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COMIRB Protocol

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COLORADO MULTIPLE INSTITUTIONAL REVIEW BOARD
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Project Title: Control Of Major Bleeding After Trauma (COMBAT): A prospective, randomized comparison of fresh frozen plasma versus standard crystalloid intravenous fluid as initial resuscitation fluid

Principal Investigator: Ernest E. Moore, M.D.

This study is sponsored by the DOD's Telemedicine & Advanced Technology Research Center (TATRC).

I. Study Main Objective: The main objective of the study is to determine the efficacy of administering plasma (specifically AB-FP24) during field resuscitation compared to current treatment policy or standard of care (SOC) in which normal saline is infused in the pre-ED phase. This **Phase 2 trial** will provide preliminary evidence to potentially support Phase 3 trials with large study populations.

Study aims:

1) To determine if plasma-first resuscitation of severe hemorrhagic shock attenuates the acute coagulopathy of trauma.
2) To determine if plasma-first resuscitation of severe hemorrhagic shock improves metabolic recovery.
3) To determine if plasma-first resuscitation of severe hemorrhagic shock decreases overall blood product transfusion and reduces the incidence of acute lung injury, multiple organ failure, and mortality. In relation to the efficacy of plasma-first resuscitation, we will assess whether waveform information can help predict activation of the massive transfusion protocol.

II. Background and Significance:

A. **Background**: Hemorrhage is the most preventable cause of death in trauma patients. However, despite improvements in patient transport via EMS, survival in the first hour post-injury has changed little over the past 40 years.[1-4] Epidemiologic studies at trauma centers have revealed hemorrhage to be responsible for up to 50% of trauma related mortality.[1-4] In parallel, military trauma data from the current war on terror, and extending back to Vietnam, identify hemorrhage as a leading cause of preventable death in combat casualties, estimated to represent >80% of potentially survivable injuries.[5-8] Recent studies have documented coagulopathy in up to a third of trauma patients upon arrival to the emergency department (ED) prior to resuscitation, which correlates with the severity of tissue injury and magnitude of hemorrhagic shock.[9-12] Collectively, these findings have

stimulated a resurgent interest in developing an improved resuscitation fluid for severe hemorrhagic shock.[13]

The pathogenesis of the acute coagulopathy of trauma is undergoing intense worldwide investigation, but is undoubtedly multi-factorial and, at this moment, remains largely ill-defined.[14-19] The most recent emphasis has been on the complex interactions between post-injury inflammation and coagulation.[20-24] Irrespective of the ongoing debate, most authorities agree there is a component of disseminated intravascular coagulation (DIC), as well as thrombin generation for tissue hemostasis, that depletes circulating clotting factors.[16-19] The existence of DIC was recognized from Vietnam combat data and verified experimentally,[25-27] but its potential role in depleting coagulation factors was not evident until whole blood transfusion was abandoned in favor of component therapy in the early 1980s. This policy change stimulated several trauma centers to recommend presumptive administration of fresh frozen plasma (FFP) with initial packed red blood cell (pRBC) transfusion.[28-30] In fact, as far as we can ascertain, our report to the American Association for the Surgery of Trauma (AAST) in 1981[29] was the first to advocate pre-emptive FFP in the severely injured patient. Furthermore, by the late 1980s, anesthesiologists documented clotting factor deficiency associated with pRBC transfusion during elective surgery.[31, 32]

B. **Current Advanced Trauma Life Support guidelines**: Current guidelines from the American College of Surgeons recommend two liters of crystalloid, such as normal saline or lactated ringers, as the initial resuscitation fluid. DHMC paramedic protocol for traumatic shock states that IV NS bolus should be given. (See Appendix 0-DHMC Paramedic Prehospital Care) If the patient remains hemodynamically unstable with SBP<90 and HR>100 after the two liters of crystalloid fluid in the ED, the trauma team will start blood transfusion with “trauma blood,” which is group O, and Rhesus D negative if female. The Massive Transfusion Protocol is initiated by the attending trauma surgeon when a) a severely injured patient arrives in profound shock believed to be due to acute blood loss, b) when a severely injured patient remains in shock (SBP<90 mmHg) despite crystalloid resuscitation and is believed to have acute major blood loss, or c) a severely injured patient who requires massive RBC transfusion (>2 units PRBC per 30 minutes) to maintain hemodynamic stability and is believed to have ongoing acute blood loss. Once AB plasma is available, it is sent from the blood bank and infused. The Massive Transfusion protocol at DHMC is initiated at a 1:2 FFP: pRBC ratio (See Appendix 1 for DHMC Massive Transfusion Protocol).

III. Preliminary Studies/Progress Report:

A. **Preliminary studies**: As outlined in the background, the need for early infusion of FFP in patients requiring pRBC transfusion has been recognized from the time of blood component use in the early 1980’s. Retrospective studies have shown increased survival with early transfusion of FFP in combat patients.[33, 34] Furthermore, there are now multiple retrospective clinical studies showing an increased ratio of FFP to pRBC during massive transfusion is warranted.[35-39] We initially suggested on a ratio of 1:4 in 1982[29], and consequently others have shown improved 30-day mortality with ratios greater than 2:3.[35] Furthermore, retrospective studies from European trauma registries have shown that FFP

to pRBC ratios closer to 1:1 improved 24-hour mortality.[37] This has been further demonstrated in the US with patients receiving higher FFP: pRBC ratios (mean, 1:1.3) within the first 24 hours of admission had a 63% lower risk of death than those with a lower ratio (mean, 1:3.7),[40] and our most recent clinical investigation suggested presumptive FFP: pRBC ratios of 1:2 improves survival and coagulopathy of trauma.[36, 41] Early and aggressive FFP administration at a ratio of 1:1 in severely injured patients, even in patients that are not massively transfused has also been reported to improve mortality.[42] A recent multi-institutional preclinical trial in a large animal model of severe polytrauma supports this concept.[43] Using an established swine model, it was shown that the animals developed coagulopathy after inducing a femur fracture, hemorrhagic shock, and hypothermia. Treatment with whole blood, FFP only, and FFP with pRBC all attenuated the coagulopathy, suggesting that FFP alone can effectively correct the coagulopathy.

The infusion of FFP in severe trauma patients with coagulopathy is further supported in the concept of damage control resuscitation (DCR).[44, 45] DCR occurs during damage control surgery where the goal of surgery is to minimize blood loss and contamination, with the plan to return to the operating room for definitive repair when the patient is resuscitated and hemodynamically stable, and hemostatically secure. DCR involves minimizing the use of crystalloid, infusion of blood products, especially plasma, and permissive hypotension to limit blood loss, yet maintain perfusion to critical organs. In a retrospective study comparing outcomes of a cohort after the implementation of a DCR protocol to a historical cohort, patients in the DCR period received less crystalloid and more FFP with a FFP: pRBC ratio of 1:1.4, but had significantly improved 30 day survival and significantly shorter ICU and hospital stays.[46]

In a review article evaluating the available data on the effects of resuscitation fluids, including isotonic crystalloids and plasma, Rhee et al. concluded that isotonic crystalloids, such as normal saline and lactated ringer, led to activation of neutrophils and increased markers of cellular injury. They also concluded that plasma had favorable effects on immune function.[47]

Recent studies are showing improved outcomes with plasma-first resuscitation. In a trauma/hemorrhagic shock rabbit model with uncontrolled bleeding, initial resuscitation with plasma had improved coagulation function, as measured by thrombelastography, compared to Hextend (the standard initial resuscitation fluid in the military).[48] Another study demonstrated that having thawed plasma available in the emergency department lead to a faster infusion of plasma on arrival to the emergency department, a reduction in overall blood product use, and a 60% increase in 30-day survival.[49]

In a retrospective review recently accepted for publication, Kim et al. examined the effects of helicopter transport, pre-hospital plasma first resuscitation protocol in patients with hemorrhagic shock.[50] There were 9 who received plasma first, of 59 patients who met criteria for the pre-hospital massive transfusion protocol. Patients that received plasma first had greater baseline and post plasma INR values, but the decrease in INR was greater in those who received plasma first. Regarding the FFP:pRBC resuscitation ratio, the plasma first group received a ratio near 1:1 compared to a ratio of 0.45:1 in the control group.

Based on the current literature and despite no previous prospective clinical trials, the University of Texas at Houston has adopted early FFP administration in their trauma protocol and is currently administering thawed FFP in the field for critically injured patients transported by helicopter. (J. Holcomb personal communication with E.E. Moore)

B. Description of Plasma: Fresh frozen plasma (FFP) is defined in the United States as plasma that is obtained from whole blood and frozen within eight hours of collection (or six hours if acid-citrate-dextrose is the anticoagulant/preservative) per AABB Standard 5.7.5.9 and FDA 21CFR640.34 (b).[51] Interestingly, FFP is regulated by the FDA under 21CFR640,[51] but unlike other blood products, there is no quality control requirement for FFP in the USA regarding the contents, especially coagulation factors and protein concentrations. However, to decrease the burden on blood centers to process blood rapidly and freeze the blood within 6-8 hours, the vast majority of the fresh frozen plasma is frozen within 24 hours and denoted FP24. Additionally, the use of FP24 allows the blood center to confirm which plasma units meet criteria of antibody negative or type AB, potentially reducing adverse transfusion reactions such as transfusion-related acute lung injury (TRALI). Plasma represents a third generation resuscitation fluid. Like first generation crystalloids, FP24 is iso-osmolar with blood and contains all of the cations (e.g. Na, K, Mg, Ca) and anions (e.g. Cl, PO_4) present in blood. Like the second-generation colloid resuscitation fluids based on albumin alone, or non-human polysaccharides such as large dextrans and starches, it has high oncotic pressure (28mmHg vs. 3mmHg in 0.9% saline). Furthermore, FP24 contains hundreds of proteins at concentrations of mg/L. In fact, after albumin (40gm/L), the top 10 proteins add another 9.6g/L, when measured biochemically. Although FP24 does contain carbohydrate, these are glyco-conjugates to proteins and structurally quite different and more complex that dextrans or starches.[13, 52, 53] As summarized in Table 1, there are only small differences in the coagulation factors between thawed FFP and FP24 and similar concentrations of these proteins are present in the freeze-dried preparations (LysoPlas N-W, Resusix).[54-56]

Table 1: Coagulation Protein Profiles of Thawed vs. Lyophilized Plasma Products						
Coagulation Factor	Thawed FFP	Thawed Plasma (FFP at 120 hr)	Thawed FP24	Thawed Plasma (FP24 at 120 hr)	LysoPlas N-W	Resusix
aPTT (sec)					38	43.2
Fibrinogen (g/L)	2.8±52	2.78±50	3.09±70	3.03±50	3.4	2.19
Factor II (%)	97±10	95±10	97±8	96±11	88.8	75
Factor V (%)	85±13	67±19	86±16	59±22	101.5	75
Factor VII (%)	105±25	70±18	89±22	77±27	103.2	108
Factor VIII (%)	81±19	43±10	66±17	48±12	0.96	108
Factor IX (%)	82±13	80±12	88±13	84±12	0.92	86
Factor X (%)	94±10	87±11	94±11	91±12	95.5	98
Factor XI (%)					91.7	107
Factor XII (%)					82	94
AT III (%)					96.3	
Protein C (%)	107±20	107±19	88±16	89±17	103.8	
Protein S (%)	97±18	90±22	92±18	78±19	73.7	

Recent proteomic analyses of injured patients have demonstrated that following injury these patients have decreased levels of circulating anti-proteases and increased levels of intracellular enzymes, which may directly affect coagulation and influence organ injury through the protease-activated receptors on endothelial cells, and common intracellular proteins, like actin, which, if polymerized, may cause acute lung injury (ALI).[57-59] Interestingly, our recent proteomic analysis of FP24 demonstrates an abundance of numerous anti-proteases, α_2-macroglobulin and α_1-antitrypsin, which have the ability to oppose protease effects of coagulation and PARS as well as gelsolin, an extracellular protein that "solubilizes" actin filaments that could induce endothelial injury.[57, 58, 60]

Overall, there is, however, a limited supply of AB type plasma. AB plasma is the universal plasma donor for patients that have not yet had blood typing and will be the only type of plasma used for this study regardless if it is FFP or FP24. According to the DHMC adult transfusion guidelines, thawed plasma is given in patients with massive transfusion or life threatening bleeding with clinical evidence of coagulopathy, regardless of PT/PTT levels and only AB plasma will be infused.

The Circular of Information for the Use of Human Blood and Blood Components, prepared by the AABB, American Red Cross, America's Blood Centers, and the Armed Services Blood Program and approved by the FDA, states the indications for infusion of FP24 are the same as FFP except for treatment of deficiencies of Factor VIII and Factor V.[61] Under the section for Dosage and Administration, "FFP must be thawed in a water bath at 30 to 37 degrees Celsius or in an FDA-cleared device."

In the traditional method of thawing plasma, the blood bank often uses a water bath at 37 degrees Celsius and takes an average of 20 minutes for 2 units.[62] After thawing, the thawed plasma must be kept at 4 degrees Celsius up to 24 hours. It has

been shown that plasma thawed and refrigerated for more than 24 hours are not considered to be equivalent to thawed FFP or FP24 due to loss of labile proteins. Relying on the water bath method would make the plasma unavailable at the time of initial resuscitation or we would have to discard refrigerated frozen plasma every 24 hours, regardless of use. We plan to use an FDA –approved dry thaw device called Plasmatherm (510K# BK100063) to thaw the AB plasma. In order to use the AB plasma in a timely manner, we plan to store the plasma in a larger bag to increase the surface area to volume ratio. After extensive testing, we determined that utilizing a 2L bag to store the plasma allows for a thaw time that is faster than the microwave plasma defroster due to an increase in the surface area for a given volume

In the trauma setting, we have determined that an independent risk factor for mortality is the number of blood products received in the first 12 hours post-injury.[63] The acute coagulopathy of trauma perpetuates the need for blood products during resuscitation and may lead to increased organ failure and mortality. It has been recommended to give FFP earlier in resuscitation to correct coagulopathy during massive transfusion.[41] Furthermore, the war in Iraq prompted the military to refocus on presumptive plasma for resuscitation of soldiers with severe blast injuries and retrospective data analysis produced compelling evidence of its merit.[33, 34, 64] These results and associated discussions at academic meetings stimulated a worldwide resurgent interest in the role of pre-emptive plasma replacement at the time of pRBC transfusion.[35, 37-39, 41, 42, 65, 66]

FP24 and FFP are FDA approved and regulated as biologics under 21 CFR 640 Subpart D and G and The Circular of Information for the Use of Human Blood and Blood Components.[51, 61] In this study, we are not altering the plasma in any way. Rather, it is the infusion in the field with the exception from informed consent that requires an Investigational New Drug application, and not the product itself.

The AB-FP24 that will be used is from never transfused male donors, and never pregnant female donors. Most of the AB plasma is FP24 in order to verify the donors and the AB type of plasma as a universal donor to increase the supply of AB Plasma for transfusion for those who have not yet been blood typed. This is the standard used for plasma transfusion in the emergent setting for America's Blood Centers. The DHMC Adult Transfusion Guidelines specifies the universal plasma donor is AB and indications for plasma transfusion include patients with massive transfusion or life-threatening bleeding with clinical evidence of coagulopathy regardless of PT/PTT values. (See Appendix 2.

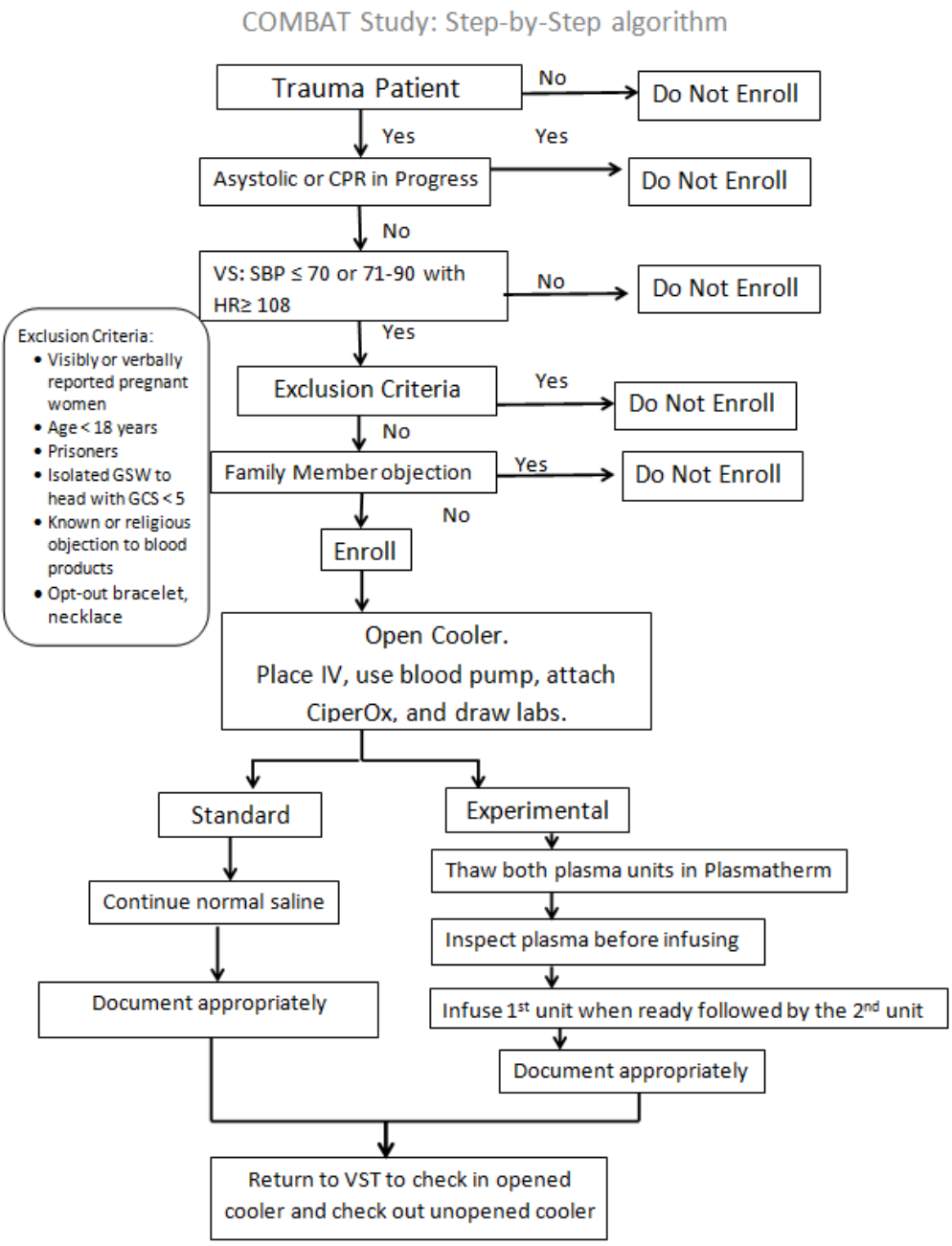
IV. Study Design and Research Methods

A. Overview of Study Design: This Phase 2 trial is a randomized, controlled trial in which eligible injured patients will be randomized in the field into two groups, an **experimental group** who will receive plasma (AB-FP24) first or a **control group** who will receive SOC resuscitation with normal saline first. Randomization will be determined by trained paramedics assisting the patient in the field, as described below in IV.A.1. From this point forward in this document, the names **Experimental group** and **Control group** will be used to designate these two groups.

1. **Rationale for Study Design:** The DOD contract specifically requires the study to emphasize testing in the field. The Denver Health Paramedic system uses the dynamic dispersal model such that ambulances are placed at certain locations and then mobilized according to shortest distance to the accident[4]. As a result, one ambulance in a given shift may cover various regions of the city and are mobilized randomly based on need. Seven to twenty eight ambulances may be deployed to cover the city depending on the time of day and day of the week. Regardless, to minimize all possible sources of confounding and bias, thus all ambulances will be equipped with Plasmatherm devices, coolers, and AB-FP24 in all ambulances.

   For randomization, half of the coolers will contain AB-FP24 (experimental) the other half will contain frozen water in a ziplock bag (or similar) that is incompatible with IV tubing (control). We chose this method of randomization for several reasons. First, it will avoid protocol violation if the randomization group is determined by the product that is in the cooler. It also does not require paramedics to open an envelope and read the randomization, allowing for faster enrollment, randomization and transport to the hospital for definitive care. This method also reduces the number of AB-FP24 units that can expire and be discarded; thus conserving the valuable resources of the blood bank. The units of AB-FP24 and frozen water will be placed in an opaque wrap or case. Only study personnel will know the type of fluid placed in each cooler prior to randomization. We chose frozen water that cannot be infused to be a dummy weight for the control coolers because we did not want to infuse cold saline that had been in a cooler for an extended period of time as this may cause further coagulopathy. If the subject is randomized to the standard group, paramedics will continue with the standard of care and begin infusing normal saline. If the subject is randomized to the experimental group, paramedics will proceed with thawing the plasma as described in Procedures involving plasma. (Section IV.K.1) Once the cooler has been opened, the paramedics will return to the Vehicle Support Technician (where paramedic equipment and medications are typically stored and distributed as well as the coolers) and obtain a fresh, unopened randomized cooler. If the cooler is not opened for the shift, it will be returned to the VST. The coolers will be assigned to paramedic teams at random. Our paramedic workforce has previous experience with field enrollment and randomization with the Polyheme study and is already on board with this study. Of note, our institution had only 4 protocol violations of the 128 patients enrolled during the Polyheme study, which relied heavily on participation of the paramedic team. (EE Moore, MD, personal communication, July 17, 2012)

COMBAT Study: Step-by-Step algorithm



Blinding is not possible because AB-FP24 is processed separately by a blood center through specific procedures. AB-FP24 is opaquely distinctly different. Colored bags would not suffice because tubing would also have to be colored, and it would interfere with urgent care. It could complicate the logistics of the clinical scenario since pre-hospital personnel and nurses use the drip chamber to ensure that the fluid is infusing thus they must be able to see the fluid, which will appear different.

2. **Exception of Informed Consent**: This study will require a waiver of consent under 21 CFR 50.24 for patient enrollment as described in the section Special Consent Issues (Section IV.M)
3. **Duration of study:** 5 years.
4. **Description of population to be enrolled:** Enrollment will include acutely injured trauma patients in severe, presumed hemorrhagic shock. Patients will be enrolled based on initial field vital signs. ***Inclusion criteria***: Age≥18 years, acutely injured, with presumed hemorrhagic shock from acute blood loss defined as SBP≤70 mmHg or SBP 71-90 mmHg with HR≥108 beats per minute. ***Exclusion criteria****:* Visibly or verbally reported pregnant women, age<18 years, known prisoners, unsalvageable injuries (defined as asystolic or CPR prior to randomization), isolated GSW to the head with a GSW < 5 (a highly lethal injury that is not primarily due to acute blood loss), known or religious objection to blood products, the patient has an opt-out bracelet or necklace, or a family member present at the scene objects to the patient's enrollment in research. ***Pregnant Women****:* It is not feasible to perform a pregnancy test after determination of eligibility because of life threatening injuries. In the community consultation, we will advise nonvisibly pregnant women opt out of the study. We will exclude any women of childbearing age that is visibly pregnant. ***Prisoners***: Only known prisoners at the time of enrollment will be excluded. Patients who are under a police hold or become a prisoner during hospitalization will be included in the study. In the acute trauma setting, patients may be placed on a police hold at any time during hospitalization. The hold may be temporary for a short time or for an indeterminant length of time. Paramedics may not know who will be placed under a police hold. Thus, ethically, these patients cannot be coerced for enrollment,. Patients placed on a police hold during hospitalization have already been randomized to the standard or experimental group. The study team would collect serial blood samples and follow up with the patient at 28 days. ***Rationale for hemorrhagic shock entry criteria***: The criteria were based on the last ROC trial[67], which in turn were chosen based on a previous trial[68] that showed that SBP≤90mmHG was not as "specific marker of hypovolemic shock", while the alternative criteria led to a larger proportion of patients requiring massive transfusion. Thus, although the traditional marker of severe shock, SBP<90, we plan to use the same entry criteria as the ROC trauma trial so the populations of the two studies may be compared, which will enhance generalizability of the findings.

Table 2 below shows the number of patients received by our trauma center from Jan 2009-Dec 2010, by different eligibility criteria and respective mortality rates. These numbers exclude patients who died within 10 minutes of arrival (a group of patients with potentially unsalvageable injuries). These are conservative estimates;

our trauma volume has increased over 20% in 2012 and we anticipate maintaining this growth.

Table 2. Trauma registry data Jan 2009-Dec 2011 (3 years), excludes DOA (patients who died within 10 minutes of arriving at ED)

Field Entry Criteria	N	Mortality
SBP<90	241	24%
SBP<80	175	32%
SBP≤70 OR (SBP 71-90 + HR≥108)	172	31%
SBP<70	109	44%

5. **Gender, race, ethnic origin restrictions:** There are no subject restrictions based upon gender, race, or ethnic origin unless the patient has a known objection to blood products. The research design will include sufficient enrollment of persons of both genders and diverse racial/ethnic backgrounds to ensure that the benefits and burdens of research participation are distributed in an equitable manner. There are no religious restrictions, but people of the faith of Jehovah's Witnesses typically object to transfusion of blood products and will be excluded from the study.

B. Detailed description of study design:

1. Procedure for enrollment, randomization and intervention:

a) Determination of eligibility of enrollment in this stage is based on initial vital signs from EMS in the field. (See Population to be Enrolled-Section IV.A.4 and Paramedic Training-Section IV.J)

b) Trained paramedics will look for opt out specific bracelets or necklace ID. If any of these opt out items are found or there is evidence of refusal of blood products, then the patient will not be enrolled.

c) If a family member is present at the scene and not in shock or severely injured, easily accessible to paramedics, and the patient is not in imminent danger of death, the paramedics will state "We are enrolling him/her in a study where we are giving plasma for its clotting factors. We cannot explain the study at this time. Is this okay?" The paramedics will not be able to look for family members among a crowd of bystanders given the acute setting and the importance of transporting the patient to the hospital as soon as possible.

d) Once determined to be eligible, the paramedics will open the cooler, remove the two cassettes from the sealed bag, and open both cassettes to determine their contents. Once the cassettes are opened, the patient will be randomized to one of the 2 arms. If the cooler contains two units of AB-FP24, the patient is randomized to the experimental group. If the cooler contains non-infusable frozen water, the patient is randomized to the control group. All ambulances will be equipped with a Plasmatherm and a cooler storage box containing either plasma or noninfusable

frozen water. Before transfusing any fluids (likely immediately before drawing blood so as not to hinder patient care), paramedics will apply the noninvasive device for recording continuous waveform data. This device will remain on the patient for 24 hours and will begin obtaining critical physiological information as soon as the monitoring begins. Since this is an observational component of the study, there will be no changes made to the patient's care during this portion. Waveform data will not be used in the provision of subsequent care.

e) If the patient is randomized to the control arm, the patient will be given intravenous normal saline as the initial resuscitation fluid with 2 large bore IVs based on the current ATLS guidelines. Subsequent care will proceed per institutional, ATLS guided resuscitation with acute pRBC administration determined by hemodynamic response and additional blood component administration guided by rTEG and coagulation panel assessment in conjunction with clinical scenario. The Massive Transfusion Protocol may be initiated using the ABC score[69] and heuristic evaluation of uncontrolled hemorrhage of the attending surgeon (See Appendix 1 for DHMC Massive Transfusion Protocol)

f) If the patient is randomized to experimental arm, 2 units of frozen AB-FP24 will be thawed in the Plasmatherm thawing device according to the operator manual as approved by the FDA (see Plasmatherm- section IV.L.2) in the ambulance and infusion will commence as soon as the AB-FP24 is ready, and will continue during transport to the ED. If the AB-FP24 is not ready, but IV access is acquired, normal saline will be infused and the volume recorded. Once the AB-FP24 is ready, the normal saline infusion will be stopped and the AB-FP24 will be started. If the patient has 2 lines, both lines will be used for AB-FP24. If only 1 IV is available, one unit of AB-FP24 will be infused until it either is completed or another line is available for infusion. The experimental group will receive at least the standard of a resuscitation fluid, regardless if the AB-FP24 is ready for infusion. After infusion of 2 units of AB-FP24 is completed, subsequent care will proceed per institutional, ATLS guided resuscitation with acute pRBC administration determined by hemodynamic response and additional blood component administration guided by rTEG and coagulation panel assessment in conjunction with clinical scenario. The Massive Transfusion Protocol may be initiated using the ABC score[72] and heuristic evaluation of uncontrolled hemorrhage of the attending surgeon, similar to the standard arm. (See Appendix 1 for DHMC Massive Transfusion Protocol)

g) Both groups will be managed based on the critical care guidelines for trauma patients as published in the Journal of Trauma, which is the routine hospital care for trauma patients at DHMC.[70-78] These are Standard Operating Procedures for critical care management specifically developed and peer-reviewed during our NIH sponsored Glue Grant studies

h) In the unlikely event multiple patients are enrolled in this study, all patients will be maintained as study patients despite the fact that there will be logistic barriers to obtaining a complete laboratory assessment. The rationale for this policy is the primary endpoint of this study is mortality; whereas, laboratory assessments are secondary endpoints.

i) There will be no ceiling dose for the total amount of intravenous fluids that will be administered in the field. Based on our experience, no more than 700cc of normal saline on average is infused prior arrival to the ED.

C. Data Collection and Storage

1. **Data Collection:** Each patient enrolled will be given a unique patient identifier that will be kept in a separate database to protect heath information.
    a. Laboratory studies will be performed to evaluate the acute coagulopathy of trauma from blood samples collected in the field, upon ED arrival, at 2, 4, 6, 12, 24, 48 and 72 hours, and day 5 and day 7 post-injury as shown below in the figure titled “Biological Sampling and Study Timeline." Standard coagulation assays (prothrombin time/INR, activated partial thromboplastin time, fibrinogen levels, and platelet counts), ROTEM (rotational theomboelastography) and TEGs, [rapid, tissue factor-activated thrombelastograms (r-TEG), classic kaolin-activated TEGs with platelet mapping of the response to arachidonic acid (AA) and adenosine di-phosphate (ADP)] will all be completed in the Clinical Laboratories or Trauma Research Laboratory at Denver Health Medical Center in addition to CBC, serum chemistries, hepatic profile, lactate, and arterial blood gases. Crystalloid volume, resuscitation fluids and number of blood products infused will be recorded hourly.
    b. In the event that multiple patients are enrolled in the study at the same time, due to logistical restrictions, samples may be delayed or not collected. Sample collection and processing will be resumed according to the protocol at the earliest opportunity.
    c. The clinical data both at presentation and throughout hospitalization will be obtained and recorded in the COMBAT/Trauma Activation Database. Additional clinical information specific to the study including blood product transfusion will be recorded in the REDCap data collecting system. Clinical data entered will include a summary of injuries on admission, illness during the index admission, medical history, medications, and infectious and non-infectious complications as well as time and cause of death. Patient data entry will end with the index hospital stay. Outpatient information will not be included.
    d. Patients will be contacted after hospital discharge on day 28 primarily for 28-day mortality.
    e. Each field, ED arrival, 2, 4, 6, 12, 24, 48, 72 hour, day 5 and day 7 time point blood samples obtained will be banked by a unique patient identifier for further research and for determination of proteomics and

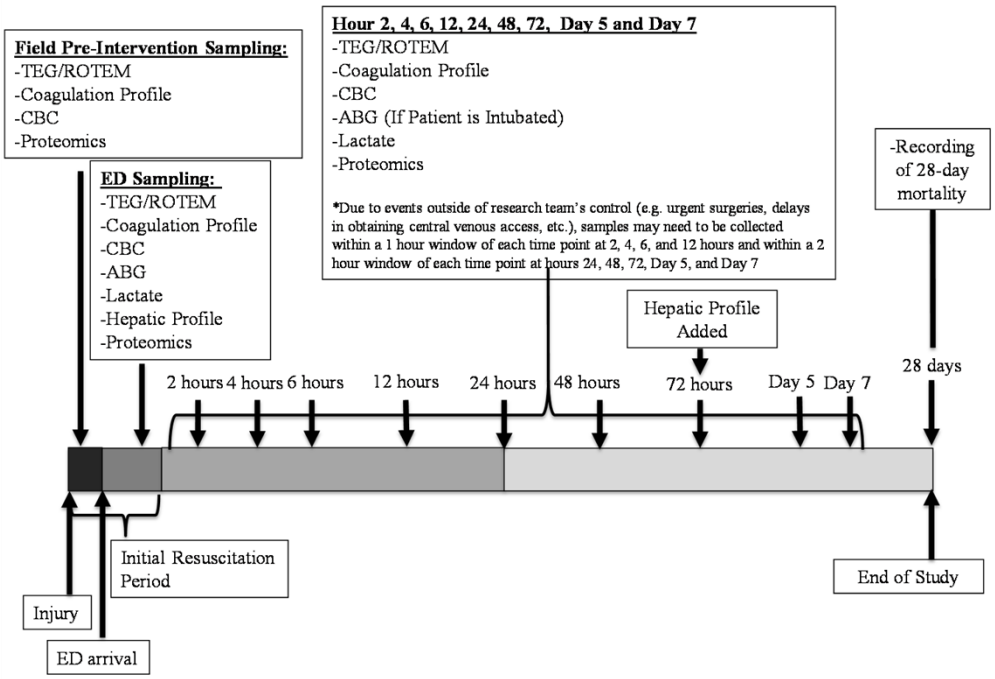
cytokines. These blood samples will also be used for panomics, such as metabolomics. Clinical data will also be kept for future research to correlate clinical outcomes with banked samples.

f. Samples from a limited data set including dates and times of events will be sent to TACTIC (Trans-Agency Consortium for Trauma-Induced Coagulopathy) for validation of inflammatory cytokine and chemokine data and to Children's Hospital of Colorado for measurements of coagulation factors.

g. Admission laboratory values obtained more than 60 minutes from ambulance arrival to the scene will be considered a missing value.

h. We will do our best to obtain blood samples at the time points indicated in this protocol. However, due to events outside of research team's control (e.g. urgent surgeries, delays in obtaining central venous access, etc.), samples may need to be collected within a 1 hour window of each time point at 2, 4, 6, and 12 hours and within a 2 hour window of each time point at hours 24, 48, 72, Day 5, and Day 7.

Biological Sampling and Study Timeline



Time Point	Amount Drawn	Total Volume
Field	29.5 mL	29.5 mL
ED	40.5 mL	40.5 mL
Hour 2, 4, 6, 12, 24, 48	33.0 mL	198.0 mL
Hour 72	37.0 mL	37.0 mL
Day 5, Day 7	33.0 mL	66.0 mL
TOTAL AMOUNT DRAWN		371.0 mL

2. **Data Storage:** Electronic case report forms and study data management will be performed using the REDCap Study Data Management System through the CCTSI Informatics Core. REDCap is a HIPAA compliant research data management system developed at Vanderbilt University and deployed at over 52 institutions. All study data are stored on a secure database server, which is separate from the web-facing server - a best practices for internet-based security. All user access requires user accounts and passwords. All user actions are recorded in a secure audit log. The database server is routinely backed-up. All security patches and application updates are applied immediately upon release. Any paper records, which will be a minimum, will be kept in a locked location, only accessible by study personnel. Investigators, wishing to develop separate studies, not described above, using these data, will be required to submit a new IRB approval to allow the use of the data. Accurate and complete study records will be maintained and made available to representatives of the U.S. Army Medical Research and Materiel Command. These representatives are authorized to review research records as part of their responsibility to protect human research volunteers. Research records will be stored in a secure manner to protect the confidentiality of subject information.
3. Banked blood samples will be de-identified and kept for future research only for subjects who regain cognitive capacity and provide written consent or their LAR has given written consent if subjects do not regain cognitive capacity. No blood samples or data will be banked for those who withdraw from the study.
4. A limited data set will be uploaded to the edcCloudShare system. The edcCloudShare is a communication tool and vehicle used for secure and timely information exchange across participating centers. It is a proprietary service that operates locally on the Epidemiology Data Center's (EDC) LAN, and it is a subset of the University of Pittsburgh network. Several security features are utilized, such as named user accounts, user roles (i.e. Viewer/Reader, Editor/Manager, Owner/Administrator), and 2048-bit AES encryption to protect the privacy and confidentiality of the information being exchanged. The edcCloudShare system uses a Windows Server 2008 R2 Enterprise VM Server. It is protected by the University of Pittsburgh's enterprise firewall system in addition to a local firewall and Symantec Endpoint protection. Updates are applied as needed, and other updates are applied quarterly during the standard change management.The data destruction plan for paper and electronic data will be per COMIRB protocol.
5. CRI Devices Data Collection

    a. Continuous waveform data will not contain any patient identifiers or protected health information (PHI). Clinical data that is collected will be de-identified prior to data analysis. Data will be locked up and will only be accessed by members of the research team. Data will be backed up to a secure server using secure transfer protocols such as the Secure Copy Protocol, which uses Secure Shell (SSH) for data transfer and utilizes the same mechanisms for authentication to ensure authenticity and confidentiality of the data in transit.
    b. Paper documents (the case report forms and data collection sheets) will be locked up with the data collection equipment and will only be

accessed by members of the research team. The medical record number (MRN) will be written on one of these documents, but upon completion of these documents, the MRN will be blacked out to de-identify the document and, thus, is confidential. These documents will be scanned as PDF's and uploaded to a secure server along with each subject's waveform data.

c. The waveform data will be synchronized and subject to data analysis at a later time, looking specifically at waveform changes in response to fluid resuscitation types, rates and volumes, response to fluid therapy, types of injuries, operative timing and management, length of ICU and hospital stay, as well as survival. These results will be compared to existing LBNP waveform data. LBNP is a laboratory model of human hemorrhage that redistributes intravascular volume to the pelvis and lower extremities, thereby inducing central hypovolemia

D. Data Analysis

1. All analyses will be conducted using SAS for Windows vs. 9.3 (SAS Institute Inc., Cary NC, USA) on de-identified data. Outcome and effect variables that are not normally distributed will be either categorized or analyzed using non-parametric methods. Missing data will be managed during analysis using the method proposed by Sauaia et al.[4]

2. Randomization effectiveness: Effectiveness of randomization will be examined by comparing the two groups regarding demographic variables (age, gender), injury severity (Injury Severity Score, blunt versus penetrating mechanism), degree of shock (Field SBP, Field Heart rate, Field Hematocrit), and a field coagulation measure (Field INR). We will adjust all analyses of endpoints for the covariates showing different distribution (either statistically significant difference at $p<0.05$ or clinically significant difference as determined by the DSMB). Specifically, adjustment for these pre-specified, potential confounders will be performed using three different methods according to the type of endpoint:

a. Categorical variables: we will use multiple logistic regression (polytomous logistic regression will be used for non-binary variables);

b. Continuous normally distributed endpoints: we will utilize mixed linear models to adjust for confounders, assuming an unstructured covariance structure.

c. Continuous non-normally distributed endpoints: if the endpoint is non-normally distributed, we will attempt first to transform the variable to approximate normality (e.g., log transformation), and if this fails, we will resort to categorization based on previously determined, clinically relevant cutoffs.[79-83]

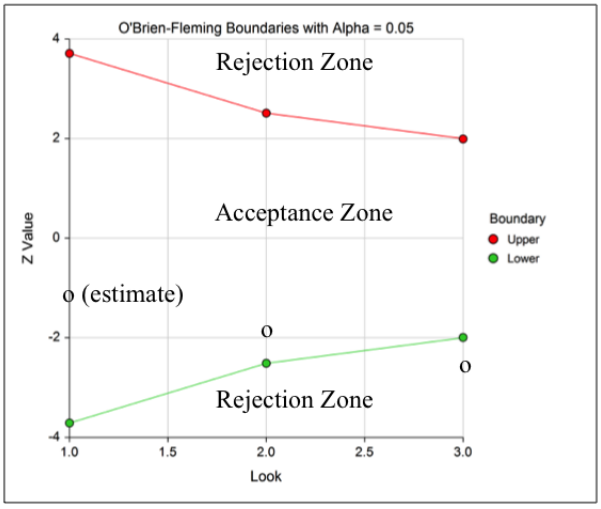
3. Intent to treat analysis: primary and secondary outcome data will be collected in all patients regardless of treatment received.[84] An "intent-to-treat" approach will be used for all primary/secondary outcome analyses, i.e., we will compare the outcomes of the two groups according to the group assignment at time of randomization, regardless of what treatment participants actually received. Phase 2 trial, this approach will allow a preliminary evaluation of the

effectiveness of the proposed treatment (in addition to the efficacy assessment), since the health care of the subjects reflects the health care available to civilian trauma. More details are given in the sections describing statistical analysis as well as the missing data prevention and treatment.[84]

In addition to the "intent to treat" approach, this study design also supports that exploratory analyses assess the association between the dose of plasma received in the field and the primary/secondary outcomes, an equivalent to the estimand "Outcome Improvement in Tolerators" described by the Panel on Handling Missing Data in Clinical Trials.[84] This will provide information on possible "dose-response" patterns for Phase 3 trials.

4. **Power analysis:** Power analyses for each primary endpoint in Stage I and II were performed using Pass 11 (Hintze, J. (2011). PASS 11. NCSS, LLC. Kaysville, Utah, USA. www.ncss.com). All power analyses account for two interim analyses for a total of three sequential, equally spaced analyses, assuming attrition rates of 0%, 10%, and 20%.
5. **Interim analyses:** We planned 5 analyses (4 interim, 1 final), equally spaced, using the O'Brien-Fleming method to calculate two-sided boundaries, assuming a 95% confidence and 80% power. Spacing will be defined by number of enrolled patients. The first look at the data will be done when one third of the predicted total enrollment has been reached and all patients accrued have been observed for the total 28-day period or till death, whatever occurs earlier. The first interim analysis will be conducted when n=50, the second at n=100, the third at n=150, the fourth at n=200 and the final at end of the 5-year period of accrual.

Boundaries will be used to declare a statistically significant difference between the two groups at an earlier stage without inflating the overall experiment errors. These interim analyses will be conducted by Dr. Sauaia and Dr. Chin using SAS vs. 9.3 data and reported to the DSMB, both in table format and in user-friendly graphs illustrating the test score, rejection and acceptance regions, as in the example below:



At each interim stage, if the test statistic falls into a rejection zone, the null hypothesis may be rejected and the DSMB will use this as one of the criteria for recommending early interruption of the trial. Otherwise, the trial continues to the next stage. At the final stage, the null hypothesis is rejected if Z falls into a rejection region. Otherwise, the null hypothesis is not rejected. In the example illustrated in the figure, the test statistic does not fall into the rejection regions for looks 1 and 2, and so the trial continued to the final analysis 3 when the test statistic fell into the rejection region, and the null hypothesis was rejected.

The results of the interim analyses described above will be just one of the criteria used by the DSMB to recommend early interruption of the trial as explained in more detailed later in this document.

6. **Covariates for analysis:** Please see description of randomization effectiveness for more details. (Section IV.D.2) In brief, potential covariates include: demographic variables (age, gender), injury severity (Injury Severity Score, blunt versus penetrating mechanism), degree of shock (Field SBP, Field Heart rate, Field Hematocrit), and a field coagulation measure (Field INR). We will adjust the analyses of endpoints only for the above-mentioned covariates showing different distribution (either statistically significant difference at $p<0.05$ or clinically significant difference as determined by the DSMB).
7. **Waveform Data:** We would like to collect more CRI data from very sick individuals to see how the algorithm captures their clinical presentation and resuscitation. We also want to see if a certain minimal CRI or low CRI for a period time triggers the acute coagulopathy of trauma. Just as other vital signs can be used to indicate certain medical conditions, we would analyze the data to see if the acute coagulopathy of trauma correlates with either a minimal CRI or low CRI for a certain period of time. We will also collate the CRI data with the clinical timeline of interventions, medications and fluids given. This will help us correlate the effects of the interventions, medications and fluids with changes in CRI.
    a. Data analytics will be used to analyze the waveform data in relation to fluid resuscitation types, rates and volumes, response to fluid therapy, types of injuries, operative timing and management, length of ICU and hospital stay, as well as overall outcomes. We will compare the waveform features from trauma patients to those features collected from research subjects with analogous levels of LBNP applied.

E. Primary Study Objectives and Endpoints

1. **Primary Study Objective:** to determine the efficacy, as measured by 28-day mortality reduction, of using postinjury field-resuscitation with plasma first compared to standard of care (SOC).

   Primary Endpoint: 28-day mortality
    a. **Definition:** 28-day mortality is defined as death within 28 days post injury (death of any cause except for death due to a second, clearly unrelated traumatic injury suffered after discharge). Research coordinators will verify the vital status of all patients at day 28. Patients

discharged home before day 28 will be reached using contact information obtained at discharge. In case of transfer or discharge to another facility before day 28, research coordinators will coordinate with the other facility to ascertain vital status, verify contact information at discharge or determine patient disposition at day 28 postinjury

b. **Hypothesis**: 28-day mortality will be significantly higher* in control patients compared to experimental patients.
c. **Statistical Analysis**: The Fisher Exact test will be use to compare 28-day mortality in the two groups. Statistical significance will be determined according to the interim analyses procedures describe below. If adjustment for pre-defined confounders is needed, we will use the above-defined method for categorical, binary variables.
d. **Interim analyses:** We planned 5 analyses (4 interim, 1 final), equally spaced, using the O'Brien-Fleming method to calculate two-sided boundaries, assuming a 95% confidence and 80% power. All patients entered in each interim analyses will have complete data on 28-day mortality (i.e., the analyses will be conducted only once the 28-day observation period for all survivors have been completed).
e. **Sample Size and Power:** This is a Phase 2 trial and is powered to detect large (17 to 19 percent-point) differences in mortality between the two groups.

Primary endpoint power calculations: 28-day mortality

Assumptions: 80% power, 95% confidence, 2-tailed test, P2=mortality in control group, P1=mortality in experimental group; 3 equally spaced data analyses, as determined by number of patients enrolled (2 interim when 1/3 and 2/3 of the predicted sample or 20 and 40 patients were enrolled, and a final when 60 to 75 patients were enrolled), using the O'Brien-Fleming method; control group 30-day mortality of 26% (based on control arm of ROC HS trial[67]) and three attrition rates (0, 10%, 20%)

Power	N1	N2	Alpha	Beta	P1	P2	Difference	Attrition Rate
0.800000	60	60	0.050000	0.200000	0.07	0.26	0.19	20%
0.800000	68	68	0.050000	0.200000	0.08	0.26	0.18	10%
0.800000	75	75	0.050000	0.200000	0.09	0.26	0.17	0%

Sample size of 60 and 60 achieves 80% power to detect a difference of 0.19 between the group proportions at a significance level (alpha) of 0.05 using a two-sided test. These results assume that 3 sequential tests are made using the O'Brien-Fleming spending function to determine the test boundaries, as seen below.

Boundaries (Bndry) for data looks
Details when Spending = O'Brien-Fleming, N1 = 60, N2 =60, P1 = 0.07, P2 = 0.26

	Lower	Upper	Nominal	Inc	Total	Inc	Total

* Although the hypothesis is worded as "higher", all tests will be two-tailed to control for differences in either direction (higher or lower than the other group).

Look	Bndry	Bndry	Alpha	Alpha	Alpha	Power	Power
1	-3.71030	3.71030	0.000207	0.000207	0.000207	0.018650	0.018650
2	-2.51142	2.51142	0.012025	0.011890	0.012097	0.398801	0.417451
3	-1.99302	1.99302	0.046259	0.037903	0.050000	0.382549	0.800000

Details when Spending = O'Brien-Fleming, N1 = 68, N2 =68, P1 = 0.08, P2 = 0.26

Look	Lower Bndry	Upper Bndry	Nominal Alpha	Inc Alpha	Total Alpha	Inc Power	Total Power
1	-3.71030	3.71030	0.000207	0.000207	0.000207	0.018650	0.018650
2	-2.51142	2.51142	0.012025	0.011890	0.012097	0.398801	0.417451
3	-1.99302	1.99302	0.046259	0.037903	0.050000	0.382549	0.800000

Details when Spending = O'Brien-Fleming, N1 = 75, N2 =75, P1 = 0.09, P2 = 0.26

Look	Lower Bndry	Upper Bndry	Nominal Alpha	Inc Alpha	Total Alpha	Inc Power	Total Power
1	-3.71030	3.71030	0.000207	0.000207	0.000207	0.018650	0.018650
2	-2.51142	2.51142	0.012025	0.011890	0.012097	0.398801	0.417451
3	-1.99302	1.99302	0.046259	0.037903	0.050000	0.382549	0.800000

Addendum Nov 16, 2016

At the second interim analyses, we had enrolled 100 patients (2/3 of the 150 total sample size), of whom 88 (12% attrition rate) patients were eligible for analysis (please, see Interim Analyses report for details). This attrition rate was similar to the lowest anticipated attrition rate (see first table above). Yet we noticed several important differences regarding the assumptions used to estimate the sample size. In brief, the cohort enrolled based on the entry criteria (field systolic blood pressure, SBP<70mmHg or SBP <90mmHg AND heart rate>108bpm) was less severely injured than anticipated (as measured by base deficit, INR and TEG G) and had lower mortality than anticipated.

Variable	Anticipated (sample size/power analyses assumptions)	COMBAT group A	COMBAT group B
INR	1.47	1.32	1.38
INR>1.5	24% to 36% *	18%	16%
Base deficit (mEq/L)	5.15	11.2	9.2
TEG G dynes/cm^2	4.9	7.3	7.5
Mortality	26%	12%	13%

* {MacLeod, 2003 #1415;Maegele, 2007 #905;Niles, 2008 #4410}

In addition, a slightly lower proportion of patients required transfusions (55% of group A, 61% of group B) compared to our anticipated proportion based on trauma registry data encompassing the period of January 2009-December 2011 (3 years) and excluding patients who died within 10 minutes of arriving at ED (64%).

Although we enrolled a cohort with less physiologic and coagulation derangement than anticipated, which decreased our statistical power to detect differences in the primary and secondary outcomes, we detected some promising trends, as follows:

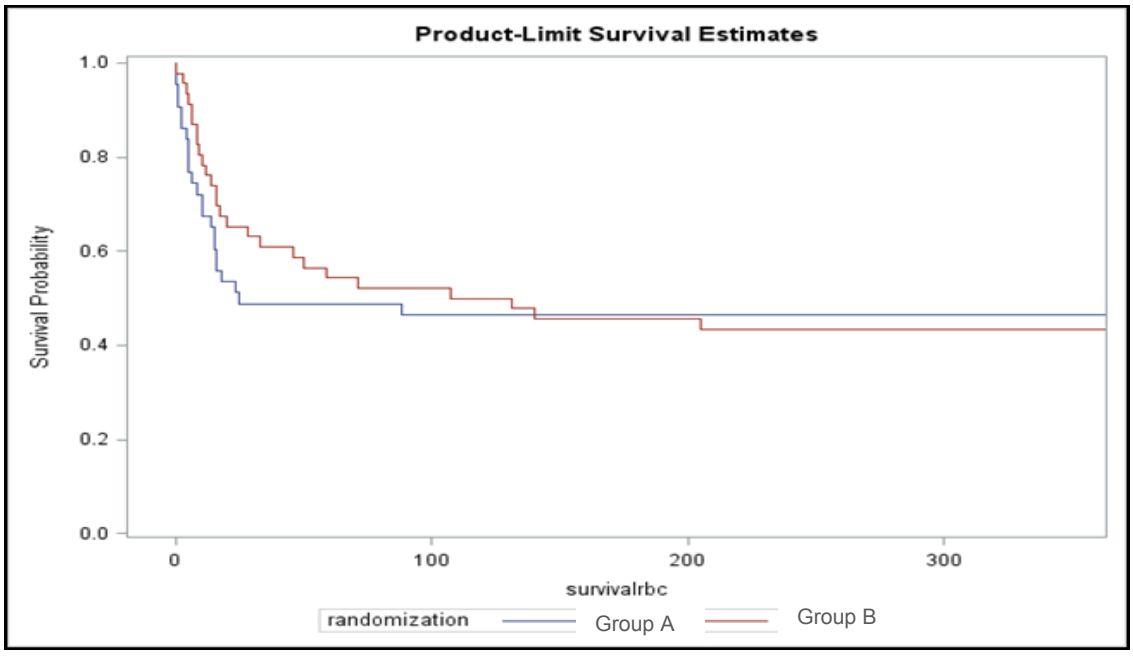
1) Group B experienced larger improvement in coagulopathy as measured by fibrinolysis from Field to ED (one of our secondary objectives, see below): as demonstrated by a repeated measures mixed linear model, the interaction between time and randomization approached significance, suggesting that group assignment modified the temporal trends of fibrinolysis as

measured by rTEG Ly30. Limiting the model to patients who presented in the Field in severe shock further increased the significance.

Linear Models for Repeated Measures including only Field and ED times for TEGs						
Model for LY30	ALL PATIENTS			SEVERE SHOCK ONLY (Field SBP<=70mmHg)		
	Beta	SE	P-VALUE	Beta	SE	P-VALUE
Intercept	6.7484	2.8959		6.2593	3.7915	
Time (ED, Field)	1.5018	2.9601	0.2528	4.9843	4.1149	0.5087
Randomization (groups A, B)	3.3722	4.165	0.8321	7.0309	5.9547	0.9767
Randomization*time (Interaction)	-8.1625	4.4787	0.0721	-14.3628	6.5971	0.0348

2) Toleration of longer time to RBC **without** any adverse event, which was another of our secondary objectives (an objective of major importance to the military, and consequently to our funder, the Dept. of Defense)

There seemed to be a difference in time from injury and from ED arrival to the first required RBC favoring group B, who tolerated longer time without increasing mortality or any other adverse event. A survival analysis, however, did not reach significance.



More important, there has been no increase in adverse events or any suggestion of harm to enrollees and enrollment has been on target.

There are several explanations for the differences in the expected and observed values. First, for the most common injury mechanisms, the case-fatality rate is decreasing in our trauma center (Sauaia et al. JAMA, 2016). Second, the wide use of TEG-guided hemostatic resuscitation in our institution has been associated with a dramatic decrease in post-injury mortality (Gonzalez, Ann Surg, 2016). In addition, our findings are not isolated. A recent multicenter RCT (Connelly et al., JAMA Surgery, 2016) also noted that the patients enrolled were less severely injured than anticipated based on previous experiences. Given the time required to establish such intense trials, usually one to two years, the assumptions used in the calculation of the sample size can understandably become outdated. This has prompted proposals of alternative designs for RCTs, such as adaptive and Bayesian RCTs as recently described by an NIH-funded workshop (Coffey et al. Clinical trials, 2012).

Based on the explanations above, we propose a continuation of the trial to increase sample size and make it adequate to detect differences in the proposed outcomes (mortality, admission acidosis and coagulation, hemorrhage as well as requirement for blood products), as follows:

Hypothesis for primary endpoint above: 28-day mortality will be significantly higher in control patients compared to experimental patients.

With 80% power, 95% confidence and assuming 12% mortality rate in the control group (based on the second interim analyses data), with 5 interim analyses (4 interim and 1 final, of which 3 already occurred), we will require 112 patients in each group (assuming the attrition rate observed in this RCT of 12%) to detect a difference of 10 percent points. This results in a total of 224 patients, which corresponds to an increase of 74 patients over the current sample size of 150 patients. See below the significance level for the 5 analyses.

Spending = O'Brien-Fleming, N1 = 112, N2 =112, P1 = 0.12, P2 = 0.02, Continuity Correction.

Look	Time	Lower Bndry	Upper Bndry	Nominal Alpha	Inc Alpha	Total Alpha	Inc Power	Total Power
1	0.20	-4.87688	4.87688	0.000	0.000	0.000	0.00015	0.000
2	0.40	-3.35695	3.35695	0.001	0.001	0.001	0.05885	0.059
3	0.60	-2.68026	2.68026	0.007	0.007	0.008	0.25750	0.317
4	0.80	-2.28979	2.28979	0.022	0.017	0.024	0.28890	0.605
5	1.00	-2.03100	2.03100	0.042	0.026	0.050	0.19460	0.800

Hypothesis 5: Admission clot strength (as measured by TEG G) will be significantly lower in control patients compared to experimental group patients

With 80% power, 95% confidence and assuming mean admission TEG G = 7 dynes/cm^2 with standard deviation of 3 in the control group (based on the second interim analyses data), with 5 interim analyses (4 interim and 1 final, of which 3 already occurred), we will require 112 patients in each group (assuming the attrition rate observed in this RCT of 12%) to detect a difference of 1.1 dynes/cm^2 (from 7 to 5.9 dynes/cm^2) between the two mean TEG Gs. This results in a total of 224, which corresponds to an increase of 74 patients over the current sample size of 150 patients.

F. Secondary Study Objectives and Endpoints

1. **Secondary Objective 1:** To determine the efficacy of postinjury field – resuscitation with plasma first compared to the current standard of care on

decreasing the incidence of adverse outcomes (as measured by a composite outcome including multiple organ failure and/or 28-day death).

Secondary Endpoint 1: Composite outcome of 28-day in-hospital mortality and postinjury multiple organ failure (MOF) incidence

a. **Definition:** This outcome is defined as the occurrence of in-hospital death or MOF within the first 28 days postinjury. MOF is defined using the validated Denver MOF score (Denver MOF score>3 of simultaneously obtained scores after 48 hours postinjury).[80] Postinjury MOF remains the leading cause of late death among trauma patients, thus having a strong association with mortality.[85]
b. **Hypothesis 1:** The incidence of the composite outcome (28-day in-hospital death or MOF) will be significantly higher† in control patients compared to experimental group patients.
c. **Statistical Analysis**: Fisher Exact Test will be used to compare the incidence of this composite outcome in the two groups. Statistical significance will be determined according to the interim analyses procedures describe below. If adjustment for the pre-defined confounders (see Randomization Effectiveness in Section IV.D.2) is needed, we will use the above-defined method for categorical, binary variables.
d. **Interim analyses:** We planned 5 analyses (4 interim, 1 final), equally spaced, using the O'Brien-Fleming method to calculate two-sided boundaries, assuming a 95% confidence and 80% power. All patients entered in each interim analyses will have complete data on 28-day outcomes (i.e., the analyses will be conducted only once the 28-day observation period for all survivors have been completed).
e. **Sample size and Power calculations:** This is a Phase 2 trial, powered to detect differences in this outcome as follows:

Power calculations: composite 28-day in-hospital death or MOF incidence

Assumptions 1: 80% power, 95% confidence, 2-tailed test, 3 equally spaced data analyses (2 interim, 1 final), using the O'Brien-Fleming method; control group 28-day composite outcome of mortality or MOF of 46% (based on control arm of ROC HS trial[67] and Denver MOF database data from 2005-2008).

Power	N1	N2	Alpha	Beta	P1	P2	Difference	Attrition Rate
0.800000	60	60	0.050000	0.200000	0.22	0.46	0.24	20%
0.800000	68	68	0.050000	0.200000	0.23	0.46	0.23	10%
0.800000	75	75	0.050000	0.200000	0.24	0.46	0.22	0

Sample sizes of 60 and 60 achieve 80% power to detect a difference of 0.24 between the group proportions of 0.22 and 0.46 at a significance level (alpha) of 0.05 using a two-sided test. These results assume that 3 sequential tests are made using the O'Brien-Fleming spending function to determine the test boundaries.

Boundaries (Bndry) for data looks

Details when Spending = O'Brien-Fleming, N1 = 60, N2 =60, P1 = 0.22, P2 = 0.46

	Lower	Upper	Nominal	Inc	Total	Inc	Total

† Although the hypothesis is worded as "higher", all tests will be two-tailed to control for differences in either direction (higher or lower than the other group).

Look	Bndry	Bndry	Alpha	Alpha	Alpha	Power	Power
1	-3.71030	3.71030	0.000207	0.000207	0.000207	0.018650	0.018650
2	-2.51142	2.51142	0.012025	0.011890	0.012097	0.398801	0.417451
3	-1.99302	1.99302	0.046259	0.037903	0.050000	0.382549	0.800000

Details when Spending = O'Brien-Fleming, N1 = 68, N2 =68, P1 = 0.23, P2 = 0.46

Look	Lower Bndry	Upper Bndry	Nominal Alpha	Inc Alpha	Total Alpha	Inc Power	Total Power
1	-3.71030	3.71030	0.000207	0.000207	0.000207	0.018650	0.018650
2	-2.51142	2.51142	0.012025	0.011890	0.012097	0.398801	0.417451
3	-1.99302	1.99302	0.046259	0.037903	0.050000	0.382549	0.800000

Details when Spending = O'Brien-Fleming, N1 = 75, N2 =75, P1 = 0.24, P2 = 0.46

Look	Lower Bndry	Upper Bndry	Nominal Alpha	Inc Alpha	Total Alpha	Inc Power	Total Power
1	-3.71030	3.71030	0.000207	0.000207	0.000207	0.018650	0.018650
2	-2.51142	2.51142	0.012025	0.011890	0.012097	0.398801	0.417451
3	-1.99302	1.99302	0.046259	0.037903	0.050000	0.382549	0.800000

Addendum November 16, 2016

Using the results of the first and second interim analyses, a new calculation for the sample size demonstrates: with 80% power, 95% confidence and assuming a 14% rate of the composite outcome in the control group (based on the second interim analyses data), with 5 interim analyses (4 interim and 1 final, of which 3 already occurred), we will require 112 patients in each group (assuming the attrition rate observed in this RCT of 12%) to detect a difference of 11 percent points. This results in a total of 224 patients, which corresponds to an increase of 74 patients over the current sample size of 150 patients.

2. **Secondary Objective 2**: To determine if early administration of plasma in the field will improve the incidence of early postinjury coagulopathy on admission compared to the current standard of care.

   Secondary Endpoint 2: Admission coagulopathy. This is an endpoint for which there is ample evidence of a strong association with mortality.[11, 86, 87]

   a. **Definition:** Admission coagulopathy will be measured by the admission international normalized ratio for pro-thrombin time (INR). INR will be defined as the first INR obtained upon ED arrival. The target time point is 30 minutes after ambulance arrival to injury scene. Our institution's average time from arrival of ambulance to scene till arrival in ED is 28 minutes [88]. INR obtained more than 60 minutes from arrival of ambulance to scene will not be used as the endpoint.

   b. **Hypothesis 2.1:** Admission INR will be significantly higher‡ in control patients compared to experimental group patients.

   c. **Statistical test 2.1:** Linear regression will be used to compare the mean admission INR of the two groups. Statistical significance will be determined according to the interim analyses procedures describe

‡ Although the hypothesis is worded as "higher", all tests will be two-tailed to control for differences in either direction (higher or lower than the other group).

below. If adjustment for pre-defined confounders is needed, we will use the above-defined method for continuous, normally distributed variables.

d. **Sample size and Power calculations:** This is a Phase 2 trial, powered to detect differences in this outcome as follows:

Power calculations: Admission INR

Assumptions 1: 80% power, 95% confidence, 2-tailed test, 3 equally spaced data analyses (2 interim, 1 final), using the O'Brien-Fleming method; control group mean admission INR=1.47 and standard deviation (SD) =1.0 (based on ROC trial control arm[67]).

Power	N1	N2	Alpha	Beta	Mean 1	Mean 2	SD	Difference	Attrition Rate
0.800000	60	60	0.050000	0.200000	1.50	1.00	1.00	0.50	20%
0.800000	68	68	0.050000	0.200000	1.50	1.02	1.00	0.48	10%
0.800000	75	75	0.050000	0.200000	1.50	1.04	1.00	0.46	0

Sample sizes of 60 and 60 achieve 80% power to detect a difference of 0.50 between the two means, a clinically relevant difference, at a significance level (alpha) of 0.05 using a two-sided test. These results assume that 3 sequential tests are made using the O'Brien-Fleming spending function to determine the test boundaries.

Boundaries (Bndry) for data looks

Details when Spending = O'Brien-Fleming, N1 = 60, N2 =60, S1 = 1.00, S2 = 1.00, Diff = 0.50

Look	Lower Bndry	Upper Bndry	Nominal Alpha	Inc Alpha	Total Alpha	Inc Power	Total Power
1	-3.71030	3.71030	0.000207	0.000207	0.000207	0.018650	0.018650
2	-2.51142	2.51142	0.012025	0.011890	0.012097	0.398801	0.417451
3	-1.99302	1.99302	0.046259	0.037903	0.050000	0.382549	0.800000

Details when Spending = O'Brien-Fleming, N1 = 68, N2 =68, S1 = 1.00, S2 =1.00 , Diff = 0.48

Look	Lower Bndry	Upper Bndry	Nominal Alpha	Inc Alpha	Total Alpha	Inc Power	Total Power
1	-3.71030	3.71030	0.000207	0.000207	0.000207	0.018650	0.018650
2	-2.51142	2.51142	0.012025	0.011890	0.012097	0.398801	0.417451
3	-1.99302	1.99302	0.046259	0.037903	0.050000	0.382549	0.800000

Details when Spending = O'Brien-Fleming, N1 = 75, N2 =75, S1 = 1.00, S2 = 1.00, Diff = 0.46

Look	Lower Bndry	Upper Bndry	Nominal Alpha	Inc Alpha	Total Alpha	Inc Power	Total Power
1	-3.71030	3.71030	0.000207	0.000207	0.000207	0.018650	0.018650
2	-2.51142	2.51142	0.012025	0.011890	0.012097	0.398801	0.417451
3	-1.99302	1.99302	0.046259	0.037903	0.050000	0.382549	0.800000

Addendum November 16, 2016

Based on the interim analyses, a proposed new sample size is: with 80% power, 95% confidence and assuming mean admission INR = 1.32 with standard deviation of 0.5 in the control group (based on the second interim analyses data), with 5 interim analyses (4 interim and 1 final, of which 3 already occurred), we will require 112 patients in each group (assuming the attrition rate observed in this RCT of 12%) to detect a difference of 0.19 (from 1.32 to 1.13) between the two mean INRs. This results in a total of 224, which corresponds to an increase of 74 patients over the current sample size of 150 patients.

e. **Hypothesis 2.2:** The incidence of admission coagulopathy as measured by INR>1.5 will be significantly higher[§] in control patients compared to experimental group patients.

f. **Statistical test 2.2:** Logistic regression will be used to compare the incidence of admission coagulopathy between the two groups. Statistical significance will be determined according to the interim analyses procedures describe below. If adjustment for pre-defined confounders is needed, we will use the above-defined method for categorical, binary variables.

g. **Sample size and Power calculations:** this is a Phase 2 trial, powered to detect differences in this outcome as follows:

Power calculations: Admission coagulopathy

Assumptions 1: 80% power, 95% confidence, 2-tailed test, 3 equally spaced data analyses (2 interim, 1 final), using the O'Brien-Fleming method; control group admission coagulopathy incidence 24%, 28% and 36% (based on [11, 86, 87]).

Power	N1	N2	Alpha	Beta	P1	P2	Difference	Attrition Rate
0.800000	60	60	0.050000	0.200000	0.06	0.24	0.18	20%
0.800000	68	68	0.050000	0.200000	0.07	0.24	0.17	10%
0.800000	75	75	0.050000	0.200000	0.07	0.24	0.17	0
0.800000	60	60	0.050000	0.200000	0.08	0.28	0.18	20%
0.800000	68	68	0.050000	0.200000	0.09	0.28	0.17	10%
0.800000	75	75	0.050000	0.200000	0.10	0.28	0.16	0
0.800000	60	60	0.050000	0.200000	0.14	0.36	0.22	20%
0.800000	68	68	0.050000	0.200000	0.15	0.36	0.21	10%
0.800000	75	75	0.050000	0.200000	0.16	0.36	0.20	0

Sample sizes of 60 and 60 achieve 80% power to detect a difference of 0.18 between the group proportions at a significance level (alpha) of 0.05 using a two-sided test. These results assume that 3 sequential tests are made using the O'Brien-Fleming spending function to determine the test boundaries.

Boundaries (Bndry) for data looks (worst case scenario)

Details when Spending = O'Brien-Fleming, N1 = 60, N2 =60, P1 = 0.14, P2 = 0.36

Look	Lower Bndry	Upper Bndry	Nominal Alpha	Inc Alpha	Total Alpha	Inc Power	Total Power
1	-3.71030	3.71030	0.000207	0.000207	0.000207	0.018650	0.018650
2	-2.51142	2.51142	0.012025	0.011890	0.012097	0.398801	0.417451
3	-1.99302	1.99302	0.046259	0.037903	0.050000	0.382549	0.800000

Details when Spending = O'Brien-Fleming, N1 = 68, N2 =68, P1 = 0.15, P2 = 0.36

Look	Time	Lower Bndry	Upper Bndry	Nominal Alpha	Inc Alpha	Total Alpha	Inc Power	Total Power
1	0.33	-3.71030	3.71030	0.000207	0.000207	0.000207	0.018650	0.018650
2	0.67	-2.51142	2.51142	0.012025	0.011890	0.012097	0.398801	0.417451
3	1.00	-1.99302	1.99302	0.046259	0.037903	0.050000	0.382549	0.800000

Details when Spending = O'Brien-Fleming, N1 = 75, N2 =75, P1 = 0.16, P2 = 0.36

Look	Lower Bndry	Upper Bndry	Nominal Alpha	Inc Alpha	Total Alpha	Inc Power	Total Power
1	-3.71030	3.71030	0.000207	0.000207	0.000207	0.018650	0.018650
2	-2.51142	2.51142	0.012025	0.011890	0.012097	0.398801	0.417451
3	-1.99302	1.99302	0.046259	0.037903	0.050000	0.382549	0.800000

[§] Although the hypothesis is worded as "higher", all tests will be two-tailed to control for differences in either direction (higher or lower than the other group).

Addendum November 16, 2016

Based on the interim analyses, a proposed new sample size is: with 80% power, 95% confidence and assuming a 18% rate of INR>1.5 in the control group (based on the second interim analyses data), with 5 interim analyses (4 interim and 1 final, of which 3 already occurred), we will require 112 patients in each group (assuming the attrition rate observed in this RCT of 12%) to detect a difference of 13 percent points. This results in a total of 224 patients, which corresponds to an increase of 74 patients over the current sample size of 150 patients.

3. **Secondary Objective 3**: To determine if early administration of plasma in the field will improve postinjury clot strength upon admission compared to the current standard of care.

   Secondary Endpoint 3: Admission clot strength. This is an endpoint for which there is evidence of association with postinjury mortality, coagulopathy and use of blood products [79, 83, 89, 90].

   a. **Definition:** Admission clot strength will be measured by **thrombelastography G-value** upon ED arrival. The target time point is 30 minutes after ambulance arrival to injury scene. Our institution's average time from arrival of ambulance to scene till arrival in ED is 28 minutes.[88] If value is obtained more than 60 minutes from arrival of ambulance to scene, it will not be used to define the endpoint.
   b. **Hypothesis 3.1:** Admission clot strength will be significantly lower[**] in control patients compared to experimental group patients.
   c. **Statistical test 3.1:** Multiple linear regression will be used to compare the mean clot strength in the two groups. Statistical significance will be determined according to the interim analyses procedures describe below. If adjustment for pre-defined confounders is needed, we will use the above-defined method for continuous, normally distributed variables.
   d. **Interim analyses:** We planned 5 analyses (4 interim, 1 final), equally spaced, using the O'Brien-Fleming method to calculate two-sided boundaries, assuming a 95% confidence and 80% power.
   e. **Sample size and Power calculations:** This is a Phase 2 trial, powered to detect differences in this outcome as follows:

Power calculations: Admission Clot Strength (TEG G-Value)

Assumptions 1: 80% power, 95% confidence, 2-tailed test, 3 equally spaced data analyses (2 interim, 1 final), using the O'Brien-Fleming method; control group admission clot strength (based on Pezold et al. [83]).

Power	N1	N2	Alpha	Beta	P1	P2	S1	S2	Difference	Attrition Rate
0.800000	60	60	0.050000	0.200000	4.90	3.72	2.30	2.30	1.18	20%
0.800000	68	68	0.050000	0.200000	4.90	3.79	2.30	2.30	1.11	10%
0.800000	75	75	0.050000	0.200000	4.90	3.84	2.30	2.30	1.06	0

[**] Although the hypothesis is worded as "lower", all tests will be two-tailed to control for differences in either direction (higher or lower than the other group).

Sample sizes of 60 and 60 achieve 80% power to detect a difference of 1.18 dynes/cm^2 between the two groups,

which is smaller than a clinically relevant difference based on previous clinical trials aiming at addressing early postinjury coagulopathy [79, 89, 90] at a significance level (alpha) of 0.05 using a two-sided test. These results assume that 3 sequential tests are made using the O'Brien-Fleming spending function to determine the test boundaries.

Boundaries (Bndry) for data looks

Details when Spending = O'Brien-Fleming, N1 = 60, N2 =60, S1 = 2.30, S2 = 2.30, Diff=1.18

Look	Time	Lower Bndry	Upper Bndry	Nominal Alpha	Inc Alpha	Total Alpha	Inc Power	Total Power
1	0.33	-3.71030	3.71030	0.000207	0.000207	0.000207	0.018650	0.018650
2	0.67	-2.51142	2.51142	0.012025	0.011890	0.012097	0.398801	0.417451
3	1.00	-1.99302	1.99302	0.046259	0.037903	0.050000	0.382549	0.800000

Details when Spending = O'Brien-Fleming, N1 = 68, N2 =68, S1 = 2.30, S2 = 2.30, Diff=1.11

Look	Time	Lower Bndry	Upper Bndry	Nominal Alpha	Inc Alpha	Total Alpha	Inc Power	Total Power
1	0.33	-3.71030	3.71030	0.000207	0.000207	0.000207	0.018650	0.018650
2	0.67	-2.51142	2.51142	0.012025	0.011890	0.012097	0.398801	0.417451
3	1.00	-1.99302	1.99302	0.046259	0.037903	0.050000	0.382549	0.800000

Details when Spending = O'Brien-Fleming, N1 = 75, N2 =75, S1=2.30, S2 = 2.30, Diff=1.06

Look	Time	Lower Bndry	Upper Bndry	Nominal Alpha	Inc Alpha	Total Alpha	Inc Power	Total Power
1	0.33	-3.71030	3.71030	0.000207	0.000207	0.000207	0.018650	0.018650
2	0.67	-2.51142	2.51142	0.012025	0.011890	0.012097	0.398801	0.417451
3	1.00	-1.99302	1.99302	0.046259	0.037903	0.050000	0.382549	0.800000

f. **Hypothesis 3.2:** The incidence of admission high clot strength defined as TEG G-value>5.0 dynes/cm^2 will be significantly lower†† in control patients compared to experimental group patients.

g. **Statistical test 3.1:** Multiple logistic regression will be used to compare the incidence of high clot strength (TEG G-value >5.0dynes/cm^2) in the two groups. Statistical significance will be determined according to the interim analyses procedures describe below. If adjustment for pre-defined confounders is needed, we will use the above-defined method for continuous, normally distributed variables.

h. **Interim analyses:** We planned 5 analyses (4 interim, 1 final), equally spaced, using the O'Brien-Fleming method to calculate two-sided boundaries, assuming a 95% confidence and 80% power.

i. **Sample size and Power calculations:** This is a Phase 2 trial, powered to detect differences in this outcome as follows:

Power calculations: Admission High Clot Strength (TEG G-value>5dynes/cm^2)

Assumptions 1: 80% power, 95% confidence, 2-tailed test, 3 equally spaced data analyses (2 interim, 1 final), using the O'Brien-Fleming method; control group admission acidosis (based on [83]).

Power	N1	N2	Alpha	Beta	P1	P2	Difference	Attrition Rate
0.800000	60	60	0.050000	0.200000	0.25	0.50	0.25	20%
0.800000	68	68	0.050000	0.200000	0.27	0.50	0.23	10%
0.800000	75	75	0.050000	0.200000	0.28	0.50	0.22	0

†† Although the hypothesis is worded as "lower", all tests will be two-tailed to control for differences in either direction (higher or lower than the other group).

Sample sizes of 60 and 60 achieve 80% power to detect a difference of 0.25 between the group proportions at a significance level (alpha) of 0.050000 using a two-sided test. These results assume that 3 sequential tests are made using the O'Brien-Fleming spending function to determine the test boundaries.

Boundaries (Bndry) for data looks

Details when Spending = O'Brien-Fleming, N1 = 60, N2 =60, P1 = 0.25, P2 = 0.50

Look	Time	Lower Bndry	Upper Bndry	Nominal Alpha	Inc Alpha	Total Alpha	Inc Power	Total Power
1	0.33	-3.71030	3.71030	0.000207	0.000207	0.000207	0.018650	0.018650
2	0.67	-2.51142	2.51142	0.012025	0.011890	0.012097	0.398801	0.417451
3	1.00	-1.99302	1.99302	0.046259	0.037903	0.050000	0.382549	0.800000

Details when Spending = O'Brien-Fleming, N1 = 68, N2 =68, P1 = 0.27, P2 = 0.50

Look	Time	Lower Bndry	Upper Bndry	Nominal Alpha	Inc Alpha	Total Alpha	Inc Power	Total Power
1	0.33	-3.71030	3.71030	0.000207	0.000207	0.000207	0.018650	0.018650
2	0.67	-2.51142	2.51142	0.012025	0.011890	0.012097	0.398801	0.417451
3	1.00	-1.99302	1.99302	0.046259	0.037903	0.050000	0.382549	0.800000

Details when Spending = O'Brien-Fleming, N1 = 75, N2 =75, P1 = 0.28, P2 = 0.50

Look	Time	Lower Bndry	Upper Bndry	Nominal Alpha	Inc Alpha	Total Alpha	Inc Power	Total Power
1	0.33	-3.71030	3.71030	0.000207	0.000207	0.000207	0.018650	0.018650
2	0.67	-2.51142	2.51142	0.012025	0.011890	0.012097	0.398801	0.417451
3	1.00	-1.99302	1.99302	0.046259	0.037903	0.050000	0.382549	0.800000

Addendum November 16, 2016

Based on the interim analyses, a proposed new sample size is: with 80% power, 95% confidence and assuming mean admission TEG G = 7 dynes/cm2 with standard deviation of 3 in the control group (based on the second interim analyses data), with 5 interim analyses (4 interim and 1 final, of which 3 already occurred), we will require 112 patients in each group (assuming the attrition rate observed in this RCT of 12%) to detect a difference of 1.1 dynes/cm2 (from 7 to 5.9 dynes/cm2) between the two mean TEG Gs. This results in a total of 224, which corresponds to an increase of 74 patients over the current sample size of 150 patients.

4. **Secondary Objective 4**: To determine if early administration of plasma in the field will improve the incidence of early postinjury acidosis compared to the current standard of care.

   Secondary Endpoint 4: Admission acidosis. This is an endpoint for which there is ample evidence of a strong association of postinjury mortality and serious complications. [91-100]

   a. **Definition:** Admission acidosis will be defined through base deficit (BD) or lactate levels upon ED arrival. The target time point is 30 minutes after ambulance arrival to injury scene. Our institution's average time from arrival of ambulance to scene till arrival in ED is 28 minutes. [88] If both base deficit and lactate are obtained more than 60 minutes from arrival of ambulance to scene, they will not be used to define the endpoint.

b. **Hypothesis 4.1:** Admission BD will be higher‡‡ among control patients compare to the experimental group patients.
c. **Statistical test 4.1:** Linear regression will be used to compare the mean admission base deficit of the two groups. Statistical significance will be determined according to the interim analyses procedures describe below. If adjustment for pre-defined confounders is needed, we will use the above-defined method for continuous, normally distributed variables.
d. **Interim analyses:** We planned 5 analyses (4 interim, 1 final), equally spaced, using the O'Brien-Fleming method to calculate two-sided boundaries, assuming a 95% confidence and 80% power.
e. **Sample size and Power calculations:** This is a Phase 2 trial, powered to detect differences in this outcome as follows:

Power calculations: Admission Base Deficit

Assumptions 1: 80% power, 95% confidence, 2-tailed test, 3 equally spaced data analyses (2 interim, 1 final), using the O'Brien-Fleming method; control group mean admission BD=5.15 and standard deviation (SD) =5.1 (based on[92])

Power	N1	N2	Alpha	Beta	Mean 1	Mean 2	SD	Difference	Attrition Rate
0.800000	60	60	0.050000	0.200000	5.15	2.52	5.10	2.63	20%
0.800000	68	68	0.050000	0.200000	5.15	2.68	5.10	2.47	10%
0.800000	75	75	0.050000	0.200000	5.15	2.80	5.10	2.35	0

Sample sizes of 60 and 60 achieve 80% power to detect a difference of 2.63 between the two means, a clinically relevant difference, at a significance level (alpha) of 0.05 using a two-sided test. These results assume that 3 sequential tests are made using the O'Brien-Fleming spending function to determine the test boundaries.

Boundaries (Bndry) for data looks

Details when Spending = O'Brien-Fleming, N1 = 60, N2 =60, S1 = 5.10, S2 = 5.10, Diff = 2.63

Look	Lower Bndry	Upper Bndry	Nominal Alpha	Inc Alpha	Total Alpha	Inc Power	Total Power
1	-3.71030	3.71030	0.000207	0.000207	0.000207	0.018650	0.018650
2	-2.51142	2.51142	0.012025	0.011890	0.012097	0.398801	0.417451
3	-1.99302	1.99302	0.046259	0.037903	0.050000	0.382549	0.800000

Details when Spending = O'Brien-Fleming, N1 = 68, N2 =68, S1 = 5.10, S2 = 5.10, Diff = 2.47

Look	Lower Bndry	Upper Bndry	Nominal Alpha	Inc Alpha	Total Alpha	Inc Power	Total Power
1	-3.71030	3.71030	0.000207	0.000207	0.000207	0.018650	0.018650
2	-2.51142	2.51142	0.012025	0.011890	0.012097	0.398801	0.417451
3	-1.99302	1.99302	0.046259	0.037903	0.050000	0.382549	0.800000

Details when Spending = O'Brien-Fleming, N1 = 75, N2 =75, S1 = 5.10, S2 = 5.10, Diff = 2.35

Look	Lower Bndry	Upper Bndry	Nominal Alpha	Inc Alpha	Total Alpha	Inc Power	Total Power
1	-3.71030	3.71030	0.000207	0.000207	0.000207	0.018650	0.018650
2	-2.51142	2.51142	0.012025	0.011890	0.012097	0.398801	0.417451
3	-1.99302	1.99302	0.046259	0.037903	0.050000	0.382549	0.800000

‡‡ Although the hypothesis is worded as "higher", all tests will be two-tailed to control for differences in either direction (higher or lower than the other group).

Addendum November 16, 2016

Based on the interim analyses, a proposed new sample size is: with 80% power, 95% confidence and assuming mean admission BD = 11mEq/L with standard deviation of 6.3 in the control group (based on the second interim analyses data), with 5 interim analyses (4 interim and 1 final, of which 3 already occurred), we will require 112 patients in each group (assuming the attrition rate observed in this RCT of 12%) to detect a difference of 2mEq/L (from 11 to 9mEq/L) between the two mean BDs. This results in a total of 224, which corresponds to an increase of 74 patients over the current sample size of 150 patients.

f. **Hypothesis 4.2:** Admission lactate will be higher[§§] among control patients compare to the experimental group patients. **Statistical test 3.2:** Linear regression will be used to compare the mean admission lactate of the two groups. Statistical significance will be determined according to the interim analyses procedures describe below. If adjustment for pre-defined confounders is needed, we will use the above-defined method for continuous, normally distributed variables.

g. **Interim analyses:** We planned 5 analyses (4 interim, 1 final), equally spaced, using the O'Brien-Fleming method to calculate two-sided boundaries, assuming a 95% confidence and 80% power.

h. **Sample size and Power calculations:** This is a Phase 2 trial, powered to detect differences in this outcome as follows:

Power calculations: Admission Lactate

Assumptions 1: 80% power, 95% confidence, 2-tailed test, 3 equally spaced data analyses (2 interim, 1 final), using the O'Brien-Fleming method; control group mean admission lactate=2.0 mmol/L and standard deviation (SD) =2.0 (based on [67, 81, 91])

Power	N1	N2	Alpha	Beta	Mean 1	Mean 2	SD	Difference	Attrition Rate
0.800000	60	60	0.050000	0.200000	2.00	0.97	2.00	1.03	20%
0.800000	68	68	0.050000	0.200000	2.00	1.03	2.00	0.97	10%
0.800000	75	75	0.050000	0.200000	2.00	1.08	2.00	0.92	0

Sample sizes of 60 and 60 achieve 80% power to detect a difference of 1.03 mmol/L between the two means, a clinically relevant difference, at a significance level (alpha) of 0.05 using a two-sided test. These results assume that 3 sequential tests are made using the O'Brien-Fleming spending function to determine the test boundaries.

Boundaries (Bndry) for data looks

Details when Spending = O'Brien-Fleming, N1 = 60, N2 =60, S1 = 2.00, S2 = 2.00, Diff = 1.03

Look	Lower Bndry	Upper Bndry	Nominal Alpha	Inc Alpha	Total Alpha	Inc Power	Total Power
1	-3.71030	3.71030	0.000207	0.000207	0.000207	0.018650	0.018650
2	-2.51142	2.51142	0.012025	0.011890	0.012097	0.398801	0.417451
3	-1.99302	1.99302	0.046259	0.037903	0.050000	0.382549	0.800000

Details when Spending = O'Brien-Fleming, N1 = 68, N2 =68, S1 = 2.00, S2 = 2.00, Diff = 0.97

[§§] Although the hypothesis is worded as "higher", all tests will be two-tailed to control for differences in either direction (higher or lower than the other group).

Look	Lower Bndry	Upper Bndry	Nominal Alpha	Inc Alpha	Total Alpha	Inc Power	Total Power
1	-3.71030	3.71030	0.000207	0.000207	0.000207	0.018650	0.018650
2	-2.51142	2.51142	0.012025	0.011890	0.012097	0.398801	0.417451
3	-1.99302	1.99302	0.046259	0.037903	0.050000	0.382549	0.800000

Details when Spending = O'Brien-Fleming, N1 = 75, N2 =75, S1 = 2.00, S2 = 2.00, Diff = 0.92

Look	Lower Bndry	Upper Bndry	Nominal Alpha	Inc Alpha	Total Alpha	Inc Power	Total Power
1	-3.71030	3.71030	0.000207	0.000207	0.000207	0.018650	0.018650
2	-2.51142	2.51142	0.012025	0.011890	0.012097	0.398801	0.417451
3	-1.99302	1.99302	0.046259	0.037903	0.050000	0.382549	0.800000

Addendum November 16, 2016

Based on the interim analyses, a proposed new sample size is: with 80% power, 95% confidence and assuming mean admission lactate=5 mg/dL with standard deviation of 2 in the control group (based on the second interim analyses data), with 5 interim analyses (4 interim and 1 final, of which 3 already occurred), we will require 112 patients in each group (assuming the attrition rate observed in this RCT of 12%) to detect a difference of 1 mg/dL between the two mean lactates. This results in a total of 224, which corresponds to an increase of 74 patients over the current sample size of 150 patients.

G. Exploratory analyses

1. **Exploratory analysis 1: 24-hour mortality** Based on the control arm of the recent ROC trial[67] and our trauma registry data, the control group 24-hours mortality is estimated to be between 19% and 26%.
    a. **Definition:** 24-hours mortality is defined as death within 24 hours post injury (death of any cause). Research coordinators will verify the vital status of all patients 24 hours postinjury and determine the time of death in relationship to injury.
    b. **Hypothesis:** 24-hour mortality will be significantly higher*** in control patients compared to experimental group patients.
    c. **Statistical test:** Logistic regression will be used to compare the 24-hour mortality in the two groups. Statistical significance will be evaluated at each interim analyses for the primary outcomes and p-values, as well as 95% and 99% confidence intervals will be reported to the DSMB for analyses. If adjustment for pre-defined confounders is needed, we will use the above-defined method for categorical, binary variables. Time from injury to death will be evaluated using the Kaplan Meyer curves, which will be compared using SAS PROC LIFETEST. We will use the Wilcoxon test (which places more weight on shorter survival times) and the log-rank test (which privileges longer survival times). SAS PROC LIFETEST also constructs statistics to test for

*** Although the hypothesis is worded as "lower", all tests will be two-tailed to control for differences in either direction (higher or lower than the other group).

association between covariates and the lifetime variable through rank tests for the association of survival time with covariates, if there are potential confounders detected using the above-described procedures.

2. **Pre-planned subgroup analyses:**
    a. **Blunt trauma mechanism:** There is evidence to suggest that the coagulation and inflammatory responses to injury differ by trauma mechanism, as they cause different tissue damage.[101, 102]
        1. **Hypothesis:** Blunt trauma patients will have different effect sizes than penetrating trauma victims regarding the primary endpoint and secondary endpoints
        2. **Statistical tests:** Same tests described for primary and secondary endpoints stratified by mechanism of injury (blunt vs. penetrating). We will compare the 95% confidence intervals to determine overlap between the blunt and penetrating groups for each endpoint.
    b. **Absence vs. presence of serious traumatic brain injury (TBI):** there is evidence to suggest a differential coagulation and inflammatory response by the presence of TBI.[101, 103, 104]
        1. **Hypothesis:** Patients with associated TBI (defined as Abbreviated Injury Score, AIS, for Head >3) will have different effect sizes than their counterparts without TBI regarding the primary endpoint and secondary endpoints.
        2. **Statistical tests:** Same tests described for primary and secondary endpoints stratified by presence of TBI. We will compare the 95% confidence intervals to determine overlap between the two groups for each endpoint.
    c. **Presence of very severe hemorrhagic shock (HS, defined as SBP≤70 mmHg) versus severe HS (defined as SBP 71-90mmHg + Heart rate>=108bpm):** There is evidence that the patients with very severe HS shock are more likely to require massive transfusion and present severe coagulopathy, thus potentially having differential mortality.[105]
        1. **Hypothesis:** Patients with very severe hemorrhagic shock (defined as SBP≤70mmHg) will have different effect sizes than their counterparts with severe hemorrhagic shock (defined as SBP 71-90mmHg +heart rate>=108bpm) regarding the primary endpoint and secondary endpoints.
        2. **Statistical tests:** Same tests described for primary and secondary endpoints stratified by degree of hemorrhagic shock. We will compare the 95% confidence intervals to determine overlap between the two groups for each endpoint. Overall experiment error will be adjusted for the number of subgroups being analyzed using the Bonferroni method.

3. **Adverse-outcome free days:** There is ample evidence suggesting a strong association with these outcomes and mortality. These outcomes were devised by the ARDS Net group to overcome survivor bias, initially for mechanical ventilation and quickly expanded to include other outcomes potentially biased by survivorship. Outcome-free-days (OFD) as proposed by the NIH sponsored ARDSnet trials,[106] takes the observation time into account. We apologize for not providing more information on this measure; we assumed the reviewers were familiar with it as it is so often used in recent clinical trials. Indeed, ALI-free days, MOF-free days and ventilator-free days (VFD) were proposed exactly to deal with the survivor –bias stated by the reviewers.
    a. **Definition:** VFDs can be arbitrarily defined as the number of days between successful weaning from mechanical ventilation and day 28 after study enrollment. The 28-day landmark was suggested both because interventional trials in acute lung injury typically involve a 28-day treatment/follow-up period after the patient enrolls in the trial and because most, but not all, patients with acute lung injury have either died or been successfully weaned from mechanical ventilation by day 28. VFDs are defined as follows:

        VFDs = 0: If the patient dies before 28 days.

        VFDs = (28 - x): If the patient is successfully weaned from mechanical ventilation within 28 days, where x is the number of days spent receiving mechanical ventilation.

        VFDs = 0: If the patient requires mechanical ventilation for 28 days or more.

        For example, VFD=2 means that the patient spent at least 26 days on the ventilator or, less likely, died within 2 days without being on a ventilator, both of which are considered poor outcomes.
    b. We will assess two adverse-outcome-free days measured over 28 days, as follows:
        1. **Ventilation free days**
        2. **Acute Lung Injury (ALI) free days (as defined by the validated Denver Lung Dysfunction score)**
    c. **Hypothesis:** Experimental group patients will have longer †††adverse-outcome-free-days than control patients.
    d. **Statistical test:** these outcomes are often skewed and non-normally distributed. Log transformation has been shown to be a good alternative to approximate normality for them. Thus, we will utilize linear regression to examine differences between the two groups. If adjustment for covariates is needed, we will proceed with the method for continuous normally distributed variables. In the case, normality cannot be achieved (as determined by the Shapiro-Wilk test for

††† Although the hypothesis is worded as longer, the tests will be two-tailed to examine association in either direction.

normality), and there is no need to adjust for confounders, we will report median and interquartile ranges as measures of data central tendency and dispersion and use the Wilcoxon test to evaluate differences. If adjustment for covariates is needed, then we will resort to categorization of the outcomes, based on previously established, clinically meaningful cutoffs.[80] Overall experiment error will be adjusted for the number of adverse-outcomes-free-days parameters being analyzed using the Bonferroni method.

4. **Temporal trends:**
    a. **Coagulation Profile:** We will assess temporal trends from pre-hospital coagulation variables (as described below) at 2, 4, 6, 12, 24, 72 hours and 28 days post-injury. The proposed coagulation measurements will also test the efficacy of plasma first resuscitation, since plasma is primarily employed for restoring levels of Factors II, V, VII, X and possibly XIII with a goal of greater than 20%, which is known to be effective for the cessation of surgical bleeding[107]. In addition, there are major changes in the post-shock plasma affecting levels of proteins involved in regulating proteolysis, membrane lipid binding and stabilizing potentially cytotoxic intracellular components released after tissue injury.

    Our recent investigations of the proteome of three patients with significant hyperfibrinolysis, two who had ALI but survived and one who developed ARDS and did not, were compared to healthy controls of the same gender. The hyperfibrinolytic state of these injured patients was confirmed by thrombelastograms (TEG) prior to blood component resuscitation (results not shown). The proteins that significantly increase in the patient plasma vs. normal males include: actin (15-28-fold), a cytoplasmic protein that has been implicated in ALI when free in the circulation, von Willebrand Factor (15-24-fold), a necessary coagulation factor, α-enolase (10-28-fold)[59] a lyase with protease activity, perioxiredoxins 2 & 6 (7-14-fold), proteins which are atypical phospholipases, adiponectin (8-19-fold), which is a hormone from adipocytes that is important in glucose uptake & insulin sensitivity, lipid oxidation and is a PPAR1/PPAR2 ligand, matrix metalloproteinase-9 (MMP-9) (3-5-fold) that is also known as gelatinase, which breaks down the extracellular matrix and is a sensitive indicator of leukocyte activation and, lastly, carboxypeptidase β2, also known as TAFI. Plasma contains many proteins which can oppose the actions of these proteins, including: anti-proteases (α1-antitrypsin, α2-macroglobulin, and other globulins), lipid carriers (albumin), and gelsolin which solubilizes and inhibits actin induced ALI.[57, 58, 60, 108]

        1. **Hypothesis**: Experimental group patients will demonstrate earlier and larger improvement in coagulation variables compared to control patients.

2. **Statistical analysis**: variables will be examined for normality through the Shapiro-Wilk test and log-transformed if necessary to approximate normality. Statistical analyses will be performed by using mixed linear models (SAS Proc Mixed), with an unstructured covariance structure between the different time measures, and if needed, adjustment for potential confounders in case randomization fails to account for differences. Overall experiment error will be adjusted for the number of coagulation parameters being analyzed using the Bonferroni method. Multiple comparison adjustment within each coagulation variable temporal trend will be done using the Tukey's method.

   Mixed linear modeling with SAS Proc Mixed allows for incomplete data, thus all observed points will be used in the analysis. Missing data in temporal trends will be dealt with differently depending on the reason for missing. If MAR (loss to followup, withdrawn from study), multiple imputation methods will be used, as recommended by the Panel on Handling Missing Data in Clinical Trials. However, as described in previous sections, a major reason for missing data in these cases is early death, resulting in data MNAR, for which we will use the same approach to deal with the missing data as described for the secondary endpoints.

   A similar analysis will be carried out for temporal trends in Proteomic Profile, Actin and Neutrophil Elastase inhibitors.

5. Blood components transfusion in the first 12 hours postinjury

a. **Packed Red Blood Cells (RBC) volume**

   1. Hypothesis: The volume of RBC transfused in the first 12 hours postinjury will be larger among control patients compared to the experimental group patients.

b. **Plasma volume**

   1. Hypothesis: The volume of plasma transfused in the first 12 hours postinjury will be larger among control patients compared to the experimental group patients.

   2. **Statistical test for a.1 and b.2:** These variables are usually non-normally distributed, thus the Wilcoxon non-parametric test will be used to compare the two groups and median/interquartile range will be used to express data central tendency and dispersion. If adjustment for covariates is needed, we will follow the procedure described for continuous, not normally distributed variables. Overall experiment error will be adjusted for the number of blood transfusion parameters being analyzed using the Bonferroni method.

c. **Time to first RBC:** This is a variable of high importance for DOD, which leads to direct application in war theater trauma.

1. **Hypothesis:** Time to the first RBC will be longer among patients in the experimental group compared to control patients.
2. **Statistical test:** Kaplan Meyer curves will be compared using SAS PROC LIFETEST, which provides nonparametric k-sample tests based on weighted comparisons of the estimated hazard rate of the individual population under the null and alternative hypotheses. A variety of tests can be specified, of which we will use the Wilcoxon test (which places more weight on shorter survival times) and the log-rank test (which privileges longer survival times). SAS PROC LIFETEST also constructs statistics to test for association between covariates and the lifetime variable through rank tests for the association of survival time with covariates

H. Missing data prevention and treatment procedures:

We will follow the recommendations for prevention and treatment procedures outlined in the recent report by the National Research Council Panel on Handling Missing Data in Clinical Trials.[84] Missing data prevention is crucial in the primary endpoint to avoid any reduction in statistical power and the need for statistical analyses to account for the missing data. To minimize bias by missing data, research coordinators will collect data on the primary and secondary endpoints of all patients who were randomized in the field (including vital status at day 28 postinjury), including data on those patients who did not complete the protocol or were excluded for protocol violations or other reasons. Patients who withdraw from the study will be asked to allow research coordinators to collect SOC data from their medical record and, at a minimum, to provide contact information (and permission to be contacted) for verification of the primary endpoint mortality.

Based on previous trials in our institution, we predict that mortality will be missing due to loss of follow up in less than 2% of the sample. This is comparable to the loss to follow-up observed in the ROC trial (2 out 895 field-randomized patients). Several prevention procedures are in place to prevent missing data in our institution. At least two other data collection mechanisms (in addition to the COMBAT trial) will be capturing data from these patients: the Trauma Registry and the COMBAT/Trauma Activation database, which include information on the primary and secondary endpoints for COMBAT-eligible patients.

However, we are aware that avoiding missing data completely is not always possible. Thus, as in the ROC trial, we will assume that patients for whom vital status (the primary endpoint) could not be verified despite reasonable efforts to ascertain it were alive at day 28.[67] Patients lost to follow up will be fully described in terms of group assignment and covariates. Mortality data will most likely be “missing at random” (MAR). This is a reasonable assumption since patients with missing data on day 28 vital were those discharged alive before day 28 and lost to follow-up. Mortality in this group is most likely unrelated to the trial group assignment. In the unlikely event that vital status data are missing in over 5% of the sample, we will conduct a sensitivity analysis assuming

extreme case scenarios (i.e., all missing vital status did not survive, all missing survived). [84]

For the secondary endpoint 1, missing data in the composite outcome of 28-day mortality or MOF incidence will be dealt with by using the method proposed by the authors of the validated Denver MOF score for scoring patients with missing data.[80, 81]

Because the secondary endpoints 2, 3 and 4 (admission coagulopathy, acidosis and clot strength) are obtained at 30 minutes postinjury in all trauma activation patients, we anticipate very few missing data. The main and most complicated reason for missing data in these secondary endpoints is death before test was obtained. These data are clearly "missing not at random" (MNAR), as they are likely to represent the most injured patients and those more likely to have abnormal test values. This survivor-bias plagues emergency and trauma research and it is difficult to manage in the analysis period. [109-111] As recommended by the Panel on Handling Missing Data in Clinical Trials, missing data both due to death and related to death must be treated as a special case. [84] We will proceed as follows for these analyses: 1) the primary analysis will focus on all patients with complete data; 2) the second analysis will focus on those who remained alive in either group; 3) a third approach will fold death occurring within 1-hour between ambulance arrival at scene into the two binary endpoints 2, 3 and 4 to form composite outcomes (admission coagulopathy or 1-hour death; admission acidosis or 1-hour death). For the continuous secondary endpoints 2, 3, and 4, we will limit the analyses to approaches 1 and 2 and as a third approach, multiple imputations will be used.

Missing data for the variables chosen to assess effectiveness of randomization are anticipated to be less than 5% given the mechanisms described above. For the variables MAR with >5% missing data, we will use the multiple imputation method. [81, 84] If the variable is MNAR, the missing indicator method, as proposed by Sauaia et al., which is equivalent to the inverse probability weighting method, will be employed. In brief, the variable is categorized in missing, normal value and abnormal value, using established cutoffs for normal and abnormal values.

Missing data procedures for exploratory analysis 1: By the very nature of the study, missing data for this outcome (24-hour mortality) as a binary outcome (death yes or no) is likely to be null; while missing data for time from injury to death within 24 hours can be missing, which will be censored in the above-described survival analysis.

I. Trial assessment

1. **Assessing Phase 2 trial results for promising benefits of the therapy:** Because the trial has low power for the primary endpoint and some of the secondary endpoints, it is unlikely that we can detect a statistical significant difference. Thus, it is important to pre-determine the findings that we believe will indicate promising results that must be further investigated in larger Phase 3 trials. We will specifically look at the following measures to declare that the therapy is promising:
    a. Statistical significant difference in primary endpoint or secondary endpoints
    b. Strong, yet not statistically significant association (e.g., p-value = 0.10 to 0.05) with improved primary and secondary endpoints. The direction

of the association must indicate therapy benefit in ALL primary and secondary endpoints. Associations indicating harm will be reviewed by the DSMB for possible early termination.

c. Exploratory analyses of variables with supporting evidence for association with improved patient outcomes with statistically significant results (respecting the boundaries determined by the interim analyses methods, and adjusted for multiple comparisons as detailed in the description of the exploratory analyses).

The DSMB will also be in charge to making recommendations regarding the promising nature of the therapy and whether Phase 3 trials are warranted.

2. Interrupting the trial early:

The DSMB will be responsible for making a recommendation for early stopping of the trial. The DSMB will be asked to address early termination during each and every time they are activated, as described in the DSMB roles and responsibilities. In brief, the DSMB will be activated in case of a unanticipated problem, or during the interim analyses. Drs. Sauaia and Chin will prepare the materials for DSMB review containing:

a. blinded results (experimental and control groups will be referred as A and B) of effectiveness of randomization, primary and secondary outcomes, as well as exploratory analyses (most especially the two subgroup analyses regarding blunt versus penetrating mechanism, presence of traumatic brain injury and Field SBP≤70mmHg versus SBP 71-90mmHg), any potential adverse events, and missing data description (to include number missing and their characteristics regarding outcomes and covariates).

b. predicted probabilities of all previous events and outcomes based on the Denver MOF database, the ROC trial, the National Trauma Data Bank and the Trauma registry: these statistics will assist the DSMB in determining harm based on larger numbers than those provided by the comparisons between control and experimental groups

c. graphs containing the statistical scores, interim analyses boundaries and prediction of future statistical scores based on observed data.

d. pertinent studies published since the initiation of the trial.

e. any feedback received from community members, enrolled patients, paramedics, research coordinators and attending health care providers.

Based on the above elements, the DSMB will make a recommendation on early termination of the trial.

J. Paramedic training and ambulance equipment

1. Paramedics will be trained utilizing a PowerPoint presentation that emphasizes the protocol, study background, inclusion-exclusion criteria and transfusion reactions. All trainees will have ample opportunity to ask questions and offer comments. Commencement of study enrollment will begin only after training is concluded. Paramedics authorized to perform study related procedures will be

required to not only have completed and documented this mandatory training, but must provide certification of their credentials, prior to study initiation. All documentation will be reviewed and stored by the study coordinator. In addition, paramedics hired after this training has been completed will be required to review study training material and will be guided by veteran paramedics as well as the previously mentioned study staff. Study staff will meet with the trainees specifically to discuss the study and answer any questions.

2. As requested by the Jehovah's Witness Hospital Liaison (JWHL) Committee during the Community Consultation, the JWHL Committee will have the opportunity to educate and train the paramedics how to seek Jehovah's Witnesses.
3. For the study, all the DHMC paramedic ambulances will be equipped with validated coolers, (See Section IV.L.1 Dry Ice Plasma Storage Box) to store the 2 units of AB-FP24. The Thermal Isolation Chamber (TIC) panels will be exchanged every 48 hours and the AB-FP24, if not used, will be replaced every 11.5 months. According to the 28th Edition of the AABB Standards for Blood Banks and Transfusion Services, plasma frozen within 24 hours of phlebotomy expires at 12 months from collection. (See Appendix 12) Type AB plasma is a valuable resource that we do not want to waste or discard unless it is expired by the AABB standards. To be conservative, we will replace AB-FP24 at 11.5 months from collection. This method was deemed to be the most cost effective and logistically feasible in the ambulances. When the frozen AB-FP24 is needed, it will be rapidly thawed in a FDA approved dry warming device called Plasmatherm powered by a 12V to 120V AC inverter (see section IV.L.2 Plasmatherm).
4. The AB-FP24 will be infused once ready with the standard Y-set with blood transfusion filter (170-260 micron). If IV access has been obtained and the AB-FP24 is not ready to be infused, fluid resuscitation will not be withheld and crystalloid fluid will be infused until the AB-FP24 is thawed. This will minimize the possibility that subjects in the study group will receive inferior care if the AB-FP24 is not ready for infusion.
5. Paramedics will briefly look for necklace ID or bracelet. If the patient has any of these, he/she will not be enrolled.
6. If the patient has a durable power of attorney that reports Jehovah's Witnesses or no blood products, regardless if fractions are accepted, we will not enroll the patient in the study. The degree that fractions will be accepted varies among members of the Jehovah's Witnesses congregation. Thus to be conservative and minimize the chance of enrolling patients that do not accept plasma fractions, we will not enroll these patients.
7. If a family member is present at the scene and not in shock or severely injured, easily accessible to paramedics, and the patient is not in imminent danger of death, the paramedics will state "We are enrolling him/her in a research study where we are giving a blood product. We don't have time to explain the study at this time. Is this okay?" The paramedics will not look for family members among a crowd of bystanders given the acute setting and the importance of transporting

the patient to the hospital as soon as possible. Paramedics cannot assess competency of family members in this time-sensitive setting; thus family members that are not in shock or severely injured will be assumed to be competent.

8. It will be assumed that patients will not be competent to give an assent since by nature of the eligibility criteria, the patient will be in severe shock.
9. If the patient will not be transported to Denver Health, he/she will not be enrolled. If the patient is enrolled, but due to unforeseen circumstances (such as impending airway), must be transported to a nearby facility that is not Denver Health, then the patient will be unenrolled. If possible, every effort will be made to contact the family and follow the patient for any adverse effects. The consent form will be used to verify that the patient (or family) was informed.

K. Procedures involving plasma

1. **Procedure for thawing frozen AB-FP24:** AB-FP24 will be thawed in the Plasmatherm (see section IV.L.2) and in accordance to the instructions accompanying the product and as approved by the FDA.

Plasma is thawed but not transfused: If the AB-FP24 is thawed but not transfused for unforeseen reasons, such as discovery of Jehovah's Witnesses, then the unit(s) will be discarded or used for research purposes. It will not be transfused into humans.

2. **Procedure for Issuing 2 units of AB-FP24:** Bonfils Blood Center will be providing the AB plasma for the study.. This is modified from the DHMC policy for Blood Transfusions to be applicable to this study.
    a. AB-FP24 will be administered through the standard Y-set with blood transfusion filter (170-260 micron).
    b. Vital signs are required before the transfusion of all components and again after 15 minutes and at the completion of the transfusion. Transfusion rate will be compatible with the patient's condition. The patient will be monitored closely during the entire transfusion. The documented start and stop times are directly related to the actual transfusion of the component. Paramedics will document vital signs and start times in the field. Study coordinators will assume responsibility of additional vital signs and stopping time of the AB-FP24.
    c. The patient medical record shall include the following:
        1. Name of the components transfused
        2. Donor identification number of components
        3. Date and time of transfusion (Start and Stop time)
        4. Pre and post transfusion vital signs
        5. The volume transfused
        6. The transfusionist's name (paramedic)
        7. Documentation of related adverse events
3. **Rescue procedure for transfusion reactions:** This is modified from the DHMC policy for Blood Transfusions to be applicable for this study.

a. Careful observation throughout the transfusion allows for early detection of adverse reactions and optimal treatment, if necessary. All reactions should be handled initially as possible hemolytic reactions and the transfusion must be stopped. Any adverse events associated with the transfusion of blood or blood components should be documented in the patient's Medical Record and reported to the Blood Bank.
b. The most common clinical events accompanying or announcing transfusion reactions are, in order of decreasing frequency:
    1. Fever, with or without chills
    2. Skin symptoms, hives and/or itching or rash
    3. Chest pain
    4. Hypotension
    5. Nausea
    6. Flushing
    7. Respiratory Distress (wheezing, coughing or dyspnea)
    8. Bleeding at infusion site
    9. Hemoglobinuria
    10. Circulatory overload
    11. Anaphylaxis
c. If an adverse reaction is suspected, follow the procedure below:
    1. Stop the transfusion
    2. Maintain IV access with Normal Saline and change the tubing.
    3. Notify the patient's physician upon arrival to the ED and initiate immediate treatment as ordered.
    4. For all other blood products involved in a reaction, the transfusion shall be stopped and a Transfusion Reaction Investigation (F20-251) shall be initiated. This form can be obtained from the Blood Bank.
    5. Notify the Blood Bank (303) 436-6929 of the suspected transfusion reaction.
    6. Collect a sample drawn from the patient as soon after the reaction was detected. Send a 6 mLs pink top tube, labeled with a new Blood Bank armband to the Blood Bank along with the unused blood, blood bag with attached hard back copy of the transfusion tag, the IV tubing used and the top 2 copies of the Transfusion Reaction Investigation 3 part form. The back copy of the Transfusion Reaction Investigation form should remain in the patient's chart as the initial report. A post transfusion reaction Urinalysis with Microscopic may also be ordered by the patient's physician.
    7. The Blood Bank will complete the Transfusion Reaction initial report and notify the caregiver of the critical results. Pathology

will evaluate the patient's reactions, Blood Bank's initial report, culture when indicated, and report will be documented in the patient's medical record. Consultation between the Medical Director of the Transfusion Service, the patient's physician and Risk Management is required when a fatal hemolytic transfusion reaction occurs. Further evaluation and FDA notification may be indicated. The Hospital Transfusion Committee is responsible for peer review and blood utilization practice.

4. Look back procedures: Since the plasma will be tracked through the DHMC Blood Bank, look back/product recall procedures will be conducted as per DHMC standard protocol. (See appendix 3: Look Back Procedures/Product Recall)

L. Devices used in this study:

1. **Cooler box**: Bonfils Blood Center, US License #0166, CFN#1723428, validated a cooler box, an army surplus ammo can with 2" foam insulation and phase change material (PCM) for temperature control, has undergone intensive in-house validation such that the summer temperature test proved that the above mentioned container would maintain a temperature of -18°C or colder for 27.8 hours. Therefore two units of plasma will remain frozen for at least 24 hours when properly packed with PCM as also mentioned in the validation report (appendix 12). Furthermore, this precise packing methodology per Bonfils Blood Center standard operating procedures will be implemented without deviation. In addition, the PCM will be changed at least every 24 hours to ensure that the plasma remains frozen.
    a. 2 units of frozen AB-FP24 will be stored in the half of the cooler boxes. PCM bottles will be exchanged every 24 hours. If the stored frozen AB-FP24 is not used within 11.5 months of collection, it will be replaced. This allows a wide buffer to ensure plasma that is beyond 12 months of collection is not infused. If there is any possibility of compromise to the plasma, study personnel and Dr. Tuan Le, who is responsible for maintaining the supply of frozen AB-FP24, will be notified and the unit will be removed and labeled for research purposes or discarded. The cooler will be inspected and replaced if there is any concern of the integrity of the cooler.
2. **PlasmaTherm by Barkey** (510K# BK100063,) is approved for thawing plasma by the FDA. It is distributed by Genesis BPS. (65 Commerce Way, Hackensack, NJ 07601 Phone: 866-712-5663 201-708-1400; Fax: 201-708-1104; email: info@genesisbps.com) The device will be used as approved by the FDA (see appendix 4 for operating manual).

a. Plasmatherm functions similarly to the typical water bath, however, the water does not contact the plasma, reducing the potential for contamination in the event of back breakage. If a bag breaks, Plasmatherm is easier to clean up than a typical water bath.

b. Monthly, the Plasmatherm will be inspected for damage and cleaned. If there is any concern regarding the use of the Plasmatherm, the PI will be notified. Before each shift, the display will be tested

3. **CipherOx M1 by Flashback Technologies** is a non-FDA approved device consisting of an FDA-approved Nonin OEM III pulse-oximeter integrated with an ARM III processors, memory, a mini-USB port for charging and data off-load, as well as a high-resolution screen for indoor and outdoor use. The device collects non-invasive waveform data from acutely injured, potentially bleeding trauma patients. The device displays the date/time and an image of a finger with a sensor that is displayed in "green" if the data collection is "good." The providers are blinded to all physiological data collection by the device.

M. Special Consent Issues

1. We have requested an exception from informed consent for emergency research under 21 CFR §50.24 as the patients selected for enrollment in this clinical study will be unable to provide prospective informed consent due to the extent of their injuries and their immediate need for resuscitation from life-threatening hemorrhagic shock. These patients are often unable to give consent upon arrival, and this study cannot be carried out without an exception from informed consent.
    a. The therapeutic window dedicated to obtaining prospective informed consent from the patient or a legally authorized representative is very brief. On average, line-placement has been accomplished by experienced field care personnel in approximately one minute, and therefore, the process of patient assessment, scene care, randomization, enrollment, thawing of plasma, and the start of AB plasma infusion can be expected to occur in less than 8 min. The opportunity to obtain prospective informed consent from the patient or a legally authorized representative or to provide an opportunity for a family member to object to the subject's imminent enrollment in the study may not be possible prior to the commencement of study procedures.
    b. Once the patient arrives at the hospital, the admissions department and social workers in the ED attempt to locate family members of the patient using every resource available, including several databases, previous medical records, and cellular phones. Within the trauma population, few of the patients have advance medical directives identifying a legally authorized representative (LAR). If no LAR was previously identified and there are several interested parties, per Colorado law, the social worker meets with all interested parties to select a proxy decision maker (See Appendix 5: Proxy Decision Maker). Continued diligent attempts by social workers to contact a legally authorized representative or family member of the patient will continue if the patient remains incapacitated.
    c. Prisoners: If the patient becomes a prisoner during the study, they will be treated similar to non-prisoner subjects in accordance with DH policy of hospital care of patient-inmates. (see appendix 13)

d. Procedure for consent

1. Once a LAR/PDM is available, a member of the study team will inform him/her of the study and obtain consent for continued collection of blood samples up to day 7 and clinical data up to day 28.
2. If a LAR/PDM does not consent for continued data collection, no further blood draws and/or data collection will occur. The LAR/PDM will be asked if he/she would like the patient withdrawn from the study.
3. If a patient becomes competent to consent, a member of the study team will inform and re-obtain consent from the patient for continued data collection.
4. If a family member has consented for continued data collection, but when competent, the patient does not consent for continued data collection, no further blood draws and/or data collection will occur. The patient will be asked if he/she would like to be withdrawn from the study.
5. The LAR/PDM or the patient may withdraw from the study at any time, including at the time of consent. If the patient is withdrawn from the study, previously collected blood samples and data will be destroyed.
6. If a patient never regains decisional capacity and no LAR/proxy can be found, data and samples may be used since the subject has been enrolled under Exception for consent for emergency research. In this situation, all attempts at consenting will be documented.

e. Procedure to inform family of patients who died before consent is obtained

1. After patient death, if applicable, social work and decedent affairs/coroner will be contacted for information about the social situation and family contact information.
2. In 2-4 days after patient death, call next of kin or family contact. If family members are met in person at any time after death and the timing is appropriate, inform the family in person.
3. During the call, verify address and mail information letter as follow-up or give the letter to family if met in person.
4. If no one answers the call, leave a message, if possible. If no response in 24 hours, call back. Repeat up to 3 times.
5. The rationale of this procedure is that 21 CFR 50.24 requires that information about the clinical investigation be provided to the LAR or family member of patients who died before consent is obtained, if feasible. In certain clinical situations, the family may not be local and may not come to the hospital, thus, we will call

the family members after a brief period of grieving. However, given the sensitive situation, we prefer in-person communication, and will engage family if appropriate. We will follow up with the information letter, either by mail or in person as appropriate, allowing family members to read it at a later date. If we are unable to contact family after 3 tries, we will consider informing the family as not feasible.

2. **Community Consultation and Public Disclosure (CCPD):** Protection of the rights and welfare of patients is necessary in all clinical studies and is paramount in studies involving vulnerable populations. Because this study involves victims of trauma who likely will not be able to provide informed consent or actively refuse enrollment, patients are placed in particularly vulnerable circumstances. This lack of autonomy creates a special need for FDA, sponsors, IRBs, and clinical investigators to work closely together to ensure that the interests of this vulnerable patient population are protected to the maximum extent possible. For such studies to be conducted, investigators must provide an opportunity for dialogue with the community in which a study will be conducted and from where subjects will be drawn. The IRB, Department of Defense, and the investigators will coordinate these efforts using the plan outlined in Appendix 6 Community Consultation and Public Disclosure Plan and Appendix 7 List of Neighborhoods and HOAs around Denver Metro Area. Discussion with the information disseminated to the community will adhere to 21 CFR §50.24 and 56.115.
    a. In brief, the community consultation and public disclosure plan required by the 21 CFR §50.24 will include feedback from community meetings, online and social media feedback as well as paid advertisements, media news releases to TV, radio and newspapers, and women's health and community clinics. In addition, prior to initiation of the study, the clinical investigators and IRB will make arrangements for public disclosure of the plans for the investigation and a balanced disclosure of the risks and potential benefits to patients enrolled in the study. This disclosure will also include background information about the study, a synopsis of the protocol and study design, risks and benefits of fresh frozen plasma versus the standard crystalloid fluid, the selection of subjects, the use of provisions for exception from informed consent requirements and the process for attempting to contact a legally authorized representative, and the ways in which subjects can communicate their desire not to participate. Following the completion of the study, a summary of the findings of the clinical trial will be disseminated to the community. This information, presented to the community, is in language that is understandable and does not promote fresh frozen plasma, and will include the results of the trial and demographics of the patients enrolled.
    b. Under 21 CFR §50.24, we submitted an Investigational New Drug Application (IND# 15216) for the use of thawed plasma in the resuscitation of severely injured trauma patients in an emergency

research protocol, which is necessary to obtain a waiver of consent in emergency research.

3. Under Title 10 US Code Section 980, Department of Defense funds may not be used for human research unless an informed consent of the subject or a legally authorized representative of the subject is obtained in advance for human subjects research. The Secretary of Defense may waive this prohibition for a specific research project to advance the development of a medical product necessary to the armed forces if the research project may directly benefit the subject and is carried out in accordance with all other applicable laws.
   The contract with the Department of Defense is specific to the evaluation of the use of plasma in the field. As described above, the majority of potentially survivable injuries are due to hemorrhage. If administering plasma earlier in the field decreases hemorrhage from acute coagulopathy of trauma, injured members of the armed forces may have more time to reach a facility to be better stabilized and resuscitated. Although some institutions have adopted the principle of early plasma administration in the management of severe trauma and the acute coagulopathy of trauma, there are no prospective studies measuring the benefit and risks of administering plasma early.
   Although this is a greater than minimal risk study, subjects are likely to receive direct benefit from this clinical trial from closer evaluation and testing of coagulation status. Based on the previous retrospective studies and preclinical studies described in the Section II.A. (Background) and Section III.A. (Preliminary studies) as well as our current understanding of administering plasma earlier and more frequently in the resuscitation of patients in severe hemorrhagic shock, patients receiving early plasma are likely to directly benefit from this study.
   This study will be carried out in accordance with 21 CFR §50.24 Exception from Informed Consent for Emergency Research.

N. Description of Risks, Benefits and Justification

1. **Risk of blood transfusion:** These risks apply to any patient that receives blood products regardless of group assignment. Both the standard and experimental groups are likely to receive blood products during the resuscitation period, but the study group will receive at a minimum 2 units of AB plasma. In this study, all plasma used as an initial resuscitation fluid will be AB and hence universally infusible without the requirement for patient blood typing. This is the usual type of fresh frozen plasma used in acute trauma setting prior to obtaining type and cross match (see appendix 9 for adult transfusion guidelines). Risks of blood

Table 3: Risk of Disease from Blood Product Transfusion

Transmitted Blood Product Disease	US Transmission Risk/Unit Transfused
Hepatitis A Virus (HAV)	1:8,300,000
Hepatitis B Virus (HBV)	1:282,000
Hepatitis C Virus (HCV)	1:1,149,000
Human Immunodeficiency Virus (HIV-1, -2)	1:1,467,000
Malaria	1:4,000,000
Human T-cell lymphotrophicvirus (HTLV-I, -II)	1:2,990,000

transfusion include febrile non-hemolytic transfusion reaction (1.1% to 2.5%), hemolytic transfusion reactions (1:1,250,000), transmission of blood borne pathogens (See Table 2), transfusion-related lung injury (TRALI) (8.1 per 100,000), transfusion associated circulatory overload (TACO) (2-3 per 100).[112] A blood transfusion may also result in a reaction that includes fever, chills, or hives. Although uncommon, the patient may experience this type of reaction as the result of receiving the incorrect type of blood. By using AB type of plasma, this risk is minimized. This risk is minimized in the blood bank by a questionnaire to exclude donors who are at risk of having a blood borne pathogen as regulated by the FDA under 21CFR 640 subpart G. The donated blood is also tested in the laboratory for infectious diseases.

A blood transfusion may increase the likelihood of developing an infection while the patient is in the hospital, may exaggerate how the patient's body responds to injury, and may increase the patient's risk of internal organ failure after his/her injury.

Excessive transfusions place patients at higher risk of hypervolemia, and pulmonary edema, but this risk also exists for patients who receive excessive intravenous fluids. The risk of 2 units of AB-FP24 as initial resuscitation leading to cardiac failure, hypervolemia and pulmonary edema is low as it is a smaller volume (500ml) than the standard 2 liters of crystalloid intravenous fluid. These risks exist with transfusions of any blood products

2. **Risk of Inadvertent Release of Protected Health Information (PHI)**: This risk is minimized by using a unique patient identifier and role based security for the database. All hard copies will be kept locked in the research office or be destroyed.
3. **Venipuncture risk:** In most cases blood will be drawn from an intravenous line (I.V.) already placed as part of the patient's standard medical care. In the event the patient does not have an I.V., blood will be drawn using a method known as venipuncture. This procedure consists of placing a small needle in a vein in the patient's arm to withdraw blood. Risks associated with drawing blood from the patient's arm include pain, bruising, lightheadedness, and rarely infection.
4. **Potential Scientific Problems:** Potential scientific problems include the possibility our hypothesis will not increase survival, improve MOF, and decrease the need to blood products. We feel this possibility is low given the background and preliminary studies that have been done, however, we will evaluate the outcomes at each interim analysis to detect early if our hypothesis is incorrect. Another potential scientific problem may be the enrollment of patients who are nonvisibly pregnant, or younger than 18 years of age, during the hospital stay. Age is often estimated at the time paramedics arrive to the scene and some patients may appear to be 18 years of age or older but may truly be younger. We plan to minimize the potential to enroll pregnant or potentially pregnant women and patients less than 18 years, by recommending they opt out with the bracelet and necklace ID. Other potential scientific problems include the risk of unforeseen events such as thrombotic events of myocardial infarction, stroke, and venous thromboembolism. We will monitor for these events in the study

patients and determine if administration of plasma leads to increased thrombotic events. Upon discovery of these events, it will be reported to the Data Safety Monitoring Board. The risk of this is low since these patients are hypocoagulable and at risk for bleeding. Finally, the study is not blinded to clinicians. Thus, there may be a treatment bias to withhold blood transfusions for borderline values in the study arm for a longer time or to transfuse those in the standard arm quicker. However, the initial resuscitation occurs in the ambulance, where the patient is randomized. The majority of the resuscitation occurs in the Operating Room, often driven by the anesthesia team or the Surgical Intensive Care Unit, driven by the ICU team, who may have no knowledge which study arm the patient is enrolled in or if the patient is enrolled at all.

5. **Risk to Investigator or Institution.** There is no known direct risk to the Investigator or Institution. However, the transmission of viruses may occur through contact with contaminated needles and blood or blood products. Accordingly, all study personnel involved in the collection of blood and/or handling of specimens will employ appropriate blood and body fluid precautions in the both the clinical and laboratory settings.
6. **Benefits**: Utilizing AB plasma as the initial resuscitation fluid may increase the likelihood of survival, decrease multiple organ failure, and decrease the overall need for blood products, especially in the setting of activation of the massive transfusion protocol. Furthermore, implementation of this study may help patients in the future by giving important information about the treatment of acute blood loss and initial resuscitation in trauma. Benefits for patients in the control arm include intensive surveillance of the acute coagulopathy of trauma that may result in further appropriate medical treatment
7. **Risk-Benefit analysis:** Risks to the subject include reaction to risk of transmission of blood-borne pathogens, which is minimal, risk of reaction to blood transfusion, venipuncture, inadvertent release of PHI, or unforeseen happenings. Benefits include the potential to improve outcome in the critically injured with acute coagulopathy of trauma as well as decreased need for blood products. The risks of blood transfusion is small, but this specific trauma population that is severely injured and in hemorrhagic shock will likely receive transfusion of multiple blood products, including plasma, regardless of the type of initial resuscitation fluid. By transfusing AB plasma early as a resuscitation fluid, we may be able to slow down the ACoT, leading to a decreased number of transfused blood products. As stated above, transfusion of blood products in the first 12 hours is an independent risk factor of mortality.[63] Furthermore, using the same inclusion criteria as in the recent ROC hypertensive HTS trial,[67] mortality in this study is expected to exceed one in four. At the same time, massive pRBC infusion remains a major risk factor for MOF[36, 38, 99] while plasma transfusion does not.
8. **Adverse Events**
    a. The PRA team will be responsible for identifying and documenting adverse reactions. They will ask the principal investigator and the attending physician for the relatedness of the adverse reaction to study

and if they should be reported to the appropriate agencies. The DSMB as well as the RM will evaluate the relatedness of adverse reactions. All product related SAE's will be reported to the FDA within 15 calendar days.

b. The definitions of serious adverse events are from our MOF database and the Glue Grant study and in Table 4:

Table 4: Definitions of Serious Adverse Events

Serious Adverse Events	Presentation and Treatment
Bloodstream Infections	Bacteriologic confirmation of a recognized pathogen from one or more blood cultures and organism cultured is not related to an infection at another site. If a skin contaminant is cultured (diptheroids, Bacillus sp, Propionobacterium sp, coagulase-negative staphylococci), the organism must be cultured from at least two cultures within a 48 hour period and the patient has at least one of: fever>38.5^{o}C, WBC>10,000 or <3,000/mm^{3}, SBP<90 mmHg or >25% drop in SBP.
Myocardial infarction	Acute, irreversible myocardial injury documented by both: 1) abnormal increase in CK-MB or troponin, and 2) new, serial T-wave, S-T segment or Q wave EKG abnormalities.
Cerebral infarction	New neurologic deficit not present on admission which is sudden or rapid in onset and last >24 hours or until death and confirmed as an infarction by CT or MRI.
Lung dysfunction	Denver lung dysfunction score ≥2* (P/F ratio adjusted for altitude≤165)
Renal dysfunction	Denver renal dysfunction score ≥ 2* (serum creatinine ≤2.5mg/dL)
Multiple Organ Failure (MOF)	Denver MOF score ≥3 for 1 day*
Febrile non-hemolytic reaction	A temperature elevation of ≥ 1^{o}C or 2^{o}F occurring during or shortly after a transfusion and in the absence of any other pyrexic stimulus. Symptoms treated with antipyretics
Allergic reactions	Mild or self-limiting urticaria or wheezing that usually respond to antihistamines.
Anaphylactoid/anaphylactic reactions	Hypotension, tachycardia, nausea, vomiting and/or diarrhea, abdominal pain, severe dyspnea, pulmonary and/or laryngeal edema, and bronchospasm and or laryngospasm. Immediately treated with epinephrine.
Transfusion-related acute lung injury (TRALI)	Acute onset of hypoxemia within 6 hours of a blood component transfusion, bilateral infiltrates on chest x-ray, and the exclusion of preexisting acute lung disease or transfusion-associated circulatory overload (TACO) in a patient without clinical risk factors for the development of ALI.
Bacterial sepsis	High fever (≥2^{o}C or ≥3.5^{o}F increase in temperature), severe chills, hypotension, or circulatory collapse during or shortly after transfusion suggests possible bacterial contamination and/or endotoxin reaction. Treated with discontinuation of the transfusion and aggressive therapy with broad-spectrum antimicrobials and vasopressor agents, if necessary.

*Denver postinjury MOF Score[80]

TABLE 1. **Denver postinjury MOF score**

Dysfunction	Grade 0	Grade 1	Grade 2	Grade 3
Pulmonary PaO_2/FiO_2 ratio	>208	208–165	165–83	<83
Renal creatinine, μmol/L	<159	160–210	211–420	>420
Hepatic total bilirubin, μmol/L	<34	34–68	69–137	>137
Cardiac inotropes	No inotropes	Only 1 inotrope at a small dose*	Any inotrope at moderate dose or >1 agent, all at small doses*	Any inotrope at large dose or >2 agents at moderate doses*

*Inotrope doses (in $\mu g \cdot kg^{-1} \cdot min^{-1}$): milrinone: small <0.3, moderate 0.4–0.7, large >0.7; vasopressin: small <0.03, moderate 0.03–0.07, large >0.07; dopamine: small <6, moderate 6–10, large >10; dobutamine: small <6, moderate 6–10, large >10; epinephrine: small <0.06, moderate 0.06–0.15, large >0.15; norepinephrine: small <0.11, moderate 0.11–0.5, large >0.5; phenylephrine: small <0.06, moderate 0.06–3, large >3.

c. **Data Safety Monitoring Board (DSMB):** The DSMB will include Martin Schreiber, MD, Professor of Surgery, Chief of Division of Trauma, Critical Care and Acute Care Surgery of Oregon Health and Science University; Arthur Derse, MD, JD, Professor of Bioethics and Medical Humanities, and Emergency Medicine, Director for Medical and Legal Affairs, and Director, Center for Bioethics and Medical Humanities, Director, MCW Medical Humanities Program of the Medical College of Wisconsin;Kirk Bol, MSPH, Vital Statistics Program, Colorado Department of Public Health and Environement; and Jeannie Callum, MD, FRCPC, Associate scientist, biological sciences-Trauma, Emergency, and Critical Care Program, Sunnybrook Research Institute, Associate staff, department of laboratory hematology/transfusion medicine, University Health Network, Director of transfusion medicine and tissue banks, department of clinical pathology, Sunnybrook Health Sciences Centre, and Assistant professor, department of laboratory medicine and pathobiology, University of Toronto. Dr. Schreiber will serve as the chairperson of the DSMB. He previously served on the DSMB for the ROC clinical trial.

   1. **Role and Responsibilities:** The role and responsibilities of the follow FDA Guidance for Establishment and Operation of Clinical Trial Data Monitoring Committees.[113] A draft DSMB charter is in Appendix 10.

d. **Research Monitor:** Per DOD requirements, Michael Wang, MD, Assistant Professor of Pediatrics and Head of the Mountain States Regional Hemophilia and Thrombosis Center, will serve as the Research Monitor (RM), whose purpose is to be involved in Department of Defense (DOD)-supported research studies that are determined to pose more than minimal risk to subjects (DOD Instruction 3216.02, Nov 2011). The RM is not a member of the study team and is not affiliated with the Department of Defense. The RM's duties should be based on specific risks or concerns about the research and in relation to scrutinizing the research effort on behalf of interests of the research participants.

   1. The RM may perform oversight functions and report their observations and findings to the IRB, DSMB, investigators, DOD's HRPO, or the FDA. Functions could include observing recruitment and enrollment procedures and the consent process

for individuals, groups or units, overseeing study interventions and interactions, reviewing monitoring plans; and overseeing data matching, data collection, and analysis.

2. There may be more than one research monitor (e.g., if different skills or experiences are necessary). The monitor may be an ombudsman or a member of the data safety monitoring board. The research monitor may discuss the research protocol with the investigators, interview human subjects, and consult with the IRB, DOD HRPO and the FDA; 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; and shall have the responsibility to promptly report their observations and findings to the IRB, DSMB, investigators, DOD's HRPO or the FDA.
3. The research monitor is authorized to review research records as part of their responsibility to protect human research volunteers. Research records will be stored in a secure manner so as to protect the confidentiality of subject information

e. *All unanticipated problems involving risk to subjects or others must be promptly reported by phone (301-619-2165), by email (Usarmy.detrick.medcom-usamrmc.other.hrpo@mail.mil), or by facsimile (301-619-7803) to the HRPO. A complete written report will follow the initial notification. In addition to the methods above, the complete report can be sent to the US Army Medical Research and Materiel Command, ATTN: MCMR-RP, 810 Schreider Street, Fort Detrick, Maryland 21702-5012..*

f. Any deviation to the protocol that may have an adverse effect on the safety or rights of the subject or the integrity of the study must be reported to the HRPO and the IRB as soon as the deviation is identified.

g. A summary of the safety monitoring schedule in Table 5:

Table 5: Monitoring Schedule

Equipment	Frequency	Responsible Party	Product
Exchange of PCM bottles	Every 24 hours	Paramedics	Log of coolers
Inspection of cooler box	every shift	Paramedics	Log of coolers
Plasma replaced if not used	11.5 months from time of collection	PRA	
Plasmatherm inspection	Every shift	Paramedics	Log of checklists
Clinical Lab TEG -Manufacturer calibration -Quality control	 - every 6 months - every shift	 Clinical Lab Clinical Lab	Documented in Clinical Lab log
Research Lab TEG			Documented

-Manufacturer calibration -Quality control	- every 6 months - every week	PRAs	log
Freezer for sample storage	Connected to Sensaphone alarm system at all times and physically checked once a day M-F.		
Data Monitoring			
Evaluation of data quality	Data collectors will be fully trained and observed in the first two patients to assure correct data obtention; data entry will be verified by cross checking with the medical record the data entered for the first 5 patients. Discordances between medical record (which will be considered the "gold standard") and data entry will be adjudicated by Dr. Chin. In the case there is any discordance in the primary or secondary endpoints, data collectors will be re-trained to ensure data reliability. Discordances over 5% in the variables used in the exploratory analyses will trigger re-training. At the end of the study, a random sample of 10% of the patients will have their data cross-checked with the medical records and if there are discordances with primary endpoints, all records will be reviewed to ascertain the primary endpoints. Secondary endpoints and exploratory analyses will follow the same procedure if over 5% discordance is found. Laboratory measurements defined in exploratory analyses 4, 5, and 6 follow standard procedures in the lab to ensure data integrity	Theresa Chin, Research Fellows, PRAs	
Evaluating data for adverse events	Daily	Theresa Chin,Research Fellows, PRAs	
Verify training of newly hired paramedics	Every 4 months	Research Fellows, PRAs	
DSMB meeting	Every 6 months or sooner	DSMB	DSMB report
Interim Analysis	Per analysis plan	Angela Sauaia Theresa Chin	Interim Report

9. **Medical Care for Research Related Injury:** In the event of a research related injury, medical care will be arranged, and the patient or the patient's insurance company will cover the cost.

O. Roles and Responsibilities of Study Personnel:

1. Dr. Ernest E. Moore: As Vice Chairman of Surgery for research at the University of Colorado and Principal Investigator of this study, Dr. Moore will be responsible for overall progress of the study, especially critical aspects and in apportioning responsibilities to other senior investigators involved in the study. Dr. Moore will see identifiable data and interact with subjects.

2. Dr. Anirban Banerjee: As the Program Director of the NIH/NIGMS funded Trauma Research Center, Dr. Banerjee will be responsible for overall administration and financial oversight of the study as well as data processing and reporting. Dr. Banerjee will not see identifiable data or interact with subjects.
3. Dr. Kirk Hansen: As Assistant Professor and an internationally acclaimed expert in advanced Mass Spectrometric methods deciphering the proteomics of post-shock blood and lymph, he will have primary responsibility for quantitatively identifying proteins in blood samples. Dr. Hansen will not see identifiable data or interact with the subjects.
4. Dr. Xiayuan Liang: As an Associate Professor of pathology, and the hematopathologist at Colorado Children's Hospital, Dr. Liang will ensure that all measurements of soluble coagulation factors, anti-coagulant and the thrombolytic system are done appropriately and will aid in the analysis of these data. She is also an expert in processing very small volumes of blood to obtain the most comprehensive panel of coagulation factors using the powerful STARR instrument at nominal costs. Dr. Liang will not see identifiable data or interact with subjects.
5. Dr. Angela Sauaia: As an expert epidemiologist and biometrician, she will be responsible for coordinating data collection and quality, database management, and blinded interim data analyses and relations with IRB. Dr. Sauaia may see identifiable data.
6. Dr. Christopher Silliman: As a Professor of Pediatrics and Surgery and a board certified pediatric hematologist, Dr. Silliman will review all hematological data, coagulative proteomics data, and perform the ELISAs and other assays in his laboratory including Quality Controlled analyses and presentation. Along with Dr. Moore, he will be responsible for analysis of the TEG data. Dr. Silliman may see identifiable data.
7. Dedicated study PRAs will be responsible for providing coverage of the ER in response to trauma alerts. They will provide coverage 24/7 with available back up, especially over holiday and weekend nights when historically most such alerts occur. All will be thoroughly cross trained in specimen processing and banking; data abstraction, entry and reporting; regulatory reporting; and patient consenting. These PRAs will also be trained to conduct viscoelastic assays on sequentially obtained samples, as well as helping perform other assays (cytokines, ELISAs etc.). All PRAs will be required to undergo CITI human research training prior to viewing identifiable information and/or having interaction with subjects.
8. Dr. Chris Colwell: As the Director of Emergency Medicine at Denver Health Medical Center, he will oversee the logistics of delivering AB-FP24 to patients. Dr. Colwell will see identifiable data and may interact with subjects.
9. Dr. Tuan Lee: As the Medical Director of Bonfils Blood Center and Medical Director for Transfusion Services at Denver Health, , Dr. Lee will provide scientific and medical direction that supports high-quality blood products and components, laboratory testing, donor collections and counseling, hospital relations, product management and clinical research. .

10. Dr. Theresa Chin is a consultant on the project and a current surgical resident.
11. Arsen Ghasabyan is the safety officer of the project. He is responsible for overseeing the PRA's.
12. Dr. Steven Moulton: Will oversee aspects of the study related to the collection of waveform data.

P. Accurate and complete study records will be maintained and made available to representatives of the U.S. Army Medical Research and Materiel Command. These representatives are authorized to review research records as part of their responsibility to protect human research volunteers. Research records will be stored in a confidential manner so as to protect the confidentiality of subject information

Q. Summary of Knowledge to be Gained:

1. ***Public Purpose:*** Hemorrhage is the most preventable cause of death in trauma patients. Epidemiologic studies at civilian trauma centers have revealed hemorrhage as responsible for up to 50% of trauma related mortality. But despite improvements in patient transport via EMS, survival in the first hour postinjury has changed little over the past 40 years. Crystalloids remain the mainstay of treatment without a convincing reduction of mortality due to blood loss. Thawed plasma, as a third generation fluid for initial resuscitation, appears logical and promising, but has not been evaluated. While many urban trauma centers can provide various blood products, including thawed plasma, as required in the ED and in the field, the vast majority of rural trauma centers cannot resemble the austere conditions of the military frontline environment. Stage I will allow this study to be generalizable to the most typical scenario in the U.S., i.e., thawed plasma only available in the ED. Moreover, as we have witnessed, natural disasters and acts of terrorisms cause mass casualties that can overwhelm labile blood product resources. A stable plasma preparation that can be stored for longer periods is necessary for these situations. Civilian trauma victims will benefit from longer time for transportation as well as more time for blood banks to prepare and deploy specific blood components. Blood products, especially pRBCs have been associated with increased morbidity and mortality among civilian trauma victims who will likely experience improved outcomes with decreased blood product utilization. In addition, our already depleted blood supplies will benefit from decreased utilization.
2. ***Military Significance:*** Traumatic blood loss due to combat related injuries is the primary cause of death in field combatants. While significant advances have been made in the prevention and treatment of hemorrhage with the introduction of novel hemostatic dressings and tourniquets, little progress has been made in developing new resuscitation strategies. Primary resuscitation using crystalloids is the current standard of care. Crystalloids only provide a means to temporarily increase intravascular volume to maintain blood pressure and may not be the optimal initial resuscitation fluid in traumatic hemorrhagic shock. The proposed study will elucidate the use of plasma in the pre-hospital setting as an initial resuscitation fluid for traumatic hemorrhage. This project will also be the

gateway study to examine and compare the use of long shelf-life and more logistically accessible resuscitation fluids, such as lyophilized plasma, and, in development, the multifunctional resuscitation fluids. This study will provide an exponential leap forward in the care and development of future therapies for the wounded war fighter in the field. The data generated from this study will provide **i.** an evaluation of the safety and efficacy of the early use of plasma, **ii.** information on the mechanism(s) of acute traumatic coagulopathy, and **iii.** a functional and proteomic baseline to gauge the desired composition of future plasma-based products, such as lyophilized plasma and multifunctional resuscitation fluids. Delaying the requirement of pRBC transfusion will allow longer transport times to combat support units stocked with pRBC in the military theater. In addition, there will be more time to adequately array blood components for transfusion. Decreasing blood products transfusion in the initial postinjury period has implications for decreased need of precious blood products, as well as a decrease in the deleterious effects of blood products in the postinjury period including increased rates of MOF, ARDS, infections and death as well as hospital resources utilization (ICU stay and mechanical ventilation time)

3. ***Waveform Data:*** Previous research supports the notion that LBNP is a well tested and safe model that can be used to simulate severe hemorrhage approaching cardiovascular collapse in humans. However, data from LBNP experiments have not been directly compared to data collected from bleeding and hemodynamically unstable trauma patients in a hospital setting. If our main hypothesis is proven correct, that the physiologic changes that occur with the application of LBNP mimic those observed in bleeding and hemodynamically unstable trauma patients, then the LBNP model could be used to further explore the human response to severe hemorrhage secondary to trauma. This knowledge would facilitate future research investigating the relationship between continuous physiologic waveform features and central volume loss, and may in the future reveal additional early indicators of hemodynamic instability, cardiovascular collapse and risk of death secondary to traumatic hemorrhage.

R. Changes to the Protocol: Substantive modifications to the research protocol and any modifications that could potentially increase risk to subjects must be submitted to the HRPO for approval prior to implementation. The USAMRMC ORP HRPO defines a substantive modification as a change in Principal Investigator, change or addition of an institution, elimination or alteration of the consent process, change to the study population that has regulatory implications (e.g. adding children, adding active duty population, etc.), significant change in study design (i.e. would prompt additional scientific review), or a change that could potentially increase risks to subjects.

S. Continuing Review and Final Report: A copy of the continuing review report and the re-approval notification by the IRB must be submitted to the HRPO as soon as possible after receipt of approval Please note that the HRPO also conducts random audits at the time of continuing review and additional information and documentation may be requested at that time.

T. The final study report submitted to the IRB, including a copy of any acknowledgement documentation and any supporting documents must be submitted to the HRPO as soon as all documents become available. **DOD Funded Research Guidelines**: The protocol will not be initiated until written notification of approval of the research project is issued by the HRPO.

Suspensions, clinical holds (voluntary or involuntary), or terminations of this research by the IRB, the institution, the sponsor, or regulatory agencies will be promptly reported to the USAMRMC ORP HRPO. The knowledge of any pending compliance inspection/visit by the Food and Drug Administration (FDA), Office for Human Research Protections, or other government agency concerning this research; the issuance of inspection reports, FDA Form 483, warning letters, or actions taken by any regulatory agencies including legal or medical actions; and any instances of serious or continuing noncompliance with the regulations or requirements must be reported immediately to the HRPO.

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