction*. Each IND Safety Report will be prominently labeled, "IND Safety Report", and a copy will be provided to all participating investigators (if applicable) and sub-investigators.

Written IND Safety Reports will be submitted to the FDA as soon as possible and, in no event, later than 15 calendar days following the Investigator's receipt of the respective adverse event information and determination that it meets the respective criteria for reporting.

For each written IND Safety Report, the Investigator will identify all previously submitted IND Safety Reports that addressed a similar suspected adverse reaction experience; and will provide an analysis of the significance of newly reported, suspected adverse reaction in light of the previous, similar report(s), or any other relevant information.

Relevant follow-up information to an IND Safety Report will be submitted to the applicable review division of the FDA as soon as the information is available; and will be identified as such (i.e., "Follow-up IND Safety Report").

If the results of the Investigator's follow-up investigation show that an adverse event that was initially determined to not require a written IND Safety Report does, in fact, meet the requirements for reporting; the Investigator will submit a written IND Safety Report as soon as possible—but in no event later than 15 calendar days—after the determination was made.

Telephoned IND Safety Reports – Fatal or Life-Threatening Suspected Adverse Reactions: In addition to the subsequent submission of a written IND Safety Report (i.e., completed FDA Form 3500A), the Investigator will notify the responsible review division of the FDA by telephone or facsimile transmission of any *unexpected, fatal or life-threatening suspected adverse reaction.*
The telephone or facsimile transmission of applicable IND Safety Reports will be made as soon as possible, but in no event later than 7 calendar days after the Sponsor-Investigator's receipt of the respective adverse event information and determination that it meets the respective criteria for reporting.

Reporting Adverse Events to the Responsible IRB: In accordance with applicable policies of the Washington University, St Louis Institutional Review Board (IRB), the Sponsor-Investigator will report, to the IRB, any observed or volunteered adverse event that is determined to be: 1) *associated with the investigational drug or study treatment(s)*; 2) *serious*; and, 3) *unexpected*. Adverse event reports will be submitted to the IRB in accordance with the respective IRB procedures.

Applicable adverse events will be reported to the IRB as soon as possible and, in no event, later than 10 calendar days following the sponsor-investigator's receipt of the respective information. Adverse events which are 1) *associated with the investigational drug or study treatment(s)*; 2) *fatal or life-threatening*; and 3)

unexpected will be reported to the IRB within 24 hours of the Sponsor-Investigator's receipt of the respective information.

Follow-up information to a reported adverse event will be submitted to the IRB as soon as the relevant information is available. If the results of the Sponsor-Investigator's follow-up investigation show that an adverse event that was initially determined to not require reporting to the IRB does, in fact, meet the requirements for reporting; the Sponsor-Investigator will report the adverse event to the IRB as soon as possible, but in no event later than 10 calendar days, after the determination was made.

Human Protections: This trial will be conducted under the Department of Health and Humans Services 21 CFR 50.24 Exception from Informed Consent for Emergency Research. As such, we will submit this protocol to our local Institutional Review Board for their review and approval in concert with a Community Consultation Proposal (see Appendix D). The trial Investigators will also submit an IND application to the FDA according to applicable regulation since TXA is not FDA approved for use in trauma patients. All key personnel involved in the design or conduct of the research involving human subjects will receive the required education on the protection of human research participants prior to conducting this study. Patients' risks of participating in research are kept to a minimum with measures to protect confidentiality and safety monitoring. Benefits outweigh risks as TXA has been shown to improve survival in 2 prior studies and all patients enrolled in this study will have additional assessments performed, including a duplex ultrasound on hospital day 7 or at discharge (whichever comes first), which may identify thrombus formation in this high risk population that otherwise may not have been identified. In addition, we will disseminate the results of this clinical trial to the community and medical community as soon as possible.

Community Consultation: The content of community consultation will inform the communities that informed consent will not be obtained for most (or all) research subjects. Specifically, the goal will be to; inform members of the surrounding communities about all relevant aspects of the study including its risks and expected benefits, hear the perspective of the communities on the proposed research and address questions and concerns, and to provide information about ways in which individuals wishing to be excluded may indicate this preference (i.e. "opt out" bracelets). The type and frequency of community consultation will; provide opportunities for broad community discussion, ensure that representatives from the community(ies) involved in the research participate in the consultation process, use the most appropriate ways to provide for effective community consultation, and be based on numerous factors, including the size of the community(ies), the languages spoken within those communities, the targeted research population and its heterogeneity. We will utilize focus groups, clubs/associations that may include members at high risk for trauma with blood loss, and use web-based outreach and surveys as mechanisms to provide community consultation. Public disclosure methods will include newspaper, posters, internet based message boards, local magazines, etc. as sources. We will supply a mechanism for as many members of the community to "opt out" of participation in this trial (i.e. "opt out" bracelets). The information collected from community consultation will be compiled and reports completed and made available to trial site IRBs, DoD HRPO, and the FDA. Please see Appendix D for the complete Community Consultation Proposal. Once WU IRB approval and DoD HRPO approval have been obtained, we will implement the Public Disclosure Plan (attached in Appendix E).

DSMB: A DSMB will be established that will confidentially review interim/cumulative data for evidence of study-related adverse events and for quality, completeness, and timeliness. The DSMB established for the TAMPITI trial will monitor the implementation and safety of this study. DSMB membership of this advisory committee will consist of experts not involved in the planning or the conduct of TAMPITI and will be established by the Trial Investigators. Trial Investigators will convene the DSMB and provide an executive summary. DSMB membership will be comprised of an, anesthesiologist, emergency department intensivist, a blood bank specialist, and a pediatric hematologist/oncologist. The DSMB Chair will also serve as the Research Monitor. She will facilitate discussion, integrate differing points of view and move toward consensus on recommendations to be provided to the DoD, FDA and Trial Investigators. The Data Safety Monitoring Plan (DSMP) with the full DSMB charter is attached in Appendix B.

Research Monitor:

For research determined to be greater than minimal risk, DoDI 3216.02 requires that the IRB approve, by name, an independent research monitor with expertise consonant with the nature of risk(s) identified within the research protocol. We have appointed Jessica Zenga, MD to the role of the Research Monitor. Dr. Zenga is a physician within the Department of anesthesia. Her duties will include but may not be limited to the following:

1. Discussing the research protocol with the investigators;

2. Be the Data Safety Monitoring Board (DSMB) Chair and schedule meetings;

3. Speak with human subjects to ensure ongoing understanding of study related procedures and their continued interest in participating or withdrawing from study, serving as their advocate;

4. Shall have authority to stop a research protocol in progress, remove individual human subjects from a research protocol, and take whatever steps are necessary to protect the safety and well-being of human subjects until the IRB can assess the monitor's report;

5. Shall have the responsibility to promptly report their observations and findings to the IRB or other designated official and the HRPO.

Subject Withdrawal: Subjects will be enrolled into this study under an exception from informed consent requirement for emergency research. Informed consent will be obtained, when possible, by the subject or legally authorized representative (LAR). The subject and LAR will be advised by the research team of their right to withdraw from study participation at any time without penalty or adverse affect on their routine medical care. However, subjects will be encouraged to comply with all safety evaluations to ensure their safety. If a subject elects to withdraw from the study they will be informed that all data obtained to this point will be maintained for data analysis purposes but no further study procedures will take place.

Sponsor-Investigator Discontinuation of the Clinical Research Study: Both the Investigator and the DoD reserve the right to terminate the study at any time. Should this be necessary, the procedures will be arranged on an individual study basis after review and consultation with both parties. In terminating the study, the DoD and the Investigator will ensure that adequate consideration is given to the protection of the subjects' interests and safety. The WUSL IRB will be notified immediately (verbally and in writing) of this decision as will all study participants.

Record maintenance and retention: The Investigator will maintain all case report forms and all source documents that support the data collected from each subject; and all trial documents, as specified by applicable regulatory requirement(s). The Investigator will take measures to prevent accidental or premature destruction of these documents.

Essential documents must be retained for at least 2 years after the last approval of a marketing application worldwide, or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents will be retained for a longer period if required by the applicable regulatory requirements. If the responsible Investigator retires, relocates, or for other reasons withdraws from the responsibility of keeping the study records, custody must be transferred to a person who will accept the responsibility.

The Investigator will retain the specified records and reports for up to 2 years after the marketing application is approved for the investigational drug; or, if a marketing application is not submitted or approved for the investigational drug, until 2 years after investigations under the IND have been discontinued and the FDA so notified.

Data Handling: A Case Report Form (CRF, see Appendix 1) will be completed for each subject enrolled into the clinical study. The Investigator will review, approve and sign/date each completed CRF; the Investigator's signature serving as attestation of the Investigator's responsibility for ensuring that all clinical and laboratory data entered on the CRF are complete, accurate and authentic.

Source Data are the clinical findings and observations, laboratory and test data, and other information contained in *Source Documents*. *Source Documents* are the original records (and certified copies of original records); including, but not limited to, hospital medical records, physician or office charts, physician or nursing notes, subject diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, x-rays, etc. When applicable, information recorded on the CRF shall match the *Source Data* recorded on the *Source Documents*.

Review of Research Records:

Representatives of the Under Secretary of Defense (Personnel & Readiness) are authorized to review research records as part of their responsibility to protect human research volunteers. In the event that a HIPAA authorization is required, include the above as parties to whom private health information may be disclosed.

Appendix A

Acute Kidney Injury is defined as an abrupt (within 48 hours) reduction in kidney function based on an elevation in serum creatinine level, a reduction in urine output, the need for renal replacement therapy (dialysis), or a combination of these factors. It is classified in three stages:

STAGE	CHANGE IN SERUM CREATININE LEVEL	URINE OUTPUT	OTHER
1	Increase ≥ 0.3 mg per dL (26.52 µmol per L) or ≥ 1.5- to twofold from baseline	< 0.5 mL per kg per hour for more than six hours	—
2	Increase > two- to threefold from baseline	< 0.5 mL per kg per hour for more than 12 hours	—
3	Increase > threefold from baseline or ≥ 4.0 mg per dL (353.60 µmol per L) with an acute rise of at least 0.5 mg per dL (44.20 µmol per L)	< 0.3 mL per kg per hour for 24 hours or anuria for 12 hours	Renal replacement therapy required

NOTE: *Each stage is defined by the change in serum creatinine level, the change in urine output, or the need for renal replacement therapy*.

Mehta RL, Kellum JA, Shah SV, et al. Acute Kidney Injury Network: report of an initiative to improve outcomes in acute kidney injury. Crit Care. *2007;11(2):R31*.

Stroke defined as by the development of a new neurological deficit identified on clinical examination correlating with CT scan or MRI findings of cerebral infarction in an anatomic location corresponding to the newly identified deficit.

Myocardial infarction will be defined by the Universal Definition of Myocardial Infarction. Either of the following criteria meets the diagnosis: Detection of rise and/or fall of cardiac biomarkers (preferably troponin) with at least one value above the 99th percentile of the upper reference limit together with evidence of myocardial ischemia with at least one of the following, symptoms of ischemia, EKG changes indicative of new ischemia (new ST-T changes or new left bundle branch block (LBBB)), development of pathologic Q waves in the EKG, Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality, and identification of an intracoronary thrombus by angiography or autopsy.

Pulmonary embolism will be defined by any of the following, computed tomography angiography of the thoracic vasculature demonstrating thrombus in the pulmonary arterial vessels, a ventilation/perfusion scan interpreted as having high probability for pulmonary embolism in the setting of new onset tachycardia or hypoxemia, death with autopsy confirmation of the presence of a pulmonary arterial thrombus or embolus.

Seizure is defined by the development of a new transient or persisting neurologic deficit identified on clinical examination and accompanied by electroencephalographic abnormality in an anatomic location corresponding to the newly identified deficit.

Multiple Organ Dysfunction Syndrome (MODS): progressive but potentially reversible physiologic dysfunction of 2 or more organ systems that arises after resuscitation from acute life threatening events.

Table 5: Multiple Organ Dysfunction Score (MODS)

Organ System Values	MODS Score					Normal Value Ranges
	0	1	2	3	4	
Haematologic: Platelet Count ($x10^3/mm^3$ or $10^9/L$)	> 120	81-120	51-80	21-50	≤ 20	*> 120*
Hepatic: Serum Bilirubin (µmol/L)	≤ 20	21-60	61-120	121-240	> 240	*≤ 20*
Renal: Serum Creatinine (µmol/L)	≤ 100	101-200	201-350	351-500	> 500	*≤ 100*
Cardiovascular: PAR	≤ 10	10.1-15	15.1-20	21-30	> 30	*≤ 10*
Glasgow Coma Score	15	13-14	10-12	7-9	≤ 6	*15*
Respiratory: PO_2 / FiO_2	> 300	226-300	151-225	76-150	≤ 75	*> 300*

Sepsis: As defined by the Surviving Sepsis Campaign[70], the definition of sepsis requires a systemic response to infection i.e confirmed infection plus 2 or more of the following criteria: Temperature > 38 °C or 36 °C, Heart rate > 90 beats/min, Respiratory rate > 20 breaths/min or PaCO 2 < 32 torr (< 4.3 kPa), WBC > 12000 cells/mm 3, < 4000 cells/mm3, or > 10% immature(band) forms.

ARDS: As defined by the Berlin Criteria published in JAMA June 20, 2012, Vol 307, No. 23.

Transfusion guidelines: If class IV shock or physician determination that patient will need massive transfusion (> 10U of RBCs in 6 hours) then the MTP will be initiated that follows all DCR principles as outlined by Spinella to include a 1:1:1 ratio of RBCs:Plasma:Platelets [29]. Cryoprecipitate will be administered if fibrinogen values are < 150 mg/dl. No factor concentrates such as rFVIIa, prothrombin or fibrinogen concentrates will be administered to patients in this trial. If a patient does not meet MTP criteria the following indications will be used for the following blood products when the patient has active bleeding:

-2 units of RBCs for Hb < 9mg/dl
-2 units of plasma for INR> 1.5
-1 unit of apheresis platelets for a platelet count < 100,000
-10 units of cryoprecipitate for fibrinogen concentration < 150mg/dl

DVT prophylaxis Protocol: Patients will be placed on Lovenox 40 mg SC daily unless renal impairment CrCl of <30mL/min. Patients with renal impairment (CrCl of < 30 mL/min) will receive an alternative prophylaxis at the discretion of the clinical care team. This information will be captured in the case report form.

Appendix B **Data Safety Monitoring Plan:**

Patient safety is of paramount importance in this trial and there is an extensive set of procedures in place for monitoring adverse events. These procedures are as follows:

Data Safety Monitoring Board (DSMB)

A Data Safety Monitoring Board (DSMB) appointed by Drs. Bochicchio and Spinella, Clinical Principal Investigators of the study, will meet once a month (either by teleconference or face to face when possible) to review the progress of this study (e.g., enrollment, site performance) as well as data on the safety of each arm of the study. Before the study begins, the DSMB (in consultation with the study statistician and the PIs) will decide the content of the reports to be presented to the board. Using guidelines established by the DSMB and the investigators before the study begins, the DSMB may recommend termination of the study if either of the treatment arms (2 gram TXA or 4 gram TXA) is found to be unsafe. Additionally, the DSMB may recommend modifications to the protocol if a correctable safety issue is identified. After each meeting, the DSMB Chair will prepare a letter to the study principal investigators, Drs. Grant Bochicchio and Phillip Spinella, which will describe the safety review that took place at the meeting and that indicates whether or not there are any safety concerns. This letter will be provided to the IRB according to their policies and procedures.

We have appointed an experienced clinician for the study (Dr. Jessica Zenga). Dr. Zenga will review all adverse events in the study as they occur. Dr. Zenga will report safety concerns that arise during the trial to the PIs and study team. Dr. Zenga will lead the DSMB meetings and will discuss any concerns about adverse events. Dr. Zenga has considerable experience with clinical trial safety and is serving on other DSMBs for research trials being conducted in our institution.

Principal Investigators

Drs. Bochicchio and Spinella will be informed of the DSMB's monthly assessment of study performance and safety.

Data Safety Monitor Board Members:

* Dr. Jessica Zenga, Data Safety Monitoring Chair/Research Monitor
Dr. Enyo Ablordeppey
Dr. George Despotis
Dr. Melanie Fields

Events to be Reviewed by DSMB:

- All adverse events (AEs), regardless of relationship to study drug will be reviewed once a month by the DSMB
- All SAE's will be reviewed by the PI and Research Monitor within 24 hours of their occurrence.
- All ***Unexpected ,Serious Adverse Events*** (SUA'S) thought to be related to study drug will be reported to the PI and Data Safety Monitor and IRB immediately. Emergency medical treatment will be provided as necessary

Stopping Rules:

Subjects who experience any thromboembolic event (MI, PE, Ischemic stroke, or symptomatic DVT) or obvious seizure activity while the study drug is being infused will warrant immediate cessation of the study drug administration and a medical safety/DSMB review. The Research Monitor can temporarily place the trial on hold until the DSMB can confer to discuss any study concerns.

Appendix C TAMPITI Schedule of Events	Screening	Hour 0	Baseline	10 Min	20 Min	40 Min	1 HR	1.5 HR	2 HR	3 HR	4 HR	6 HR	8 HR	10 HR	12 HR	24 HR	72 HR	Day 7[f]	During Hospital Admission	Hospital Discharge
Informed Consent	X																			
Demography	X																			
Medical/Surgical History	X																			
Inclusion/Exclusion	X																			
Physical Exam	X															X	X	X	X	X
Vital Signs	X		X													X	X	X	X	X
Immune Parameters[a]		X										X				X	X			
Pharmacodymanic/ Pharmacokinetic Odd Schedule[b]		X		X		X		X		X		X		X		X				
Pharmacodymanic/ Pharmacokinetic Even Schedule[b]		X			X		X		X		X	X	X		X					
Repository Samples[c]		X					X					X				X	X			
Study Drug Administration			X[d]																	
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X
Adverse Events			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X[g]
Data Capture[e]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X
Clinical Outcomes																X			X	X
DVT Screen by Duplex																		X		

[a] Cytokines: TNF-α, IL-6, IL-10, and IFN-γ and leukocytes (CD66+/ROS+ , CD4+/CD69+ and CD8+/CD69+ , CD14+/HLA-DR+ , CD4+/Foxp3+) measured at time 0, 6, 24 and 72 hours. 5mL per sample for a total of 20mL

[b] 2mL per sample for a total of 16mL

[c] 15mL per sample for a total of 75mL

[d] Administered within 2 hours from known injury time. Any thromboembolic event (MI, PE, Ischemic stroke, or symptomatic DVT) or obvious seizure activity while the study drug is being infused will warrant immediate cessation of the study drug administration and a medical safety and DSMB review

[e] Daily assessments for up to 30 days after receiving study drug for any S/S of thromboembolic event (i.e. shortness of breath, chest pain, extremity swelling, fever of unknown etiology, etc. in addition to collecting data on blood product administration, clinical lab findings, and other relevant clinical data.

[f] Or hospital discharge whichever occurs first.

[g] Adverse events will only be collected through day 30 or hospital discharge, whichever occurs first.

Appendix D

TAMPITI

Community Consultation Plan for additional protections associated with 21 CFR 50.24, Exception from Informed Consent (EFIC) Requirements for Emergency Research for:

Tranexamic Acid Mechanisms and Pharmacokinetics in Traumatic Injury (TAMPITI TRIAL)

IRB PROTOCOL NUMBER: TBD

Supported by:
The Department of Defense
DoD Award: W81XWH-14-1-0373

FDA IND#: TBD

Investigators: Philip Spinella, MD
Grant Bochicchio, MD, MPH

Introduction

This proposal is intended to provide a logistical outline for the implementation of the additional protections associated with 21 CFR 50.24, Exception from Informed Consent (EFIC) Requirements for Emergency Research in communities in the St. Louis and surrounding areas. The implementation of this plan is pending review and approval from the Washington University in St. Louis Human Research Protections Office and IRB. Additional resources may be requested to assist in the review of this plan and are available upon request from the Principal Investigators, Philip Spinella, M.D. and Grant Bochicchio, MD, MPH.

Trauma is the leading cause of death in persons younger than 40 years. Hemorrhage is the etiology in 30% of these deaths, and remains the leading cause of potentially preventable mortality (66-80%) on the battlefield[1]. As a result, the prevention of death from hemorrhagic shock has been the subject of intensive research, development funding and effort. Death secondary to hemorrhagic shock occurs from both surgical bleeding and coagulopathy. Acute traumatic coagulopathy is characterized by a hypocoagulable state, where the net balance of coagulation is such that there is low clot forming capacity and strength, which does not allow for adequate hemostasis. Acute traumatic coagulopathy occurs **early** in patients who are in shock from hypoperfusion and is not due to coagulation factor consumption or dysfunction because of acidosis, moderate hypothermia, or dilution[2]. However shock (oxygen debt) itself is associated with a coagulopathy that is due to the systemic activation of anticoagulant and fibrinolytic pathways[1, 3]. We have demonstrated previously that the protein C pathway is implicated in this process, and that fibrinolysis is mediated by de-inhibition of tPA through PAI-1 consumption[2]. Low levels of PAI-1, in combination with increased plasminogen activator release from the vessel wall contributes to hyperfibrinolysis. It has been suggested that TAFI is the main driver of fibrinolysis inhibition, and that reduction in TAFI activation by the competitive binding of protein C to T-TM is the mechanism for increased fibrinolysis with activation of protein C [1].
Due to the knowledge of increased fibrinolysis promoting a hypocoagulable state in severe trauma, trials have been performed to determine if antifibrinolytics such as tranexamic acid (TXA) could reduce morbidity and mortality by reducing death from hemorrhage[4]. TXA is an antifibrinolytic that inhibits both plasminogen activation and plasmin activity, thus preventing clot break-down rather than promoting new clot formation. TXA occupies the lysine-binding sites on plasminogen, therefore preventing its binding to lysine residues on fibrin. This reduces plasminogen activation to plasmin. Similarly, blockade of lysine-binding sites on circulating plasmin prevents binding to fibrin and thus prevents clot break-down. TXA is excreted largely unchanged in urine and has a half-life of approximately 2 hours in circulation when studied in patients without traumatic injury. Intravenous administration of TXA was approved by the FDA in 1986 for the prevention or reduction of bleeding in patients with hemophilia undergoing dental procedures. The FDA approved use of the oral form of TXA to control heavy menstrual cyclic bleeding in 2009. Despite the extensive use of TXA in many elective surgical populations and an increasing use in severe trauma patients, TXA does not have an FDA approved indication for patients with traumatic injuries[1]. Unfortunately, severe trauma patients are unable to participate in the informed consent process. They must be included in this study despite their inability to provide consent for themselves as their trauma induced coagulopathy makes them unique. Nearly one in four severely injured patients arriving to the ED experiences trauma induced coagulopathy and its presence is associated with a four-fold increase in mortality.(81-83) The coagulopathy of trauma occurs due to several factors including hypothermia, acidosis, loss of clotting factors through hemorrhage and hemodilution, in addition to the body's use and subsequent depletion of both platelets and clotting factors.(79) Dilutional coagulopathy occurs when trauma patients who are bleeding are resuscitated with fluid or blood products that don't contain the same clotting factors lost in the acutely hemorrhaged whole blood.(80) Furthermore, in the critically injured trauma patient, a complex series of enzymatic reactions

occur which can cause the clotting cascade to become abnormally activated, leading to excessive clot formation and subsequent breakdown (fibrinolysis) out of proportion to the injury.(79) This abnormal and disproportionate activation of the coagulation system quickly consumes the rest of the body's clotting factors, resulting in a further lack of essential factors required to achieve hemorrhage control.

In addition, trauma patients may also have a baseline coagulopathy because of preexisting medical conditions. For example, patients may be on anticoagulant therapy such as warfarin (Coumadin) or dabigatran for stroke prevention in the setting of atrial fibrillation. These patients and those with chronic liver or renal failure have an increased risk of developing a truly life-threatening coagulopathy and hemorrhage after trauma.(84) Therefore, these unique and critical physiologic characteristics set trauma patients apart from routine and elective surgical patients and must be evaluated independently to truly determine appropriate dose treatment and outcome, such is planned in this study.

As a result, any clinical trial which aims to prospectively evaluate TXA in this population will require an Investigational New Drug application (IND) approval from the FDA before being implemented.

Widespread interest in using TXA for severely injured trauma patients occurred immediately following the published results in 2010 of the landmark CRASH-2 (Clinical Randomization of an Antifibrinolytic in Significant Hemorrhage). [5] More recently, data from the Military Application of Tranexamic Acid in Trauma Emergency Resuscitation Study (MATTERs) has been published, providing perspective from a different and likely more severely injured patient population[6]. MATTERs is a retrospective observational study that evaluated data from the Camp Bastion Surgical Hospital in Afghanistan. Despite both the CRASH-2 trial and MATTERS studies indicating TXA use reduced the risk of mortality, questions regarding mechanisms of action and optimal dosing still remain. The CRASH-2 trial reported a reduced risk of death with treatment occurring within 3 hours of injury **but increased risk of death from hemorrhage if given 3 hours after injury**. The MATTERs study demonstrated an unadjusted increased risk of thromboembolic events for those treated with TXA.

Therefore the analysis of multiple bolus doses (within range of current practice) on mechanisms and pharmacokinetics is important and especially relevant to the practical application of TXA in austere military environments. It is critical for formal and thorough pharmacokinetic analyses to be done in patients with traumatic injury with active bleeding since this population has very different physiology than elective surgery patients. The pharmacokinetic analysis of multiple bolus doses in addition to mechanistic, outcomes and safety data will inform the medical community regarding the most appropriate dosing of TXA in the acutely injured, critically ill, trauma patient.

Research involving severely injured trauma patients presents an ethical dilemma. We recognize that the informed consent process is an important mechanism in the protection of patient autonomy in the conduct of proper clinical research. However, the process of *informed consent* cannot always be conducted prior to initiation of study procedures. In cases where patients are incapable of participating in the informed consent process, consent using a legally authorized representative (LAR) has often been substituted, even though the true wishes of the patient are rarely known. This has become an accepted practice in clinical research and is often adequate for most research studies proposed. However, for cases involving emergency research, the LAR is often not readily available. The delay in obtaining informed consent can therefore significantly impact the efficacy of an intervention and often excludes patients from inclusion in time-critical studies. Therefore, as we have explained, new treatments for severely injured trauma patients must be developed and failing to conduct research on potentially beneficial treatments for this population also poses harm.

APPLICABILITY OF EXCEPTION FROM INFORMED CONSENT REQUIREMENTS FOR EMERGENCY RESEARCH (EFIC) TO THE TAMPITI TRIAL AND COMPLIANCE WITH DHHS REQUIREMENTS (21 CFR 50.24 and DoD 10 USC 980)

The specific DHHS regulations for justification of research using EFIC process are listed below. Each regulation is followed by an explanation of how this study meets these requirements.

1. Human subjects are in a life-threatening situation that necessitates urgent intervention;

Patients with severe traumatic injuries are in a life-threatening situation requiring urgent intervention

Trauma is the leading cause of death in persons younger than 40 years. Hemorrhage is the etiology in 30% of these deaths, and remains the leading cause of potentially preventable mortality (66-80%) on the battlefield[1]. As a result, the prevention of death from hemorrhagic shock has been the subject of intensive research, development funding and effort. Death secondary to hemorrhagic shock occurs from both surgical bleeding and coagulopathy. Acute traumatic coagulopathy is characterized by a hypocoagulable state, where the net balance of coagulation is such that there is low clot forming capacity and strength, which does not allow for adequate hemostasis. Acute traumatic coagulopathy occurs early in patients who are in shock from hypoperfusion and is not due to coagulation factor consumption or dysfunction because of acidosis, moderate hypothermia, or dilution[2]. However shock (oxygen debt) itself is associated with a coagulopathy that is due to the systemic activation of anticoagulant and fibrinolytic pathways[1, 3]. We have demonstrated previously that the protein C pathway is implicated in this process, and that fibrinolysis is mediated by de-inhibition of tPA through PAI-1 consumption[2]. Low levels of PAI-1, in combination with increased plasminogen activator release from the vessel wall contributes to hyperfibrinolysis. It has been suggested that TAFI is the main driver of fibrinolysis inhibition, and that reduction in TAFI activation by the competitive binding of protein C to T-TM is the mechanism for increased fibrinolysis with activation of protein C [1].

2. Available treatments are unproven or unsatisfactory;

Trauma remains the single greatest cause of mortality in individuals between the ages of 1 and 44, and hemorrhage is the leading preventable causes of death among trauma patients. Despite the enormity of the problem, scientists have not identified an effective pharmacological agent that improves outcomes following severe traumatic injuries and hemorrhage. TXA appears to have benefit in the prevention of deaths from hemorrhage following trauma, based on prospective civilian data from CRASH-2 as well as retrospective military data from MATTERs, and has been incorporated into current US Military clinical practice guidelines for trauma and combat casualty care. Despite the probable benefits, several gaps in knowledge exist with regard to the optimal use of TXA, which is of particular importance to military populations and will be directly addressed through the current study. Questions remain with regard to the mechanism of action of TXA in trauma. This has not been fully elucidated, and the specific effects of TXA on coagulation, immune and endothelial function have not been studied in depth. In addition, questions remain regarding the safe use of TXA both in terms of post-injury adverse events (thromboembolic complications, neurologic sequelae) as well as use in specific populations, namely, those patients with traumatic brain injury in the setting of hemorrhage, a pattern frequently seen in theater.

3. Collection of valid scientific evidence is necessary to determine the safety and effectiveness of the intervention;

In 2010, the results of the landmark CRASH-2 (Clinical Randomization of an Antifibrinolytic in Significant Hemorrhage) trial were published, creating widespread international interest [5]. This multicenter, multinational study randomized 20,211 adult trauma patients to either one gram of TXA infused over ten minutes followed by one gram of TXA infused over eight hours versus an equivalent volume of normal saline placebo administered within eight hours of injury. Inclusion criteria consisted of systolic blood pressure less than 90 mmHg and/or with a heart rate greater than 110 beats per minute, or patients deemed to be at risk of significant hemorrhage. The primary outcome measure was in-hospital death within four weeks of injury. Secondary outcomes included thromboembolic events (myocardial infarctions, cerebrovascular accidents, pulmonary emboli, and deep vein thromboses), surgical interventions, blood transfusions, and the total units of blood transfused. The study found a significant decrease in all-cause mortality (14.5 vs. 16.0%, p=0.0035) and deaths from bleeding (4.9 vs. 5.7%, p=0.0077) in patients receiving TXA compared to placebo. More recently, data from the Military Application of Tranexamic Acid in Trauma Emergency Resuscitation Study (MATTERs) has been published, providing perspective from a different and likely more severely injured patient population[6]. MATTERs is a retrospective observational study that evaluated data from the Camp Bastion Surgical Hospital in Afghanistan. Patients were included in the study if they sustained combat-related injury and subsequently received a minimum of one unit of packed red blood cells. The primary outcome measures were 24 and 48-hour as well as in-hospital mortality (which was inclusive of any mortality occurring within 30 days of injury). Secondary endpoints included transfusion requirement and correction of coagulopathy based on resolution of extended prothrombin and thromboplastin times between arrival in the emergency department and arrival in the intensive care unit postoperatively. Overall, 896 consecutive patients were analyzed, with 293 receiving TXA. Patients receiving TXA were more severely injured than those not receiving the drug (ISS 25.2 vs. 22.5, $p<0.001$), and a greater proportion presented with severe TBI and admission systolic blood pressure $\leq$ 90 mmHg. Patients receiving TXA were given more packed red blood cells, fresh frozen plasma, platelet, and cryoprecipitate transfusions than patients not receiving the drug. In spite of these admission characteristics, patients receiving TXA had lower overall 48-hour (11.3 vs. 18.9%, p=0.004) as well as in-hospital (17.4 vs. 23.9%, p=0.03) mortality. Additionally, although more patients who received TXA were hypocoagulable on admission, fewer TXA patients were hypocoagulable on arrival to the ICU, and there was a significant reduction in the proportion of hypocoagulable patients in the TXA group between admission and the ICU. While these data add to the beneficial profile of TXA, MATTERs also reported a greater number of pulmonary emboli (8 vs. 2, p=0.001) and deep vein thromboses (7 vs. 1, p=0.001) in TXA-treated patients.

Despite both the CRASH-2 trial and MATTERS studies indicating TXA use reduced the risk of mortality, **questions regarding mechanisms of action and optimal dosing still remain**. As a result, an in depth examination on TXA's mechanisms of action is warranted. **Since improved survival and adverse effects in both CRASH-2 and MATTERs related to death from hemorrhage and adverse thrombotic effects, a focused evaluation of the effects of TXA on coagulopathy utilizing tests designed to analyze coagulation with a detailed emphasis on fibrinolysis is essential**.

The assays proposed in this trial will offer a comprehensive analysis of potential TXA hemostatic modulation in an effort to gain a more thorough understanding of *in vivo* effects. Laboratory analysis will range from whole blood assays to quantify the speed and strength clot formation with comparison to fibrinolysis and plasma analysis aimed at establishing the balance of procoagulant to anticoagulant mediators. Investigations at the USAISR laboratory provide further insight in to the multidimensional nature of hemostasis, which involves a balance of mediators. Thus, pharmacological effects must be studied in the context of understanding the *in vivo* activity of specific mediators. Here we propose to evaluate not only procoagulant and anticoagulant levels, but also effector-inhibitor complexes, thrombin: antithrombin (TAT) and plasmin-antiplasmin complex (PAP), that measure binding and therefore indirectly reflect activity.

The CRASH-2 trial reported a reduced risk of death with treatment occurring within 3 hours of injury but

increased risk of death from hemorrhage if given 3 hours after injury. The MATTERs study demonstrated an unadjusted increased risk of thromboembolic events for those treated with TXA. In addition, TXA use in cardiac surgery has been associated with an increased risk of postoperative convulsive seizures. This finding appears to be temporally associated with TXA doses that are 2–10 fold higher than those used in CRASH-2. A proposed mechanism for seizures is the structural similarity of TXA to γ-aminobutyric acid, which has a potential to cause neurotoxicity. Therefore, the collection of safety and clinical outcome data in a RCT of TXA are critical since the CRASH-2 study did not capture detailed information for these outcomes.

The CRASH-2 study administered TXA as a 1gram IV bolus dose followed by 1gram infusion over 8 hours. However, in a trauma patient with potentially limited vascular access, a one-time IV bolus dose would be more practical (especially in austere military settings) and may facilitate increased plasma concentration in the immediate time period where hemostatic control is critical. **Therefore the analysis of multiple bolus doses (within range of current practice) on mechanisms and pharmacokinetics is important and especially relevant to the practical application of TXA in austere military environments.** It is critical for formal and thorough pharmacokinetic analyses to be done in patients with traumatic injury with active bleeding since this population has different physiology than elective surgery patients. The pharmacokinetic analysis of multiple bolus doses in addition to mechanistic, outcomes and safety data will inform the medical community regarding the most appropriate dosing of TXA in the acutely injured, critically ill, trauma patient.

4. Obtaining prospective informed consent is not feasible because the subjects are not able to give their informed consent as a result of their medical condition;

   - Subjects are not able to participate in the informed consent process due to the extent of their injury (i.e., altered mental status, shock, etc.).

5. The intervention must be administered before consent can be obtained from the subject's LAR.
   - The therapeutic window for TXA administration is within 2 hours of injury (documented by the patient's EMS report, police report or witness interview), This window was chosen based on The CRASH-2 trial which reported a reduced risk of death with treatment occurring within 3 hours of injury but increased risk of death from hemorrhage if given 3 hours after injury.

6. There is no reasonable way to identify prospectively individuals likely to become eligible for participation;

   - Acute trauma patients cannot be identified prospectively.

7. Participation in the research holds out the prospect of direct benefit to the subjects;

   Subjects may benefit from participation in the trial due to the following*:*

a) Severe traumatic injuries involving hemorrhage need better interventions than are currently available.

b) Prospective civilian data from CRASH-2 as well as retrospective military data from MATTERs suggests a benefit of TXA administration in the prevention of deaths from hemorrhage following trauma.

c) The risks of the study are reasonable given the safety of TXA as it is FDA approved for other indications (i.e. menorrhagia, hemophilia, post partum hemorrhage) and is being used "off label" without concern in elective orthopedic patients.

d) All subjects will have additional assessments performed during their participation in the study which includes a duplex ultrasound on hospital day 7 or discharge (whichever comes first) which may identify thrombus formation in this high risk population that otherwise may not have been identified.

8. <u>The clinical investigation could not practicably be carried out without the waiver:</u>

- The therapeutic window for the administration of TXA is two hours post injury (see following section for justification of this window). Since severely injured trauma patients will be unable to consent themselves, attempts to find a LAR will be made. If LAR is available, standard LAR consent procedures will be used. A written consent form that complies with the policies of the Washington University IRB will be used (see Appendix A). In cases where a LAR is not available, anyone who may have accompanied the patient to the Hospital will be asked to provide permission on behalf of the patient. If nobody is physically available to provide permission, an attempt will be made to contact a family member/friend based on any information available and permission over the phone will be gathered when applicable. We will presumptively enroll eligible patients using the exception of informed consent process if there isn't anyone available to speak to on behalf of the patient. Attempts to locate the LAR will continue until an appropriate representative is identified and consent to continue the study can be obtained. All attempts and efforts will be documented in the subject's study file to reflect the effort made to obtain proper informed consent as soon as was possible.

We are committed to obtaining consent from the LAR within the protocol time window and will document our efforts whenever the EFIC mechanism is used to enroll a patient. We will attempt to obtain consent in person, by telephone, fax, paper, and or any other communication possible on every patient prior to using EFIC. We will continue to seek consent from a legally-authorized representative after EFIC has been implemented. The LAR will be informed of the patient's inclusion into the study and of the details and risks of the study. At the time, the LAR will be given the option of allowing the patient to continue in the study, or to cease the subject's participation then or at any time throughout the course of the study. An informed consent form signed by the LAR will be obtained when possible.

ADDITIONAL PROTECTIONS

The 5 additional protections associated with conducting a trial under 21 CFR 50.24 are the following:

1. Community Consultation
2. Public Disclosure before the trial – including methods by which patients can "opt-out" or refuse participation in the trial
3. Public Disclosure after the trial
4. Plan for contact of Legally Authorized Representation (LAR) or family members or family friends to seek informed consent for the patient's participation in the trial within the therapeutic window if feasible or after enrollment as soon as possible when feasible.
5. Formation of a Data Safety Monitoring Board to oversee the trial

The plan for each of these activities will be discussed in detail. The regulatory language is included for convenience and reference as well as some test taken from the FDA Guidance document (April 2013) that offers an interpretation of the regulations to assist investigators, sponsors, and IRBs.

COMMUNITY CONSULTATION

The federal regulations for community consultation (21 CFR 50.24) state:

> 21 CFR 50.24
> (a)(7) Additional protections of the rights and welfare of the subjects will be provided, including, at least:
> (i) Consultation (including where appropriate, consultation carried out by the IRB) with representatives of the communities in which the clinical investigation will be conducted and from which the subjects will be drawn

The goals of community consultation are the following:

1. To ensure that all relevant communities have opportunity for input into the IRB's decision-making process before initiation of the study

2. To present information so that community members understand the proposed investigation, its risks & benefits, and to discuss that the investigation will take place without informed consent.

Community consultation does not necessarily imply that there will be community consent for the trial to take place. If community consultation were viewed as community consent, this would imply that the information came from a large proportion or essentially all the members of the community as opposed to individuals who are thought to be representative of the community. The process is meant to inform members of the community about the purpose and risks of the proposed study and to solicit input and answer questions from community members regarding the study. The IRB makes the final determination as to study approval based on the information obtained from the community consultation. For the purposes of EFIC, the definition of community is "the community in which research will take place," which includes both the geographic area where the hospital or study site is located and the "community from which subjects will be drawn," which include the group of patients who share particular characteristics (i.e. patients with the disease of interest or those "at-risk" for the disease or condition of interest).

In order to establish the "community" to be included in our community consultation plan, we obtained information from the Barnes Jewish Hospital Trauma Registry for the past three years (2011-2013) to identify the geographical locations contributing severely injured trauma patients to our ED. The following are the top 10 zip codes which provided severely injured trauma patients to our center:

1. **63136** (Jennings, MO) **74** patients
2. **63120** (St. Louis City, Pine Lawn, and St. Louis County, MO) **51** patients
3. **63147** (St. Louis City, St. Louis County, MO) **38** patients
4. **63121** (Normandy, MO) **23** patients
5. **63137** (Bellefontaine Neighbors, St. Louis City, Glasgow Village, Riverview, Moline Acres, Jennings, MO) **23** patients
6. **63133** (Hanley Hills, Pagedale, Wellston, MO) **21** patients
7. **63130** (University City, MO) **17**
8. **63138** (Spanish Lake, MO) **16**
9. **63135** (Ferguson, MO) **16**
10. **63134** (Berkeley, MO) **10**

**The following zip codes provided 9 or fewer trauma patient admissions: 63123, 63640, 63376, 63628, 63601, 63379, 63129, 63124, 63122

**45 additional zip codes from the surrounding area contributed 1 trauma patient admission each.

Further details regarding the above mentioned zip codes may be provided upon request.

The **content** of community consultation will inform the communities that informed consent will not be obtained for most (or all) research subjects. Specifically, the goal will be to:

- Inform the communities about all relevant aspects of the study including its risks and expected benefits
- Hear the perspective of the communities on the proposed research
- Provide information about ways in which individuals wishing to be excluded may indicate this preference

The **type and frequency** of community consultation will:

- Provide opportunities for broad community discussion
- Ensure that representatives from the community (ies) involved in the research participate in the consultation process
- Use the most appropriate ways to provide for effective community consultation
- Be based on numerous factors, including the size of the community (ies), the languages spoken within those communities, the targeted research population and its heterogeneity

The following is a list of activities we plan to employ for community consultation:

1. **PRESENTATION TO AN EXISTING GROUP AT REGUARLY SCHEDULED MEETING**
   ****This will occur as a <u>two-step</u> consultation process whereby we plan to meet 1st with key stakeholders who serve as "gatekeepers" to the larger community they serve. Once/if we obtain their buy in and they agree to provide access to their constituents and other community members who represent our "community", we will revise our Consultation Plan to include information regarding the specific**

meeting places and times that will focus on potential future subjects themselves/the community. We will provide a summary of the feedback we receive from the key stake holders to the IRB in an updated consultation plan prior to proceeding with Step 2.
The specific meetings we are then able to arrange with the key stakeholders' assistance (including focus groups) will be considered Step 2 of our two-step community consultation process.

In this method of community consultation, members of the study team will present the study and lead discussion about the study at a regularly scheduled meeting of a relevant community group. We have not requested permission to attend the following meetings as of yet, but plan to do so if advised accordingly by the WU HRPO.

a) Aldermanic Full Board Meetings (meet every Friday from 10 am-12 pm)
City Hall, St. Louis, MO. (step 1)

b) Normandy City Council Meetings (meet first Tuesday of each month at 7:30 pm)
http://www.cityofnormandy.gov/index.aspx?nid=319 (step 1)

c) Pasadena Hills City Board Meetings (meet the 2nd Wednesday of each month at 7 pm)
http://www.cityofnormandy.gov/index.aspc?nid=319 (step 1)

d) Green Practices Commission Meeting of University City (Scheduled to meet November 13, 2014, January 8, February 12, March 12, and April 9, 2015 from 6-7:30 pm), Herman Park Community Center: 975 Pennsylvania Ave, University City (step 1)

e) University City Council Meeting held the last Monday of each month at 6:30 pm (step 1)

f) Spanish Lake Town Hall Meeting (awaiting upcoming scheduled dates) (step 1)

g) Ferguson City Council Meetings (held the second and fourth Tuesday of each month) (step 1)

h) City Council Meeting in Berkeley (meets every other Monday at 7pm) (step 1)

i) Olivette City Council Meeting (takes place the last Tuesday of each month at 7pm) (step 1)

2. FOCUS GROUPS

We plan to arrange for several focus groups for the purpose of discussing the TAMPITI trial. The focus groups will be conducted at various locations where the following groups meet (more details and examples will be provided below regarding additional scheduled focus groups once Step 1 of our community consultation plan has taken place):

a) EMS providers in St. Louis City and surrounding counties (step 1)
b) St. Louis police and surround counties (step 1)
c) Bicycling Groups (step 2)
d) St. Louis Running Club (step 2)
e) Barnes Jewish Hospital Emergency Department (step 1)

f) YMCA of Greater St. Louis (in a variety of locations, i.e. downtown location, Carondelet Park Rec Complex, South City Family YMCA, etc.) (step 2)

3. WEB-BASED

Facebook: We plan to work with BJH and WU to create a Facebook fan wall for community members to learn about the TAMPITI (working with Joseph Ebeling JEbeling@bjc.org and Joni Westerhouse westerhousej@wustl.edu)

Posts to other group walls and fan pages will be posted by WU School of Medicine public affairs and BJH and/or study team members inviting community members to visit the website (being constructed) to learn about the trial. Posts will be placed on the following group/fan page walls (note some groups/fan pages have the same name):

Craigslist: On the St. Louis site in the community volunteer section we will place a link to our website referencing the TAMPITI study.

Twitter: We plan to coordinate efforts with WU School of Medicine public affairs and BJH for creation and maintenance of twitter posts/accounts (working with Joseph Ebeling and Joni Westerhouse on this)

4. SURVEYS (data regarding participants' anonymous demographic information and comments from the community consultation activities will be collected and will be reported once complete).

Individual surveys (surveymonkey.com) will be used as an additional way to solicit community questions and views. This method can be used to reach individuals and wide variety of respondents.

Venues for conducting individual interviews with at-risk community members and with the general public included; meetings as detailed above, hospital waiting areas, hospital staff through hospital intranet, as a link on the Wash U-TAMPITI website (being created right now)

Surveys:

- Will provide information about the EFIC regulation and the TAMPITI trial.
- Will inform the community member on how to opt-out of the study.
- Will collect information from respondents regarding their questions, concerns, and any additional feedback they want to provide.

Appendix E

TAMPITI

Public Disclosure Activity Plan for additional protections associated with 21 CFR 50.24, Exception from Informed Consent (EFIC) Requirements for Emergency Research for:

Tranexamic Acid Mechanisms and Pharmacokinetics in Traumatic Injury (TAMPITI TRIAL)

IRB PROTOCOL NUMBER: TBD

Supported by:
The Department of Defense
DoD Award: W81XWH-14-1-0373

FDA IND#: TBD

Investigators: Philip Spinella, MD
Grant Bochicchio, MD, MPH

Once the WU IRB has reviewed and approved the TAMPITI Trial and is satisfied that the Community Consultation efforts are adequate and appropriate, the IRB will provide final approval for Public Disclosure activities to commence. At this point, we will submit all WU IRB approved materials to the DoD HRPO for 2nd level review and approval. Once the DoD HRPO approves the study, Public Disclosure activities will take place as outlined below:

PUBLIC DISCLOSURE

Public Disclosure requirement of the Exception from Informed Consent (EFIC) regulations (21 CFR 50.24) for emergency research, states:

21 CFR 50.24

> *(a)(7)Additional protections of the rights and welfare of the subjects will be provided, including at least:*
>
> *(ii) Public disclosure to the communities in which the clinical investigation will be conducted and from which the subjects will be drawn, prior to initiation of the clinical investigation, of plans for the investigation and its risks and expected benefits;*
>
> *(iii) Public disclosure of sufficient information following completion of the clinical investigation to apprise the community and researchers of the study, including the demographic characteristics of the research population, and its results;*

Public disclosure is defined as the "dissemination of information about the research sufficient to allow a reasonable assumption that communities are aware of the plans for the investigation, its risks and expected benefits and the fact that the study will be conducted". It also includes "dissemination of information after the investigation is completed so that communities and scientific researchers are aware of the study's results'.

Appropriate public disclosure includes:

- Clear statement that informed consent will not be obtained for most subjects
- Information about the test articles use including a balanced description of the risks and benefits
- Synopsis of the research protocol and study design
- How potential study subjects will be identifies a
- Participating sites/institutions
- Description of attempts to contact LAR
- Suggestions for "opting out" of the study

We plan to utilize the following activities to ensure public disclosure:

1. **Broadcast (being planned) and Print Media (see Appendix for copies of hard copy)**
   a. **Local (St. Louis and surrounding areas)**

Public/Town Hall Meetings :

- We are arranging for a Town Hall Meeting to be conducted at Washington University School of Medicine (date TBD): _____

Circular and Newsletters (see *Appendix for hard copies of material)*:

- We plan to place announcements in church bulletins in as many churches as possible in the geographical areas contributing the most trauma patients

- We plan to circulate a newsletter to Wash U and BJH employees with links to our website and survey

- We plan to place an announcement in the Spanish Lake Quarterly Newsletter (next newsletter is for the winter quarter)

- We plan to send out an TAMPITI announcement with a link to our website and survey to the following organizations:
  1. BJH listserv via email within the entire BJH System
  2. WU employees listserv via email
  3. Express scripts
  4. Boeing
  5. Monsanto
  6. Anheuser Busch
  7. MoDot
  8. IDot
  9. Mastercard
  10. GM
  11. Lou Fusz
  12. Bommarito
  13. Purina

Television (see Appendix for hard copies of material)
Joni Westerhouse plans to assist us with contacting local news organizations (KSDK, KMOV, etc.) to inform them about this important clinical trial and will determine if they are interested and willing to interview Drs. Spinella and Bochicchio.

b. Regional (East St. Louis, Illinois included)

Study team members plan to obtain permission from the following businesses to hand out newsletters to patrons of the businesses who typically include patrons from areas outside of St. Louis, but who may be the target recipients of this information:

1. St. Louis Zoo
2. St. Louis Science Center
3. Six Flags
4. Scott Trade Center
5. Chaifetz Arena

c. National Media and Broadcasts

Not planned, but we will determine national interest in learning about the trial after local media reports on the trial.

2. Posters

Posters will be designed to clearly describe the study with contact information and website and will be placed in the following areas:

- Bulletin boards in Grocery Stores in the St. Louis Metro and surrounding areas. Below is a list of grocery stores within a 10 mile radius surrounding BJH (where most trauma victims come from). We did not include all grocery stores since there are 633 listed in the St. Louis Yellow Pages http://www.yellowpages.com/saint-louis-mo/grocery-stores

    - Aldi: 8445 Lucas and Hunt Rd, St. Louis, MO
      1315 Aubert Ave, St. Louis, MO
    - Shop 'n Save: 9521 Lewis and Clark Blvd, Moline Acres, MO
      10805 Old Halls Ferry Rd, Ferguson, MO
      49 N Florissant Rd, Ferguson, mO
    - Mally Supermarket: 7445 W Florissant Ave, St. Louis, MO
    - Dellwood Market: 1620 Chambers Rd, St. Louis, MO
    - Schnucks: 8037 W. Florissant Ave, St. Louis, MO
      3431 Union Blvd, St. Louis, MO
      6920 Olive Blvd, St. Louis, MO
      4137 N Grand Blvd, St. Louis, MO
      1225 S Florissant Rd, St. Louis, MO
    - Stelmacki's Super Market: 9965 Lewis and Clark Blvd, St. Louis, MO
    - Save-A-Lot: 10030 W. Florissant Ave, St. Louis, MO
      91 N Oaks Plaza, St. Louis, MO
      8960 Riverview Blvd, St. Louis, MO
    - F&G Foods: 5932 W. Florissant Ave, St. Louis, MO
    - Sam's Meat Market & More: 9241 W. Florissant Ave, St. Louis, MO
    - Northway Supermarket: 5590 W. Florissant Ave, St. Louis, MO
    - Beverly Hills Supermarket: 6714 Natural Bridge Rd, St. Louis
    - E&L Market: 4973 Emerson Ave, St. Louis, MO

- Straub's: 302 N Kingshighway , St. Louis, MO
  8282 Forsyth Blvd, Clayton, MO
- Six Stars Market: 8701 Riverview Blvd, St Louis, MO
- Yours Market: 8005 N Broadway, St. Louis, MO
- TNT: 6711 St. Louis Ave, St. Louis, MO
- North County Grocery & Liquor: 9948 Diamond Dr, St. Louis, MO

- Posters will be placed in the BJH Emergency Department, general hospital waiting rooms, out patient offices, medical school buildings, School of Pharmacy and Goldfarb School of Nursing.
- Posters will be placed in liquor stores, barber shops, salons, coffee shops, and gas stations in the St. Louis and surrounding areas.

TIMELINE FOR COMMUNITY CONSULTATION AND PUBLIC DISCLOSURE ACTIVITIES

Community consultation activities will commence upon receipt of WU IRB approval of EFIC plan. The DoD HRPO has verbally advised us to begin community consultation activities upon review and approval by the WU IRB.

Public disclosure activities will be ongoing throughout the trial, but formal efforts will begin upon full WU IRB and DoD HRPO approval, concluding when results of study are disclosed to the public. Washington University IRB and the DoD HRPO will be kept informed of specific public disclosure activities that have been performed with annual review or more often as needed and the Investigators and study team will reassess increasing public disclosure activities (with WU and DoD IRB approval) should the study take longer to complete then expected.

ANALYSIS AND PRESENTATION OF RESULTS FROM COMMUNITY CONSULTATION AND PUBLIC DISCLOSURE

We plan to report the community consultation results on our website . No protected health information will be reported.

The data being collected and reported will contain the following elements:

- Consultation methodology used
- Community type: geographic or condition-specific
- Participants involved: number and demographics
- Duration, content, format of information presented
- Free text log comments, questions, and responses to open-ended questions
- Coding of free text using qualitative research, methodologies
- Log of pre-determined closed-ended survey questions and response if used
- Log of site customized closed-ended survey questions and response if used

The information collected on these forms from community consultation will be compiled and reports be made available to the WU IRB and DoD HRPO.

Summaries of public disclosure will be reported to the IRB prior to approval, and then at least annually or upon request from the IRB. Composite reports of local and national public disclosure at the Trial-Level will be provided to the FDA at least annually.

Appendices:

- **TAMPITI and EFIC question/survey**
    - **Individual (Surveymonkey.com) TXA_Tampiti**
    - **Group (Hard copy surveys that mirror the surveymonkey.com survey)**
- **Sample flyer/poster**
- **Sample Pamphlet**
- **Sample invitations to focus groups**
- **Letter to the President of the Board of Aldermen**
- **Copy of TAMPITI trial homepage**

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