

Supplementary Online Content

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Trial protocol

This supplementary material has been provided by the authors to give readers additional information about their work.

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Protocol:

Pragmatic, Randomized Optimal Platelet and Plasma Ratios (PROPPR)

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Protocol Title: Pragmatic, Randomized Optimal Platelet and Plasma Ratios (PROPPR)

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PROTOCOL SYNOPSIS FOR PROPPR: A Resuscitation Outcomes Consortium (ROC) Protocol

Protocol Title	Pragmatic, Randomized, Optimal Platelet and Plasma Ratios
Acronym	PROPPR
Trial Phase	Phase III Trial
Study Sites	At least 12 Level I Trauma Centers in the Phase III Trial
Study Period	Expected start date: March, 2012
Study Population	Trauma subjects predicted to receive massive transfusions (MTs) and enrolled within 2 hours of Emergency Department (ED) admission to Level I Trauma Centers
Objectives	<p><i>The objective of this study is to conduct a Phase III multi-site, randomized trial in subjects predicted to have a massive transfusion, comparing the effectiveness and safety of 1:1:1 transfusion ratios of plasma and platelets to red blood cells (the closest approximation to reconstituted whole blood) with the 1:1:2 ratio. The co-primary outcomes will be 24-hour and 30-day mortality. In addition, the functional laboratory and biomarker studies will comprehensively characterize trauma induced coagulation (TIC) and inflammatory milieu providing insight into biological phenotypes, dynamic changes over time and their relationship to treatment and outcome.</i> The PROPPR Trial will be conducted under exception from informed consent ([EFIC], Appendix 1) and begin with a Vanguard Stage that will continue for up to six months to assess sites’ ability to implement the protocol and recruit subjects.</p> <p><u>Clinical Hypotheses and Aims</u></p> <p>Primary Clinical Aim: To separately compare as co-primary outcomes, 24-hour mortality and 30-day mortality between 1:1:1 and 1:1:2 groups adjusting for clinical site.</p> <p>Primary Clinical Hypothesis 1: A greater proportion of subjects who are predicted to have a massive transfusion and randomized to the 1:1:1 ratio group will survive to 24 hours after Emergency Department (ED) admission compared with subjects randomized to the 1:1:2 ratio.</p> <p>Primary Clinical Hypothesis 2: A greater proportion of subjects who are predicted to have a massive transfusion and randomized to the 1:1:1 ratio group will survive to 30 days after ED admission compared with subjects randomized to the 1:1:2 ratio.</p> <p>Ancillary Clinical Aim: To compare subjects predicted to have a massive transfusion and randomized to the 1:1:1 or 1:1:2 ratio groups on a variety of ancillary clinical outcomes measured from randomization to initial hospital discharge after adjusting for site.</p> <p>Ancillary Clinical Hypotheses 1: Subjects predicted to have a massive transfusion and randomized to 1:1:1 will differ in number of hospital-free, ventilator-free, and ICU-free days from the 1:1:2 ratio group.</p> <p>Ancillary Clinical Hypothesis 2: Subjects predicted to have a massive transfusion and randomized to the 1:1:1 and 1:1:2 ratio groups will differ in time to hemostasis, major surgical procedures, and in the incidence of transfusion-related serious adverse events during initial hospitalization; will differ in the amount of study blood products given until hemostasis and in the amount of blood products given from hemostasis to 24 hours; and will differ in functional status at initial hospital discharge and in initial hospital discharge status.</p> <p><u>Laboratory Hypotheses and Aims</u></p> <p>Overall Laboratory Hypothesis: Subjects predicted to have a massive transfusion will differ in their coagulation and inflammatory phenotypes at admission and over time which</p>

will be affected by resuscitation and affect outcome.

Laboratory Aim 1: To develop models characterizing TIC and inflammation in enrolled patients at ED admission.
Hypothesis 1: Severely injured trauma patients enrolled into PROPPR will differ in their coagulation and inflammatory phenotypes at admission by subjects' demographic and baseline injury characteristics.

Laboratory Aim 2: To develop models characterizing the dynamics of TIC in order to identify mechanistic drivers and sequelae of coagulation and inflammation, AND to characterize the natural history of the coagulation/inflammatory milieu in enrolled subjects.
Hypothesis 2: Coagulation and inflammatory phenotypes identified at admission will display dynamic changes. These phenotype changes will be driven by injury demographics and resuscitation.

Laboratory Aim 3: To assess the effect of coagulation and inflammatory models on primary and ancillary outcomes.
Hypothesis 3: Coagulation and inflammatory profiles identified in **Laboratory Aims 1 and 2** will be associated with primary and ancillary clinical outcomes.

Background

Multiple observational studies have reported that blood product component ratios (*i.e.*, plasma:platelets:RBCs) that approach the 1:1:1 ratio, as found in fresh whole blood, are associated with significant decreases in truncal hemorrhagic death and in overall 24-hour and 30-day mortality among injured patients.¹⁻¹⁷ The rationale for the 1:1:1 ratio is that the closer a transfusion regimen approximates whole blood, the faster hemostasis will be achieved with minimum risk of coagulopathy. The current DoD guideline specifies the use of 1:1:1,¹⁸ and this practice is followed in almost all combat casualties. In other observational studies, leading centers have reported good outcomes across a range of different blood product ratios.^{2-6,9,19} For example, a 1:2 plasma:RBC ratio is used with little guidance regarding platelets.^{19, 20} The American Association of Blood Banks (AABB) recently performed a meta-analysis and recommended the use of at least a 1:3 plasma:RBC ratio in Level I trauma centers until randomized trials can provide more definitive evidence.^{21, 22} The proposed randomized trial is intended to resolve debate and uncertainty regarding optimum blood product ratios.

Trauma induced coagulopathy (TIC) is the global term that describes coagulopathy after injury and the associated sequelae.²³⁻²⁵ Despite identification and quantification of this coagulopathy, the initiators of the process, underlying mechanisms, interaction of different coagulopathy phenotypes and their specific relationships to treatment and outcomes remain poorly understood and are a priority research area for the management of trauma hemorrhage. Brohi and Cohen have recently described a proposed mechanism for this TIC based on the protein C pathway.^{10, 24, 26} However, a definitive causal link has not been established. Several recent publications have documented the lack of understanding in this critical arena.^{27, 28}

Underlying the continuing controversy in trauma resuscitation research are two main concerns: transfusion-related complications²⁹ and survival/selection bias.^{30, 31} Some studies have shown decreased rates of complications from multiple organ failure (MOF) with increased ratios of blood products,^{2, 4, 5} while others have documented increased MOF rates.^{9, 19} A few studies recorded data only on patients who survived at least 48 hours, focusing on inflammatory outcomes of acute respiratory distress syndrome (ARDS) and MOF. Other studies excluded only those patients who died in the first 30 minutes after Emergency Department (ED) arrival. Because most preventable hemorrhagic deaths occur

	<p>within hours of trauma patients' ED arrival, it is critical to evaluate both the short- as well as longer-term effects of blood product transfusions. Therefore the longer a bleeding patient survives, the greater the chance to receive a cumulative ratio approaching 1:1:1 (survival bias). The proposed multi-center, randomized trial with a Vanguard Stage and intent-to-treat (ITT) analyses based on appropriate short- and long-term outcomes will 1) address the survival and selection bias that plagues previous studies, and 2) provide a more complete picture of the effectiveness and safety of 1:1:1 vs. 1:1:2 blood product ratios over the time windows of trauma patients' greatest potential benefit and risk.</p>
Study Design	Randomized, two-group, controlled Phase III trial with a Vanguard stage. Equal random allocation to treatment using stratified, permuted blocks with randomly chosen block sizes and stratification by site.
Subject Inclusion Criteria	<p><i>To be eligible, subjects must meet all of the following:</i></p> <p>1) Required the highest trauma team activation; 2) estimated age 15 years or older or greater than/equal to weight of 50 kg if age unknown; 3) received directly from the injury scene; 4) initiated transfusion of at least one unit of blood component within the first hour of arrival or during prehospital transport; 5) predicted to receive a MT by exceeding the threshold score of <i>either</i> the ABC score or the attending trauma physician's judgment criteria.</p>
Subject Exclusion Criteria	<p><i>Subjects are ineligible if they meet one or more of the following:</i></p> <p>1) Received care from an outside hospital or healthcare facility (defined as receiving a life saving intervention); 2) Moribund patient with devastating injuries and expected to die within one hour of ED admission; 3) prisoners directly admitted from a correctional facility; 4) Patients requiring an emergency department thoracotomy; 5) Children under the age of 15 years or under 50 kg body weight if age unknown; 6) Known pregnancy; 7) Greater than 20% total body surface area (TBSA) burns 8) suspected inhalation injury; 9) received greater than five consecutive minutes of cardiopulmonary resuscitation (CPR with chest compressions) in the pre-arrival or ED setting; 10) Known DNR prior to randomization; 11) Enrolled in a concurrent ongoing interventional, randomized clinical trial; 12) Have activated the "opt-out" process for the PROPPR trial.</p>
Study Intervention and Duration	A protocol using the 1:1:1 (plasma:platelets:RBCs) compared to the 1:1:2 ratio. Subjects will be followed to hospital discharge or up to the 30 th day of hospitalization (whichever comes first) and have a 30-day follow-up mortality assessment.
Primary Outcome Measures	Absolute percent (rather than relative percent) group difference in 24-hour and 30-day mortality (Co-primary outcomes)
Sample Size	Phase III: 580 subjects. 290 subjects/group provide 90% power to detect a difference as small as 10% in 24-hour mortality and 88% power to detect a 12% difference in 30-day mortality, assuming alpha=0.044 (adjusted from 0.05 for 3 interim effectiveness analyses), two sided, and assuming 24-hour and 30-day mortality in the 1:1:1 group of 11% and 23%, respectively based on epidemiologic data. At the DSMB meeting, April 25, 2013, prior to any review of unblinded data the blinded members of the DSMB reviewed a prespecified adaptive analysis conducted by blinded ROC biostatisticians and recommended that the sample size be increased from 580 to 680 to maintain a power of >85%. NHLBI approved this modification.
Analysis	The primary clinical analyses will separately compare treatment group differences in 24-hour and 30-day mortality using Mantel-Haenszel Tests with site stratification. For Laboratory Aims 1-3 we will develop models (reverse-engineered from the laboratory data) to identify drivers and sequelae of TIC and inflammation and to assess relationships among identified phenotypes and outcomes. In addition traditional regression analyses will be conducted for Laboratory Aim 3.

Monitoring Safety	<p>There will be three formal effectiveness analyses. The 2 interim analyses for the DSMB will occur after 1/3 and 2/3 of the projected 24-hour or 30-day mortality events are observed (whichever reaches its projected 1/3 and 2/3 first). The two co-primary outcomes will be separately monitored using a two-sided O'Brien-Fleming boundary with Lan-DeMets alpha spending function based on events for each of the two comparisons.³² The plan for interim analysis is suggested as a guideline for the DSMB, and could be modified by the DSMB prior to the start of the trial.</p> <p>At each DSMB meeting after the start of the trial, we will present safety data by treatment group (labeled as A,B in the same manner proposed by the 2006 FDA Guidance for Clinical Trial Sponsors on the Establishment and Operation of Clinical Trial Data Monitoring Committees, unless the DSMB requires complete unblinding). This would include, but is not limited to, total counts of all related, serious and unanticipated adverse events, including a description of the event itself. Additional safety analyses will be developed as requested by the DSMB. We will report overall mortality for the safety analysis. At the formal interim analysis we will report mortality by treatment group (or A,B).</p>
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DATA COLLECTION FLOWSHEET

ASSESSMENTS	Pre ED	ED	OR	IR	Inpatient 1st 24 hrs	Inpatient Daily Assess.	Discharge Info	30 Days
Eligibility Criteria	x	x						
Demographics		x					x	
Trauma Activation	x							
EMS Care	x							
Unit arrival information		x	x	x	x			
Informed consent process		x	x	x	x	x		
Vital Signs	x	x	x	x	x	x		
Glasgow Coma Scale	x	x	x	x	x	x	x	
Extended Glasgow Outcome Score							x	
Mortality		x	x	x	x	x	x	x
Life Saving Interventions	x	x	x	x	x	x		
Injury Information	x	x						
Blood Products (including age of product)	x	x	x	x	x	x		
Non-blood Fluids	x	x	x	x	x	x		
Medications	x	x	x	x	x	x		
Surgical Procedures			x		x	x		
Interventional Radiology Procedures				x		x		
Angiogram				x				
Lab Results		x	x	x	x	x		
Hemostasis Obtained		x	x	x	x			
*Research Lab Sample Collection		x	x	x	x	x		
Multi-Organ Failure Assessment						x		
Complications					x	x	x	
Injury Severity Score (ISS)							x	
Subject Disposition							x	
Past Medical History							x	

* Research lab samples time points:

For all subjects (screened, eligible, or randomized): 0 hour

For all randomized subjects: 2, 4, 6, 12, 24, 48, and 72 hours

1. OVERVIEW

The Pragmatic, Randomized, Optimal Platelet and Plasma Ratios (PROPPR) study design is a Resuscitation Outcomes Consortium (ROC) Protocol. ROC is funded by the National Heart, Lung, and Blood Institute (NHLBI), the United States' Department of Defense (DoD) and the Defence Research and Development Canada. ROC is a clinical trial network focusing on research primarily in the area of pre-hospital cardiopulmonary arrest and severe traumatic injury. Its mission is to provide infrastructure and project support for clinical trials and other outcome-oriented research in the areas of cardiopulmonary arrest and severe traumatic injury that lead to evidence-based change in clinical practice (<https://roc.uwctc.org/tiki/tiki-index.php>). PROPPR will be conducted as a Phase III trial at Level I Trauma Centers in North America. The Phase III trial is designed to evaluate the difference in 24-hour and 30-day mortality among subjects predicted to receive massive transfusion ([MT] defined as receiving 10 units or more RBCs within the first 24 hours). The goal of PROPPR is to improve the basis on which clinicians make decisions about transfusion protocols for massively bleeding patients.

2. SPECIFIC AIMS AND HYPOTHESES

The objective of this study is to conduct a Phase III multi-site, randomized trial in subjects predicted to have a massive transfusion, comparing the effectiveness and safety of 1:1:1 transfusion ratios of plasma and platelets to red blood cells (the closest approximation to reconstituted whole blood) with the 1:1:2 ratio. The co-primary outcomes will be 24-hour and 30-day mortality. In addition, the functional laboratory and biomarker studies will comprehensively characterize the post-trauma coagulation and inflammatory milieu providing insight into biological phenotypes, dynamic changes over time and their relationship to treatment and outcome. The PROPPR Trial will be conducted under exception from informed consent (EFIC) and begin with a Vanguard Stage that will continue for up to six months to assess sites' ability to implement the protocol and recruit subjects.

Clinical Hypotheses and Aims

Primary Clinical Aim: To separately compare as co-primary outcomes, 24-hour mortality and 30-day mortality between 1:1:1 and 1:1:2 groups adjusting for clinical site.

Primary Clinical Hypothesis 1: A greater proportion of subjects who are predicted to have a massive transfusion and randomized to the 1:1:1 ratio group will survive to 24 hours after Emergency Department (ED) admission compared with subjects randomized to the 1:1:2 ratio.

Primary Clinical Hypothesis 2: A greater proportion of subjects who are predicted to have a massive transfusion and randomized to the 1:1:1 ratio group will survive to 30 days after ED admission compared with subjects randomized to the 1:1:2 ratio.

Ancillary Clinical Aim: To compare subjects predicted to have a massive transfusion and randomized to the 1:1:1 or 1:1:2 ratio groups on a variety of ancillary clinical outcomes measured from randomization to initial hospital discharge after adjusting for site.

Ancillary Clinical Hypotheses 1: Subjects predicted to have a massive transfusion and randomized to 1:1:1 will differ in number of hospital-free, ventilator-free, and ICU-free days from the 1:1:2 ratio group.

Ancillary Clinical Hypothesis 2: Subjects predicted to have a massive transfusion and randomized to the 1:1:1 and 1:1:2 ratio groups will differ in time to hemostasis, major surgical procedures, and in the incidence of transfusion-related serious adverse events during initial hospitalization; will differ in the amount of study blood products given until hemostasis and in the amount of blood products given from hemostasis to 24 hours; and will differ in functional status at initial hospital discharge, and in initial hospital discharge status.

Laboratory Hypotheses and Aims

Overall Laboratory Hypothesis: Subjects predicted to have a massive transfusion will differ in their coagulation and inflammatory phenotypes at admission and over time which will be affected by resuscitation and affect outcome.

Laboratory Aim 1: To develop models characterizing TIC in enrolled patients at ED admission.

Hypothesis 1: Severely injured trauma patients enrolled into PROPPR will differ in their coagulation and inflammatory phenotypes at admission by subjects' demographic and baseline injury characteristics.

Laboratory Aim 2: To develop models characterizing the dynamics of TIC in order to identify mechanistic drivers and sequelae of coagulation and inflammation, AND to characterize the natural history of the coagulation/inflammatory milieu in enrolled subjects.

Hypothesis 2: Coagulation and inflammatory phenotypes identified at admission will display dynamic changes. These phenotype changes will be driven by injury demographics and resuscitation.

Laboratory Aim 3: To assess the effect of coagulation and inflammatory models on primary and ancillary outcomes.

Hypothesis 3: Coagulation and inflammatory profiles identified in **Laboratory Aims 1 and 2** will be associated with primary and ancillary clinical outcomes.

3. BACKGROUND

Injury is the leading cause of death in adults and children between the ages of 1 and 44 years. Nearly 50% of injury-related deaths occur before the individual reaches the hospital, and much of this mortality may remain difficult to prevent.^{7, 33-36} However, approximately 40% of the in-hospital deaths among injured patients involve massive truncal hemorrhage that is considered potentially salvageable.³⁷⁻⁴³ The staggering numbers of years of productive life lost due to hundreds of thousands of deaths annually from injuries (over 180,000 in the U.S. in 2007) demands more urgent attention to this major public health problem.⁴⁴⁻⁴⁸ In the late 1970s, whole blood was the primary resuscitation fluid for exsanguinating patients. Due to the concern for potential infectious diseases among donors, component therapy using separated units of RBCs, plasma and platelets, has become the standard in Level I Trauma Centers. However, no randomized trial has ever been conducted to establish which of the many different component transfusion regimens possible is best for trauma patients. Increasing knowledge of the myriad factors influencing survival and recovery following traumatic injury has focused clinical and translational research on the modifiable aspects of resuscitation and transfusion protocols.^{49, 50}

Epidemiology of Trauma

In an autopsy study from the ongoing conflicts in Iraq and Afghanistan, fully 86% of all potentially preventable deaths were from truncal hemorrhage.⁴¹ Likewise, in the civilian arena, the leading cause of potentially preventable death is early truncal hemorrhage,³⁷ with most deaths occurring within 6-12 hours of admission (Figure 1).^{6, 37}

A recent paper by Moore et al documents that the majority of massive transfusion (MT) patients traditionally receive ≥ 10 units of blood in the first 6 hours after injury and have the highest incidence of death during the same time frame (Figure 1).⁶ Likewise, civilian data from Los Angeles County document that early death is largely from hemorrhage and occurs early after admission, while late death is uncommon.³⁷ Unpublished data from University of Texas Health Science Center at Houston (UTHealth) has demonstrated that the majority of these deaths occur within the first 24 hours of admission, and very few occur after 72 hours (Figure 2). Data from Perkins and Spinella show a similar timeline in military casualties,^{51, 52} while Holcomb

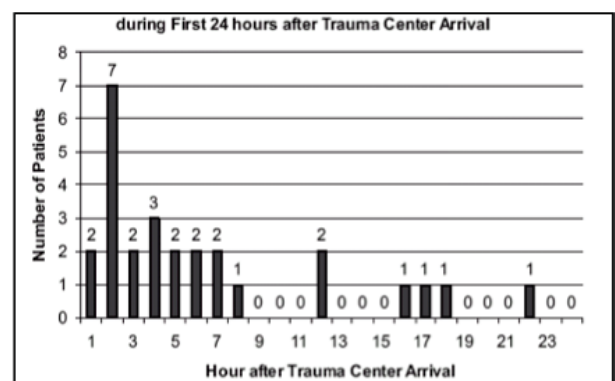


Figure 1. Mortality distribution of Massive Transfusion patients (n=27) during the first 24 hours after trauma center arrival.⁶

and Kelly have shown that truncal hemorrhage is the leading potentially preventable cause of death in U.S. military casualties.^{39,41}

Trauma Induced Coagulopathy

Coagulopathy likely plays a significant role in preventable deaths due to hemorrhage, as seriously injured patients in shock are the ones who most often present with coagulopathy in the ED. Trauma patients who are not coagulopathic rarely die. Trauma induced coagulopathy (TIC) is the global term that describes coagulopathy after injury and the associated sequelae.²³⁻²⁵ Previous studies have defined TIC as increases in plasma based coagulation tests (activated partial thromboplastin time [APTT], partial thrombin time [PTT], prothrombin time [PT] and international normalized ratio [INR]).⁵³ Identifiable coagulopathic alterations occur nearly immediately after injury and are associated with significant bleeding, morbidity, and mortality. Despite identification and quantification of this coagulopathy, the initiators of the process, underlying mechanisms, interaction of different coagulopathy phenotypes and their specific relationships to treatment and outcomes remain poorly understood and are a priority research area for the management of trauma hemorrhage. Brohi and Cohen have recently described a proposed mechanism for this TIC based on the protein C pathway.^{10,24,26} However, a definitive causal link has not been established. Several recent publications have documented the lack of understanding in this critical arena.^{27,28} Lack of a mechanistic understanding has likely contributed to the variability in transfusion practice in seriously injured patients, with survival ranging from 40-70%.⁵

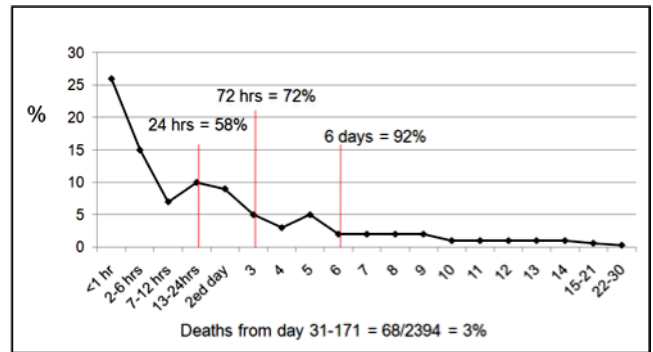


Figure 2. UTHealth-Houston 1999 – 2008 Trauma admissions = 26,028 and 2,394 deaths = 6.6%

Recently, it has been recognized that severely injured trauma patients present with early evidence of a coagulopathy that is heterogeneous based upon age, gender, physiology and mechanism of injury. Two recent studies have identified that TIC is present on arrival in the emergency department in 25% of patients with major trauma.^{54,55} It is associated with *higher* transfusion requirements, a *greater* incidence of multiple organ failure (MOF), *longer* intensive care unit (ICU) and hospital stays, and a 4x risk of mortality compared to those with normal coagulation.^{24,27,54-56}

While it is clear the coagulopathy after trauma is multifactorial and there are several acute coagulopathic phenotypes (each with different diagnoses and treatment modalities), little attention has been directed towards understanding the mechanisms involved with the early presentation of TIC. Thus, laboratory studies of coagulopathy will help define the understanding of the mechanisms of early coagulopathy associated with trauma, how best to mitigate and reverse the effects, and start describing optimal treatment regimens. Furthermore, at the TransAgency Coagulopathy meeting (April 5-6, 2010, <http://www.nhlbi.nih.gov/meetings/workshops/tactrauma.htm>), the NHLBI and DoD devoted significant time and discussion to this subject and the recommendations drawn from that two day seminar closely parallel the proposed, extensive laboratory effort described later (5.2.6 Laboratory Evaluations).

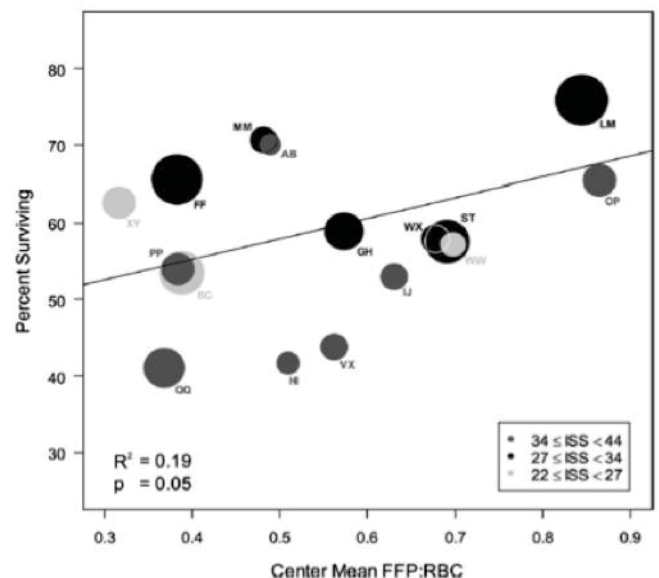


Figure 3. Bubble plot of the relationship of mean center plasma to RBC ratio to survival. Circle size represents the percentage of MT patients contributed by each center. Shades of gray indicate 3 levels of injury severity scores.⁵

Current Transfusion Practices and Ratios

Despite great advances in resuscitation practices over the course of the last half-century, recent data suggest that aggressive use of crystalloid and late and/or inadequate use of plasma and platelets may contribute to increased coagulopathic bleeding and death. A recent study of combat-injured

casualties from Iraq who received MTs revealed that those who received more plasma demonstrated much lower mortality (19%) than those who received more traditional ratios of plasma (65%).¹ Perkins, Borgman, and colleagues reported that increased platelet ratios were associated with improved survival after combat injury.^{1,7} Schnuriger and Holcomb have shown similar data in civilian trauma patients, associating improved survival with increased use of platelets.^{5,8} Holcomb et al recently conducted a multicenter retrospective study of modern transfusion practice at 16 leading civilian trauma centers.⁵ Data were collected for all trauma patients admitted in the years 2005-2006 who arrived at the hospital directly from the scene and received at least 1 unit of blood product within 24 hours of admission.⁵ From that 12 month period, 466 MT patients were analyzed and it was found that plasma:platelet:RBC ratios varied from 1:1:1 to 0.3:0.1:1, with corresponding survival rates ranging from 71% down to 41%.⁵ Importantly, at the center level, mortality was significantly correlated with mean blood product ratios (Figure 3).⁵

Increased plasma and platelet to RBC ratios significantly decreased truncal hemorrhagic death and 30-day mortality without a concomitant increase in MOF as cause of death (Table 1)⁵ These data document the relationship between increased survival and increased use of plasma and platelets; however these data may suffer from potential survival bias.⁵ Similar to the Borgman military study,¹ patients receiving increased plasma and platelets showed improved 24-hour and 30-day survival, decreased incidence of hemorrhagic death, without an increase in MOF death. Intensive Care Unit free days also were increased in the patients receiving higher plasma and platelet ratios.

	High Plasma (%)		Low Plasma (%)		P-Value
	High Platelets n = 151	Low Platelets n = 101	High Platelets n = 83	Low Platelets n = 131	
Plasma:Platelet:RBC ratio	1:1:1	1:0.2:1	0.3:1:1	0.3:0.1:1	
Survival (%)	71	52	66	41	<0.001
Survival at 6 hours	98	86	83	58	<0.001
Survival at 24 hours	87	75	77	50	<0.001
Survival at 30 days	73	54	68	43	<0.001
Median Time to death, (hours)	35	18	6	4	<0.001
Mortality Rates (%) by Cause of Death					
Truncal Hemorrhage	10	25	22	44	<0.001
Head Injury	13	15	6	14	0.3
MOF	5	7	6	3	0.45
Airway	0	1	2	2	0.24
Other	3	6	4	4	0.85
Clinical Outcomes					
Hospital free days	6 ± 8	3 ± 6	5 ± 8	3 ± 7	<0.001
ICU free days	5 ± 7	3 ± 6	6 ± 7	4 ± 7	<0.001
Ventilator free days	6 ± 8	2 ± 5	7 ± 8	4 ± 7	<0.001

Table 1. Survival, cause of death, and clinical outcomes by plasma and platelet ratio. High plasma or platelet to RBC ratio ≥ 1:2. Low plasma or platelet to RBC ratio < 1:2.⁵

Multiple observational studies have reported that blood product component ratios (*i.e.*, plasma:platelets:RBCs) that approach the 1:1:1 ratio, found in fresh whole blood, are associated with significant decreases in truncal hemorrhagic death and in overall 24-hour and 30-day mortality among injured patients.¹⁻¹⁷ The rationale for the 1:1:1 ratio is that the closer a transfusion regimen approximates whole blood, the faster hemostasis will be achieved with minimum risk of coagulopathy. The current DoD guideline specifies the use of 1:1:1,¹⁸ and this practice is followed on almost all combat casualties. In other observational studies, leading centers have reported good outcomes across a range of different blood product ratios.^{2-6, 9, 19} Additionally, little guidance regarding platelets is available.^{19, 20} The American Association of Blood Banks (AABB) recently performed a meta-analysis and recommends the use of at least 1:3 plasma:RBC ratios in Level I trauma centers until randomized trials can provide more definitive evidence.^{21, 22} The continuing debate and uncertainty regarding optimum blood product ratios reflect equipoise and support for our proposed randomized trial of the relative effectiveness of the 1:1:1 and 1:1:2 blood product ratios.

Underlying this unresolved controversy in trauma resuscitation research are two main concerns: transfusion-related complications²⁹ and survival/selection bias.^{30, 31} Some studies have shown decreased rates of complications from multiple organ failure (MOF) with increased ratios of blood products,^{2, 4, 5} while others have documented increased MOF rates.^{9, 19} A few studies recorded data only on patients who survived at least 48 hours, focusing on inflammatory outcomes of acute respiratory distress syndrome (ARDS) and MOF. Other studies excluded only those patients who died in the first 30 minutes after Emergency Department (ED) arrival. Because most preventable hemorrhagic deaths occur within hours of trauma patients' ED arrival, it is critical to evaluate both the short- as well as longer-term effects of blood product transfusions. The longer a bleeding patient survives, the greater the chance to receive a cumulative ratio approaching 1:1:1 (survival bias).

The preponderance of the recent literature suggests patients in severe HS may benefit from increased ratios of plasma and platelets to RBCs. Other reviews and single center reports from leading institutions provide an alternative view, suggesting that a 1:3 ratio be used.^{19, 20} Some of these studies have small numbers of MT patients, collected over many years, or exclude all deaths prior to ICU arrival.^{57, 58} Conflicting findings in this area are expected since all the studies are retrospective and confounded by multiple unmeasured variables. Watson and colleagues from the Glue grant consortium reported increased MOF rates with increased transfusion of plasma, when excluding patients who died in the first 24 hours.⁵⁹ However, increased plasma use was associated with improved survival when the first 24 hours was included in the analysis. Since the majority of bleeding and early death occurs within the first 24 hours, most authors have included this time frame in their analysis. It is also understood that damage control resuscitation (DCR) should not be performed in patients who are not in HS or are not at high risk of massive bleeding, as the increased plasma and platelets could increase MOF, without a survival benefit.

Defining a Massive Transfusion Study Population

The need for MT can be rapidly predicted, using data available within minutes of arrival in the ED in both blunt and penetrating military and civilian casualties (Table 2).^{49, 60-64} In combat casualties with penetrating injuries Schreiber, Wade, and McLaughlin have all documented a receiver operator characteristic area under the curve (AUC) of 0.8 using easily available variables, systolic blood pressure (SBP) < 110, heart rate (HR) > 105, hematocrit (Hct) < 32, pH < 7.25.^{49, 62, 63} Yücel and Moore showed similar results in a civilian, largely blunt injured population.^{6, 64} Nunez and colleagues have created the most rapidly acquired score, the assessment of blood consumption score (ABC score), not requiring any laboratory values and with a high value on the receiver operator characteristic curve.⁶¹

Table 2. Summary of Reported Receiver Operating Characteristic AUC Values in Trauma

Author	Variables	AUC*
McLaughlin et al. ⁴⁹	SBP, HR, pH, Hct	0.77
Yücel et al. ⁶⁴	SBP, HR, Base Deficient (BD), Hemoglobin (Hgb), Male, +FAST (Focused Assessment for the Sonography of Trauma), long bone/pelvic fracture	0.84
Moore et al. ⁶	SBP, pH, ISS>25	0.80
Schreiber et al. ⁶²	Hgb≤11, INR>1.5, penetrating injury	0.80
Wade et al. ⁶³	SBP, HR, pH, Hct	0.78
Nunez et al. ⁶¹	SBP, HR, FAST, penetrating mechanism	0.85
Nunez et al. ⁶⁰	SBP, HR, FAST, penetrating mechanism, RBC transfusion in ED	0.89

*AUC is a function of specificity and sensitivity.

Clinically, the ability to accurately predict which patients will or will not require MT is important so that increased plasma and platelet transfusions can be started early in those who will potentially benefit and avoided in those who will not.^{6, 49, 62-64} In a randomized study, this algorithm is necessary for accurate randomization and decreasing noise from minimally transfused patients. Recognizing this important issue, efforts to develop ever more accurate MT prediction algorithms are ongoing at several leading centers.

Nunez et al have recently published the simplest MT prediction model using only data routinely available within 5-10 minutes of patient arrival in any trauma center and not relying on the use of any laboratory values.⁶¹ This same group also describes the additional benefit of using a unit of RBC transfused in the ED and the ABC score to improve the prediction of MT patients,

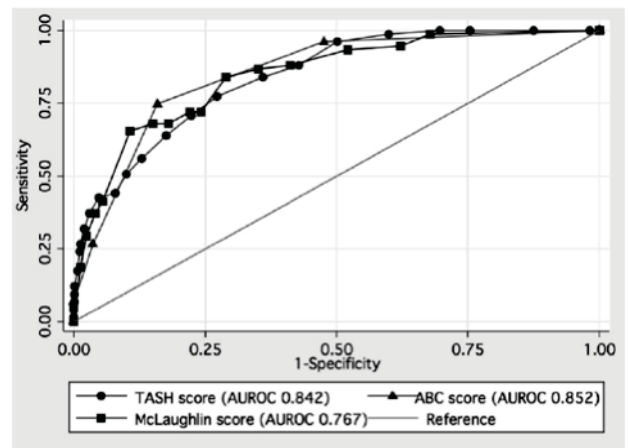


Figure 4. AUC for three different scoring systems⁵⁸

raising the AUC to 0.89.⁶⁰ The ABC score is comparable to other algorithms with more complicated and time-consuming data requirements (Figure 4); however it holds the added benefit of not being delayed by laboratory testing that could delay the correct treatment. This scoring system has also been recently been validated in a multicenter, retrospective study.⁶⁵ **PROPPR will utilize ED RBC transfusion combined with the validated ABC score or physician assessment to randomize patients.**

Risks and Complications of Transfusion

Few interventions in medicine are without risk. The risk of transfusion-related acute lung injury (TRALI) is increased as plasma and platelets use increases. Most authors estimate a risk of TRALI in 1:10,000 units of FFP transfused, which must be placed in the context of significantly improved survival reported in many recent trauma publications.^{25,66} A likely contributor to the improved outcome seen with increased plasma and platelet use is the decrease in excessive crystalloid infusion.⁶⁷ Currently, in seriously injured patients the potential benefit of increased blood product transfusion seems to outweigh the known risks.

Rationale for PROPPR Trial

In summary, it is unclear what the optimal ratio should be, and several leading centers have described good outcomes with both higher and lower ratios, confirming the presence of clinical equipoise for the proposed study groups.^{2-6, 9, 19, 49} It is critical to understand that Level I/II data from clinical trials are completely lacking in this area, and this proposal addresses the issue.

Clinical Rationale

Currently, there is no universally accepted MT guideline. Most trauma centers are using a ratio driven massive transfusion protocol for the early management of bleeding trauma patients rather than a laboratory-directed approach. This is based on the unavoidable delay in obtaining relevant clinical laboratory values.⁶⁸ This delay, which can extend up to 45 minutes, prevents reliable goal directed therapy. At least one center (Sunnybrook Health Science Center, Toronto, Canada [NCT00945542]) is studying goal directed therapy, to evaluate clinical efficacy. Based on our experience with a systematic research program starting with an international symposium focused on MT in 2006,⁶⁹ followed by a multicenter retrospective study⁵ and in 2009, a prospective, observational study (*i.e.*, Prospective, Randomized, Observational, Multicenter, Massive Transfusion sTudy [PROMMTT], Rahbar, Principal Investigator), substantial variation in mortality rates, blood product ratios and clinical practice persists across many Level I Trauma centers in the U.S., despite the call for a common massive bleeding protocol.^{28, 70} [Results from the PROMMTT study provided in this protocol are in draft stage only and have not yet undergone the signed endorsement of all co-investigators, peer-review or publication in a scientific journal.] The proposed study seeks to extend the success of the ROC and draw on important lessons learned (execution of multicenter studies, the use of public notification and community consultation, transfusion study intricacies, web-based data entry and their efficient organization) to conduct the first multi-center, randomized clinical trial (RCT) of varying blood product ratios for the treatment of massively bleeding trauma patients, starting in the ED. Unpublished data from PROMMTT reveal that the proposed ratios in this proposal are representative of current clinical practice at leading trauma centers. **Our proposed Phase III RCT is designed to 1) provide a valid and efficient clinical trial design framework for in-hospital trauma research (including a Vanguard [feasibility] stage), 2) address the survival/selection bias present in previous studies, 3) reduce the risk of post-transfusion complications and conserve resources by restricting enrollment to patients who screen positive on our predictive MT algorithm, 4) contribute to an evidence-based guideline for the treatment of massively bleeding trauma patients, and 5) elucidate the mechanisms of TIC and inflammation.**

The ED setting is a unique environment that introduces challenges to trial design and sample collection, including the use of exception from informed consent (EFIC). The ROC investigative team has extensive experience with both waiver of consent and emergency resuscitation trials. This is the first multicenter RCT of varying blood product ratios of massively bleeding patients using EFIC in the ED. The Vanguard approach is being used for the first time in a trauma trial to improve trial efficiency and increase the likelihood that the trial will be completed and informative.

Additionally, this trial includes the first use of ED RBC combined with the validated ABC score⁶¹ or clinical judgment in a prospective, randomized study to predict patients who will or will not require MT. This study will use the full potential of the ABC Score, as it is able to be obtained quickly and without delays from laboratory testing. Based on PROMMTT data, the combination of either a positive ABC score or a trauma physician's gestalt at the time of admission should provide sensitivity=85%, specificity=39%, positive predictive value (PPV) = 30%, and negative predictive value 89% for predicting a trauma patient's need for MT. An 85% sensitivity is higher than any other studies. While the PPV (and 62% AUC) based on PROMMTT data is lower than other studies, the PPV (and AUC) in PROPPR is expected to be considerably higher than 30% because potentially eligible patients who die or achieve hemostasis before the seal on the PROPPR container (containing randomized blood products from the blood bank) is broken will be excluded. The PROPPR protocol enables an unbiased exclusion of many patients who do not require an MT (false positives) and facilitates the appropriate focus on the most seriously hemorrhaging trauma patients at highest risk of mortality and with the greatest potential benefit from optimized blood product ratios. Identification of patients in need of MT is important so that increased plasma and platelet transfusions can be started early in those who will potentially benefit and avoided in those who will not.^{6, 49, 62-64} In a randomized study, this algorithm is intended to ensure accurate selection of the massively bleeding patient and increase the signal-to-noise ratio.

Research Laboratory Rationale

Successful resuscitation of massively bleeding trauma patients is constrained also by the gap in our knowledge of the complex interplay between trauma-induced coagulopathy (TIC), inflammation and blood product transfusions. Presently there is incomplete characterization of the multiple coagulopathic phenotypes, understanding of the mechanism for development of coagulopathy, and minimal prospective data to understand or target the putative benefits of early plasma based resuscitation on injured patients. Preventing TIC (e.g., with earlier plasma and platelet transfusions and less crystalloid infusions) is especially challenging when the most sensitive biomarkers of coagulation and inflammation await discovery.^{27, 70} This point is clinically important because it is impossible to optimize therapeutic effectiveness to control bleeding, impossible to understand biologically and physiologically the results of our clinical trial and impossible to minimize the risks of late thrombotic, infectious, and inflammatory complications without completely understanding the spectrum of coagulation abnormalities seen after severe injury and the effects of plasma resuscitation on mitigating those perturbations. Identification of the precise targets for the most effective therapies (e.g., an optimum combination of infusions and transfusions), will require vigilant tracking of the time-dependent perturbations in coagulation and inflammation as varying ratios are transfused, hemostasis is achieved and normal hemodynamics restored. With respect to coagulopathy, it is clear that TIC is multifactorial and there are likely several acute coagulopathic phenotypes (each with different diagnoses and treatment modalities), but little systematic attention has been directed towards understanding the mechanisms involved with the early presentation of TIC. Thus, laboratory studies of coagulopathy will help define the understanding of the mechanisms of early coagulopathy associated with trauma, how best to mitigate and reverse the effects, and start describing optimal treatment regimens. This study **will be the first** to characterize the natural history of coagulopathy and inflammation and simultaneously identifies key and novel pathways and therapeutic targets. We will collect blood from severely injured patients immediately after injury and sequentially for 72 hours, a novel venture that will provide data never before collected. Plasma will be assayed for coagulation factors and inhibitors, complement proteins and inflammatory mediators. Measures of coagulation, blood cellular populations and platelet function will be done on fresh whole blood. More specifically, we have chosen to study groups of markers in four main areas: 1) *markers of endothelial dysfunction*, 2) *cytokines and chemokines*, 3) *parameters of coagulation, including platelet function* and 4) *mobilization of progenitor cell populations and characterization of circulating cellular populations*. Analyzing these laboratory measures will answer the following questions: 1) How does plasma ratio and resuscitation regime affect TIC and clinical outcome? 2) How does resuscitation (plasma ratio) affect markers of endothelial injury, inflammation and coagulation? 3) How does resuscitation affect cell mobilization and function? 4) How do markers of vascular and circulating cell injury, coagulation, and inflammation change in severely injured patients? Additionally, clinical data that is collected will be utilized to develop a systems level natural history characterization of coagulopathy after injury and identify key and novel pathways and therapeutic targets. By comparing functional coagulation and plasma protein measurements with physiologic measures as well as outcome data we will obtain for the first time a complete picture

of the timing, severity and causes for early coagulopathy, later inflammation, infection and organ failure (Ancillary Clinical Outcomes) after severe trauma and shock.

4. RESEARCH DESIGN

PROPPR is a two-group, 580 patient, randomized, controlled Phase III trial. The rationale for the 1:1:1 ratio is that the closer a transfusion regimen approximates whole blood, the faster hemostasis will be achieved with minimum risk of coagulopathy. The current DoD guideline specifies the use of 1:1:1,¹⁸ and this practice is followed on almost all combat casualties. In other observational studies, leading centers have reported good outcomes across a range of different blood product ratios.^{2-6, 9, 19} For example, a 1:2 plasma:RBC ratio is used (albeit with little guidance regarding platelets).^{19,20}

The continuing debate and uncertainty regarding optimum blood product ratios reflect equipoise and support for our proposed randomized trial of the relative effectiveness and safety of the 1:1:1 and 1:1:2 blood product ratios. The distribution of plasma:RBC ratios among PROMMTT patients was heavily clustered around the most commonly occurring ratios of 1:1 and 1:2 (Figure 5A). The distribution of platelet:RBC ratios was more variable with less clustering around 1:1 and 1:2. The PIs of the Level I trauma centers selected for PROPPR unanimously declared equipoise and a preference for comparing 1:1:1 with 1:1:2 plasma:platelet:RBC ratios over any others (Figure 5B).

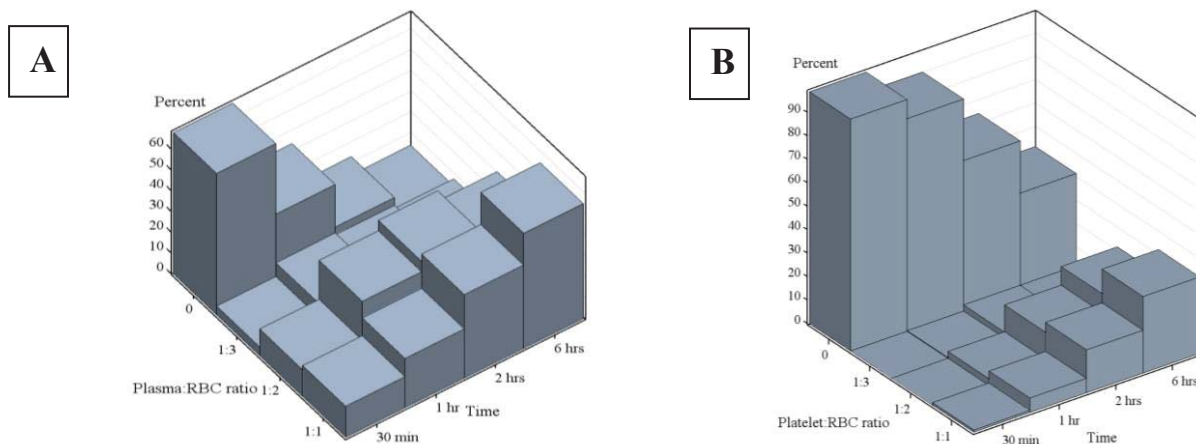


Figure 5. Distribution of plasma (A) and platelet (B) ratios given to substantially bleeding PROMMTT patients by time since admission (ratios in patients who died before the reference time are excluded from the distribution). This three-dimensional figure illustrates the time-varying nature of plasma transfusions conditional on survival. At 30 minutes after ED admission, nearly 70% of patients who have received at least 1 unit of RBCs have not yet received any plasma. In contrast, by 6 hours over 90% have received at least one unit of plasma and over 50% have achieved a 1:1 ratio of plasma to RBCs. A 1:1 ratio is defined here as greater than a 0.667 ratio of plasma to RBCs and a 1:3 ratio is defined as greater than 0 and less than or equal to 0.333.

4.1 Study Population

The target population is trauma subjects who are admitted to one of the participating sites and who meet the inclusion and exclusion criteria detailed below.

4.2 Setting

Level I trauma centers throughout North America, with previous involvement in trauma studies will participate in the trial. Each site is qualified and ready to proceed with the trial. At the site initiation visit, verification that standard operating procedures are in place and are consistent with the GLUE Grant guidelines before enrollment will begin at that site.

4.3 Inclusion Criteria

To be eligible, subjects must meet ALL of the following

- 1) Subjects who require the highest trauma team activation at each participating center,
- 2) Estimated age of 15 years or older or greater than/equal to weight of 50 kg if age unknown,
- 3) Received directly from the injury scene,

- 4) Initiated transfusion of at least one unit of blood component within the first hour of arrival or during prehospital transport, and
- 5) Predicted to receive a MT by exceeding the threshold score of *either* the ABC score or the attending trauma physician's judgment criteria (Table 3).

heart rate > 120 bpm	1 point
systolic blood pressure ≤ 90 mmHg	1 point
penetrating injury	1 point
positive FAST (intra-abdominal fluid by ultrasonography) exam	1 point

4.4 Exclusion Criteria

Subjects are ineligible if they meet one or more of the following

- 1) Received care (as defined as receiving a life saving intervention) from an outside hospital or healthcare facility (Procedures and care given at an outside health facility cannot be documented or controlled resulting in a high variability of standards of care and clinical outcomes.)
- 2) Moribund patient with devastating injuries and expected to die within one hour of ED admission; for example, those subjects with lethal traumatic brain injury deemed futile care by the neurosurgery or trauma attending prior to CT scanning or intracranial pressure monitoring, e.g. near decapitation, massive loss of intracranial contents, or transcranial gunshot wounds. Clinical assessment of severity of injury and not pupil reactivity has been found relevant in predictive models.^{71,72} Elderly subjects with massive myocardial infarction or stroke and severe injury based on the assessment of the trauma attending prior to randomization will also be excluded from randomization. (Those with non-survivable injuries or declared dead within 60 minutes of admission are unlikely to receive a MT.)
- 3) Prisoners, defined as those who have been directly admitted from a correctional facility (Prisoners are excluded because of their vulnerable population status. A free-living individual who is under police observation as a suspect will remain in the study until discharge or incarcerated.)
- 4) Patients requiring an emergency department thoracotomy (Trauma patients requiring an emergency department thoracotomy have exsanguinated from large vessel injury, have an extremely high mortality and usually do not survive, irrespective of treatment.)
- 5) Children under the age of 15 years or under 50 kg body weight if age unknown (Subjects under 15 years of age will be excluded, as the majority of adult trauma centers consider age 15 or older to be an adult and would not admit those under age 15. However, this will allow the inclusion of subjects 15 to 17 year olds that are at a high risk of motor vehicle accidents causing blunt or penetrating injuries and are admitted to Trauma Centers.)
- 6) Known pregnancy in the ED (Pregnant women have a significantly increased intravascular volume and physiologic reserve for bleeding which can require adjustments to the standard treatment protocols. Therefore for consistency for data analysis, pregnant women will be excluded.)
- 7) Greater than 20% total body surface area (TBSA) burns (Subjects with large and severe thermal injuries will require early and aggressive resuscitation to replace intra-vascular volume losses. As such, subjects with both large TBSA burns and traumatic injuries will require a resuscitation approach that is different to current isolated trauma resuscitation strategies. Additionally, in the absence of concomitant severe blunt trauma, these subjects are unlikely to receive blood products in the early resuscitative phase.)
- 8) Suspected inhalation injury
- 9) Received greater than five consecutive minutes of cardiopulmonary resuscitation (CPR with chest compressions) in the pre-arrival or ED setting (Subjects who receive greater than five consecutive minutes of CPR in the pre-hospital or initial ED setting are more likely to have non-survivable injuries and are not likely to receive a massive transfusion. Conversely, brief episodes of CPR are not unusual in severely hypotensive subjects.)
- 10) Known Do Not Resuscitate (DNR) prior to randomization

- 11) Enrolled in a concurrent, ongoing interventional, randomized clinical trial
- 12) Patients who have activated the “opt-out” process or patients/legally authorized representatives that refuse blood products on arrival to ED.

5. INTERVENTION (Figure 6)

5.1 Screening Procedures

Clinical research staff will be available in the hospital at each center on a 24/7 basis to conduct screening for PROPPR. The research staff will screen all major trauma subjects admitted to the ED with the highest acuity status. Data collection, blood draw for time 0, and subject observation will begin on the highest acuity subjects immediately upon the patient’s arrival to the ED. Once it is determined that the subject is ineligible, data collection will cease. For subjects meeting the PROPPR eligibility criteria, the research staff will perform an assessment using the validated ABC score (Table 3).⁶¹ Subjects with two or more positive variables from the ABC score on admission will be eligible to be randomized in the trial and receive the PROPPR transfusion protocol. The clinical person responsible for implementing physician orders will notify the blood bank per standard procedure at each institution. In subjects with fewer than two of these variables, the PROPPR research staff will query the trauma attending as to their clinical judgment regarding whether the patient will require a MT. If the attending responds with a “yes” the patient will be eligible for the trial. The physician can wait to respond to the gestalt question, if unsure; however, he or she must respond within one hour of ED admission to activate the protocol. If the answer, however, is “no” the patient will be considered ineligible and all study procedures will end. The data collected up to the time the patient is deemed ineligible will be kept at each site and submitted to the HDCC to allow a description of screened patients versus enrolled subjects and provide demographic data for the blood samples analyses. The clinical data required to calculate the ABC score is routinely acquired at Level I trauma centers and should be available within minutes of arrival on all potential subjects.

5.2 Study Procedures

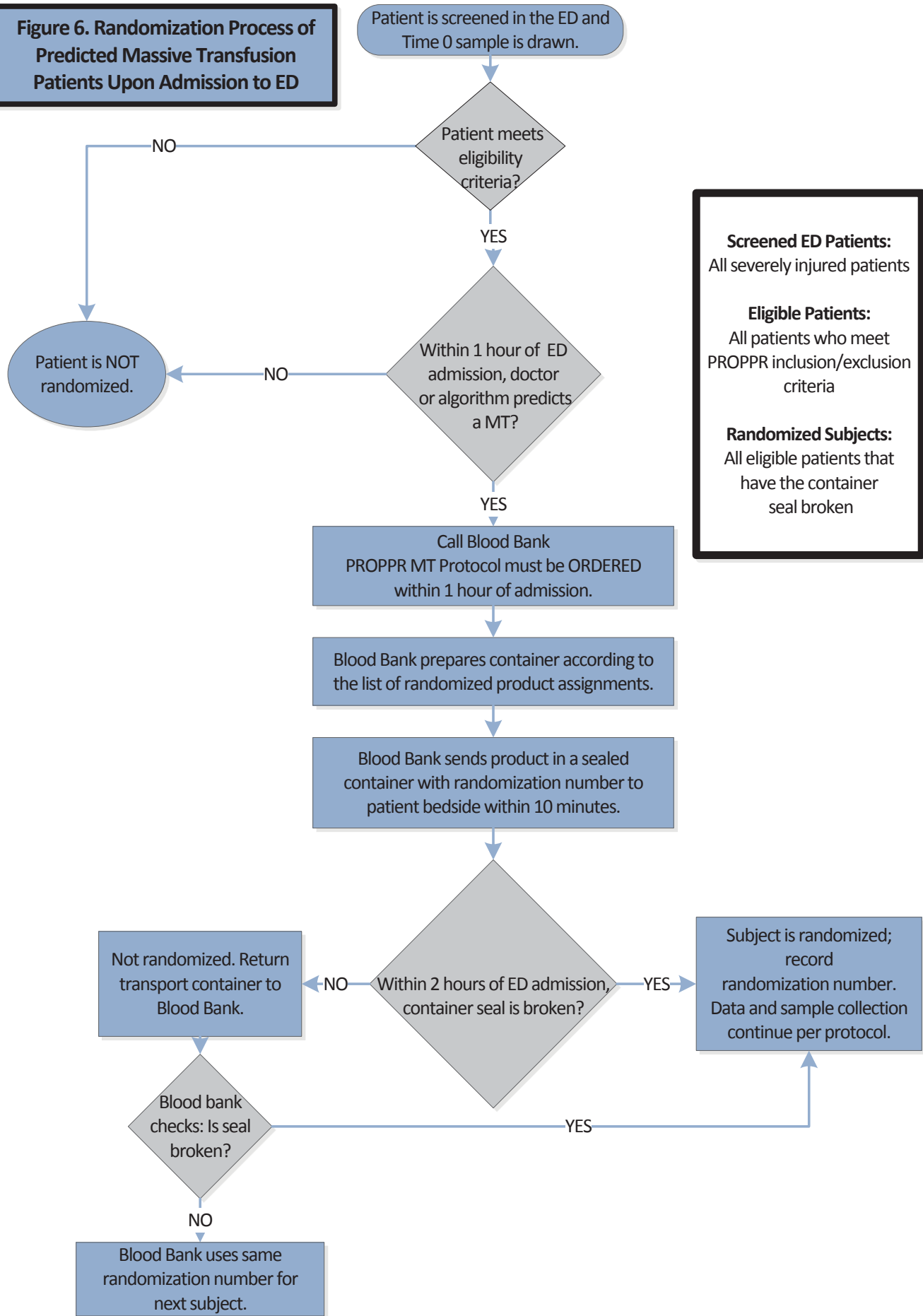
5.2.1 Randomization

A stratified, permuted blocked randomization scheme will be used to assure balance over time in the intervention groups. Block sizes will be randomly chosen to avoid revealing a treatment assignment in this unblinded trial. Randomization will be stratified by site. For consistency in all sites, randomization of blood products will be completed in the blood bank. Randomization lists will be prepared by the UTHealth Data Coordinating Center (HDCC) and sent to the contact person at the blood bank at each site who will keep the codes.

The randomization process for eligible subjects will begin when the attending trauma physician or the ABC score predicts that the patient will receive a MT (Figure 6). In eligible patients with severe injury and profound hypotension, especially with penetrating wounds, scoring systems are not required to predict the need for MT. The attending trauma physician will automatically call for a MT. The clinical staff member will then notify the blood bank to randomize the patient. The person at the blood bank who holds the randomization list will prepare the container using the next subject randomization number on the list and associated blood product assignment, seal the container, and have the container quickly delivered to where the patient is. Platelets may be harmed when placed on ice, therefore, the appropriate amount of platelets will be placed into an opaque container, attached to the transport container. The opening for this container will be sealed as well. The container will be labeled with the subject’s randomization number.

If in the opinion of the attending trauma physician, the patient has improved sufficiently to no longer require a massive transfusion, or if the patient had died and thus no longer meets eligibility criteria (and before the container seal is broken), the container will be quickly returned to the blood bank. If the seal is unbroken, the blood products will be returned to their appropriate storage location, the subject’s randomization number will be returned to the randomization list, and the next eligible subject will receive the same blood product assignment. Thus, a patient is not randomized into the trial until the container seal is broken.

Figure 6. Randomization Process of Predicted Massive Transfusion Patients Upon Admission to ED



Screened ED Patients:
All severely injured patients

Eligible Patients:
All patients who meet PROPPR inclusion/exclusion criteria

Randomized Subjects:
All eligible patients that have the container seal broken

This approach takes into account the rapidly changing physiology of these patients within the first minutes of hospital arrival, minimizes the number of ineligible subjects who will be randomized, followed and included in the intent-to-treat analysis and followed, and minimizes wastage of precious blood products. To help the enrollment and randomization process function smoothly, total quality improvement methodology, such as used in the NINDS t-PA Stroke Trial to reduce time from stroke onset to treatment, will be used in this trial to decrease time from door to randomization and receipt of study blood products.⁷³ In order to ensure the randomization process is conducted in a consistent manner at all sites, one to two blood bank technicians will be funded to assist the blood bank and enable them to meet the requirements of the clinical research team.

Once the seal on the container is broken, the subject is randomized into the assigned treatment group. The subject will continue to receive products as assigned until: (1) the PROPPR transfusion protocol has been discontinued by the trauma attending because hemostasis has been achieved, (2) the subject has died, or (3) the patient or LAR refuses continuation in the trial. While the PROPPR transfusion protocol ratio groups are ongoing, no additional plasma, platelets, or RBC will be allowed. When situation 1 is met, additional individual units of plasma, platelets, or RBCs can be transfused, based on institutional guidelines, local laboratory results, and clinical judgment. All resuscitation fluids and blood products transfused pre-hospital and within 24 hours of admission will be recorded.

In the event two or more subjects enter in the ED in close proximity and are both predicted to be a MT patient, the first patient will be randomized and followed. Notation will be made on the screening log regarding why the additional predicted MT patient was missed. In cases where products for all treatment groups are unavailable for transfusion, the blood bank will indicate the patient will not be randomized into the trial.

5.2.2 Blinding

Although it will be impossible to mask intervention assignment at the bedside in a double- or single-blinded manner, concealing the blood products in a sealed container until the moment of actual transfusion will maintain rigor and prevent bias as much as possible, while maintaining the ability to care for these critically ill subjects. To promote blinding, a “sham” platelet bag will be attached to each container that does not contain platelets. Adherence to the treatment protocol will be carefully monitored and protocol deviations will be identified through the data collected or reported to the Houston Data Coordinating Center (HDCC) by study coordinators. The co-primary outcomes, 24-hour and 30-day mortality, are endpoints making blinding less of a concern in terms of outcome assessment.

5.2.3 Initial Blood Release

Usual, approved procedures for the release of blood products will be followed according to each individual site. Rapid utilization of plasma is made possible by keeping 2-4 units of thawed AB plasma available in the ED at all times, and many trauma centers have implemented this practice. Thawed plasma may be stored in a refrigerator for up to 5 days, and in busy hospitals is rarely wasted. A recent report from leading blood banks describe decreasing plasma waste by 80% after implementing a thawed plasma program.⁷⁴

5.2.4 PROPPR Transfusion Protocol (Figures 6&7)

- a. Upon notification of a PROPPR subject for randomization, the blood bank will prepare the appropriate treatment group products in a container available for delivery to the subject’s bedside. The goal for delivery of the first container is 10 minutes after notification. Total quality improvement methodology will be used to attain this goal.⁷³ This rapid response requires thawed plasma in the blood bank. If six plasma and platelet and RBC units are not immediately available (based on blood type of patient or availability), the blood bank will issue units that are ready and notify appropriate personnel when the remainder of the units that constitute the first container are available. In the event that ABO/type-specific products are unavailable, universal donor products will be used, in accordance with each blood bank’s policy. Based on the requirement for a rapid response, the first container will likely contain uncrossmatched products, including thawed plasma.
- b. After the first container leaves the blood bank, the team will then prepare a second container of the same ratio group. This process will automatically be repeated each time the set of components is issued until the

- attending trauma physician notifies the blood bank that the PROPPR transfusion protocol is no longer needed. This process will ensure that there is no delay in availability of blood products.
- c. The blood containers should follow the subject at all times to prevent duplicate blood orders and unavailability of blood products when needed by the subject. Any subsequent container that was delivered to the subject, but was not needed, will be returned to the blood bank.
 - d. All standard blood bank laboratory documentation will be completed for all blood products.
 - e. It is recognized that randomization and organizing the transfusion container will be additional work for the blood bank personnel. Funds have been set aside for additional blood bank technicians to facilitate this process.

PROPPR Container Cycles

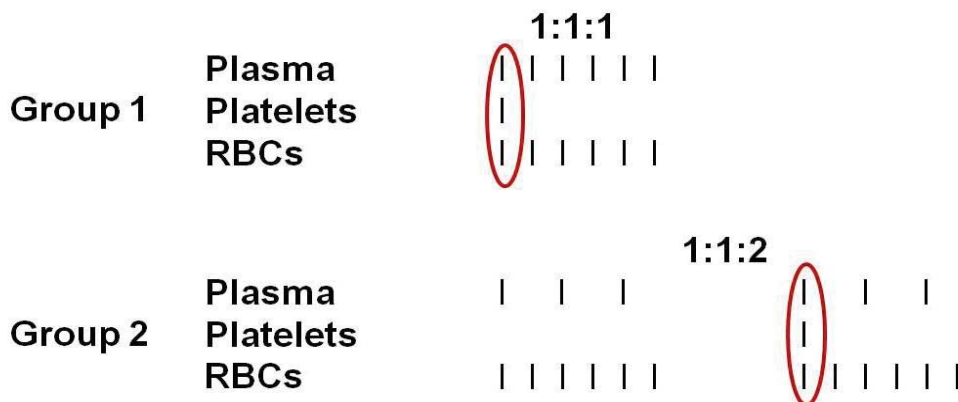


Figure 7 represents one container cycle for each ratio group. Each hash mark represents one unit of blood products. Every 6 units of RBCs represent one container. The red circles indicate when platelets are given. 1 unit of platelets is the equivalent of a pool of 6 units on average. The container cycles repeat until hemostasis is achieved

Products can be serially transfused (platelets, RBC then plasma) or products can be transfused simultaneously.

Group 1 will be randomized to receive the 1:1:1 ratio of plasma:platelets:RBC. For Group 1, the blood bank at each site will prepare the initial container containing 6 units plasma, 1 unit platelets (a pool of 6 units on average) and 6 units RBC; the blood bank will send the initial and all subsequent containers until notified of the discontinuation of the PROPPR transfusion protocol. A laminated card stating, “Transfuse Platelets First” will be attached to the unit of platelets in each container, and subjects are expected to receive one unit of blood product products before the first container arrives (from RBC and plasma available immediately upon ED arrival).

Group 2 will be randomized to receive the 1:1:2 ratio. For Group 2, the blood bank will prepare the initial container containing 3 units plasma, 0 units platelets and 6 units RBC, a second container containing 3 units plasma, 1 unit platelets (a pool of 6 units on average) and 6 units RBC, and the blood bank will send this sequence of 2 containers repeatedly, until notified of the discontinuation of the PROPPR transfusion protocol (Table 4). The laminated card stating, “Transfuse Platelets First” will be attached to the unit of platelets in every 2nd container of the sequence, and subjects are expected to receive the 1st unit of platelets with the 7th unit RBC.

Plasma	As soon as the subject is randomized for a massive transfusion Group 1 = For every 6 plasma, give 6 RBC (1:1 ratio) Group 2 = For every 3 plasma, give 6 RBC (1:2 ratio)
Platelets	As soon as the subject is randomized for a massive transfusion Group 1 = For every container, give 1 dose of platelets (1:1 ratio) Group 2 = For every other container, give 1 dose of platelets (1:2 ratio) *1 platelet dose equal to either 6 random-donor units or 1 apheresis unit

Crystalloid and artificial colloid fluid use is highly variable in clinical practice, largely because Level 1 data are not available to guide their use. Therefore, their use in PROPPR, consistent with a pragmatic trial, will not be standardized or randomized, but their use will be recorded throughout the trial and data collection period to allow for ancillary analyses taking this information into account. The use of pharmacological adjuncts (rFVIIa, amicar, tranexamic acid, PCCs, fibrinogen concentrates, etc) and cryoprecipitate is highly variable in clinical practice, also largely because Level I data are not available to guide their use. Therefore, their use in PROPPR will not be standardized or randomized, but their use will be recorded throughout the trial and data collection period to allow for ancillary analyses taking this information into account. Stratification by site, in the randomization and subsequent analysis with site as a covariate as described in the statistical analysis plan will be used to provide some adjustment for site-related variability in use of the above described products. In ancillary analyses we will adjust for pre-randomization treatments.

Subjects who have re-bleeding events or require MT *after* the PROPPR transfusion protocol has been discontinued will be managed per site-specific, laboratory-directed, or institutional guidelines. These products will be recorded in detail until hemostasis is achieved. Re-bleeding requiring arteriogram embolization or unscheduled return to the OR after the PROPPR transfusion protocol is discontinued will be recorded as an adverse event.

Any deviation from these transfusion guidelines will be recorded as such.

5.2.5 Clinical Data Collection

Direct bedside data collection will begin at time of the highest level trauma subject arrives in the ED and will continue until 1) it has been determined that the subject is not eligible for this trial, 2) the subject or LAR refuses continuation in the trial, 3) the subject has achieved hemostasis 4) the subject has expired or 5) 24 hours have elapsed, whichever comes first. Until deemed ineligible, data from subjects will be collected and reviewed for screening purposes. Data on eligibility will be submitted to the HDCC to allow a description of screened versus enrolled subjects.

At screening, in addition to collecting ABC scores, we will collect data for the Trauma Associated Severe Hemorrhage (TASH) Score⁶⁴ to allow later comparisons between the two scales (Table 2). The TASH score requires the hemoglobin results and is thus not readily available before randomization needs to occur. By collecting information on both scoring systems in the same patient population, this will allow for a direct comparison between the two methods. Direct bedside data collection will continue on all randomized subjects until 1) active resuscitation has ended, or 2) 24 hours has elapsed. Data to be collected during direct observation will include all blood product transfusion information including the start time of each unit, uncrossmatched vs. crossmatched information, leukoreduced vs. nonleukoreduced products, life saving interventions (LSI), all fluids and blood products, initial clinical laboratory results, surgical procedures and complications. For the purposes of this trial, all fluids and blood products given prior to the randomization process will be documented in the study data collection forms as pre-randomization fluids/products. All fluids and blood products given after the randomized ratios are terminated and prior to 24 hours will be documented as post-randomization fluids/products. The Data Collection Flowsheet (Appendix 3) shows a list of type of data to be collected as well as the frequency of the data collection.

Data will be collected on a daily basis for 30 days of hospitalization or until discharge/death on all subjects who have consented to continue in the trial. Information collected will include demographics, injury, blood product transfusions (including age of products), damage control and other surgical interventions, vital signs, routine daily lab results, complications such as MOF, ALI, TRALI, AKI, ARDS, transfusion-related hyperkalemia and/or hypocalcaemia, all thromboembolic complications (*i.e.*, DVT, PE, MI, stroke), sepsis, abdominal complications, compartment syndromes, and infections. Routine clinical laboratory tests will vary between sites. Common lab tests might include CBC with platelets, electrolyte panel, coagulation tests (PT/PTT/INR), TEGs, fibrinogen, blood type, arterial or venous blood gas, and urinalysis. Available lab results will be recorded. In addition to the information collected daily, the final/discharge diagnosis, discharge destination (*i.e.* home, long term acute care hospice, skilled facility, death), and discharge extended Glasgow outcome scale (GOSE) will be obtained at the time the subject is discharged from the hospital.

Data will be collected using standardized case report forms. After data collection, the data will be entered into to a secure, web-based data system designed for this trial. The web-based program will provide the flexibility of entering data from multiple locations and centralizes the data management process. To ensure security, each user will be assigned a username and password and this username, date and time of each login will be recorded in a login history file to ensure a record is maintained of each access to the system. This information will also be recorded in the change history audit logs. The data entered for the PROPPR trial will be maintained in a secure database at the HDCC.

If discharge occurs before hospital day 30 and the subject is discharged to a hospice, nursing home or other healthcare provider, research staff will contact the facility to ascertain the subject's vital status. If the subject was discharged to his/her usual residence before day 30, the research staff will contact the subject or their family/legally authorized representative (LAR). If vital status remains unknown the clinical site will request periodic searches for the subject's social security number in the Social Security Master Death Index, the respective State Health Department's vital statistics/mortality database, and the mortality databases of a credit reporting agency, e.g., Experian. For subjects not reported as deceased by these sources by day 30 following ED admission, batch searches of the mortality databases will continue every quarter until trial close-out. Date (and cause of death when available) for out-of-hospital deaths will be documented; however, underlying and contributing causes of death may not be available from these sources. A subject will be considered to be alive if they can be contacted or are reported alive by a healthcare facility, LAR, or other administrative data source at or after the 30-days from admission. Selected elements from the medical records (OR notes, patient history, morbidity and mortality notes, etc.) will be collected in a HIPPA compliant manner and presented to a death adjudication committee for all in-hospital deaths for subjects enrolled in this study. For subjects discharged to another facility, the clinical research staff should complete an authorization form to release protected health information (PHI) and obtain signatures from the subject or LAR prior to discharge. A copy of the signed authorization form and study consent will be provided to the facility for release of PHI. Clinical sites will follow local and state HIPPA guidelines for release of PHI for research.

5.2.6 Research Laboratory Data Collection

Throughout this trial, we will collect blood samples from severely injured subjects upon arrival and sequentially for 72 hours. Plasma will be assayed for coagulation mediators, complement proteins and inflammatory mediators. Functional measures of coagulation and platelet function will be assessed on fresh whole blood. **These samples are for research only and will not be available to inform clinical decisions.** These data will be utilized to develop a systems level characterization of coagulopathy in seriously injured subjects. By comparing functional coagulation and plasma protein measurements with physiologic measures as well as outcome data we will obtain for the first time a complete picture of the timing, severity and causes for early coagulopathy, later inflammation, infection and organ failure after severe trauma and shock.

Blood samples will be collected upon arrival in the ED (time 0) for all screened patients and at 2, 4, 6, 12, 24, 48, and 72 hours (or discharge from hospital – whichever occurs first) for all randomized subjects. The eight time points were selected to provide a broad temporal survey of hemostasis after injury, which is weighted toward early sampling to fully characterize the early phase of TIC. Later sampling (48 and 72 hours) will allow us to characterize the transition from a hypocoagulable to a hypercoaguable state and to fully examine the effect on resuscitation and outcome on coagulation and inflammation after injury and shock. All attempts will be made to obtain study samples at the designated time intervals. All research samples must be collected within +/- 30 minutes. In the event that samples cannot be collected in this time frame, documentation will be noted on the data collection forms. Only the 0 hour sample will be collected and processed for those subjects who are screened, determined to be eligible (at the 0 hour blood draw) but are not randomized. The 0 hour samples collected on the screened patients will be processed and stored for future analysis. The analysis will include coagulation mediators, complement proteins and inflammatory mediators similar to the serial samples collected on the enrolled subjects. The analysis will not include any genetic analysis. These 0 hour samples will also be identified by a study code number. A modified consent process will be conducted in this group of subjects. The method of consent (i.e. waiver of consent, waiver of documentation, or full consent) will be dependent on the site's local IRB policies and regulations.

Up to 23 ml of blood will be collected in addition to the clinical sampling at each time point into multiple different tubes. The blood volumes collected for research purposes are below IRB recommended 3-5% total blood volume within 24 hours. The sampling tubes will include 1) 1 citrate for for whole blood analyses, 2) 2 citrate for plasma, 3) Blood Collection Tubes 1 citrate plus a protease inhibitor for special assays and 4) 1 blood collection tube with EDTA and cell preservative for flow cytometry analyses. Samples will be collected by the clinical person responsible for implementing physician orders for laboratory testing. The clinical research staff will then be responsible for the processing and shipping at each site. All samples drawn for research purposes will be identified by the study number, the site identification number and date/time of collection. No personal identifying information will be included on the samples processed for research purposes. Assays that require immediate processing (TEG, Multiplate) will be performed at each study site by personnel trained at the core lab sites or send by an overnight courier to the central flow cytometry lab (UTHealth) for analyses. Other samples will be spun, aliquotted, frozen at -80°C, bar coded, and batch shipped by the clinical research staff to the appropriate labs (UTHealth, UCSF, and Vermont) for measurement. The samples will be disposed per appropriate biohazard guidelines.

The research laboratory data will be entered into a web based relational database created for the lab measurement component of this trial.

6. STUDY OUTCOME MEASURES

6.1 Primary Clinical Outcomes

Absolute percent (rather than relative percent) group difference in 24-hour and 30-day mortality (Separate co-primary outcomes)

Rationale for the Co-Primary Outcomes (24-hour and 30-day mortality)

Despite a consensus conference on outcomes for blood product studies, disagreement remains; thus, we chose co-primary outcomes. The two outcomes will be considered as separate study questions and both outcomes will be reported in the initial report on the PROPPR trial.

Rationale for 24-Hour Mortality: In PROMMTT, the recently completed, ten-center observational study, 297 observed patients received a massive transfusion (MT). Of the 297 observed MT patients, 117 (39%) died in-hospital within 30 days of ED admission. Of those 117 in-hospital MT deaths, 83 (71%) occurred within 24 hours of ED admission across all blood ratio groups combined. The potential benefit of transfusing optimum blood product ratios to severely injured trauma patients soon after ED admission, and reducing or preventing coagulopathy altogether will be most easily detectable after ED admission within the brief 24 hour span of highest mortality risk. Deaths among trauma patients within the first 24 hours are more often due to massive bleeding that is amenable to rapid resuscitation with an appropriate transfusion protocol than deaths occurring later in the course of an extended 30-day hospitalization that may be unrelated to the transfusion protocol.⁷⁵

Recent meetings on optimal endpoints in randomized trauma studies (February 2008, Dallas, TX & September 2009, Houston, TX) included multidisciplinary injury experts from academia, Department of Defense (DoD), industry, FDA, and academic societies. Furthermore, the PROPPR trial design was reviewed at the recently held State of the Science Transfusion meeting jointly sponsored by NHLBI and DoD.⁷⁶ The conclusion from these three meetings was that the primary outcome of future trauma trials, including PROPPR, should be 24-hour mortality reflecting the changing epidemiology of trauma.⁷⁷ Based on the time to death in recent military and civilian studies, and agreement from experts in the field, 24-hour mortality will be the co-primary outcome of the PROPPR trial.

Rationale for 30-Day Mortality: In PROMMTT, of the 117 in-hospital MT deaths, 98.7% of these deaths occurred within 30-days. PROPPR will use 30-day mortality as a co-primary outcome as the latter is a traditional trauma trial standard for evaluating delayed complications and safety of trial interventions, the benefit is durable, the outcome is important to scientists and patients and provides evidence to support the most efficient use of the nation's blood supply. All PROPPR subjects will be tracked for vital statistics for a full 30 days, whether or not they have left the hospital.

Both the 30-day and 24-hour mortality outcomes will be reported on all publications and reports that arise from the data collected in this trial.

6.2 Ancillary Clinical Outcomes

Time to hemostasis hospital-free days, ventilator-free days, ICU-free days within the first 30 days or hospital discharge, whichever comes first); incidence of major surgical procedures (e.g., thoracotomy, craniotomy, laparotomy, major amputation), complications (transfusion-related acute lung injury, acute lung injury, acute kidney infection, multiple organ failure, acute respiratory distress syndrome, sepsis, abdominal complications, infections, thromboembolic complications, rebleeding requiring an arteriogram or unscheduled return to the OR after PROPPR transfusion protocol discontinuation, transfusion-related hyperkalemia and/or hypocalcaemia during hospitalization), the number and type of blood products used from randomization until hemostasis is achieved, the number and type of blood products used after hemostasis is achieved to 24 hours post-admission and functional status at hospital discharge or 30 days, whichever comes first, as measured by discharge destination and GOSE.

Rationale for Ancillary Clinical Outcomes

These comparisons will allow assessment of other possible benefits and complications related to treatment (ratio) group. Also these data will be important in developing the models describe in 6.3 below.

6.3 Primary Research Laboratory Outcomes

Models will be developed to identify drivers and sequelae of TIC and inflammation, and to characterize the natural history of the coagulation milieu. The principal modeling approach will be reverse-engineering of the biological networks from the research laboratory data augmented by the existing expert knowledge. Both baseline (Laboratory Aim 1) and dynamic (Laboratory Aim 2) models will be developed. When interpreting the resulting models (Laboratory Aim 3), special emphasis will be put on the primary and ancillary clinical outcome measures for laboratory analyses, including mortality, time to hemostasis, incidence of coagulation abnormalities, total blood product transfusions, incidence of organ injury (i.e., acute lung injury and acute renal failure) and ventilator associated pneumonia, 30-day mortality, ventilator-free, ICU-free and hospital-free days and incidence of nosocomial infections.

7. PROJECTED ENROLLMENT

7.1 Availability of Study Population for Phase III trial

Based on unpublished data from the retrospective study and PROMMTT, the total number of subjects actually receiving MTs during the Vanguard stage data collection period (6 months) for at least 4 centers is expected to be 80 (an average of 40 MT subjects per site/per 6 months). Based on an analysis of the retrospective data using the ABC prediction algorithm,⁶¹ the Vanguard stage is planned to randomize at least 60 subjects predicted to receive MTs over the 6 month data collection period. Only 50 of the 60 (80%) subjects randomized are expected to actually receive a MT within 24 hours of admission.

7.2 Timeline for the Phase III trial

As a conservative estimate based upon the data from our site selection surveys, we expect to enroll at least 2.7 patients per site per month. Using 12 sites that initiate enrollment at a staggered rate as they complete their public notification community consultations, we project that we will enroll the required 580 patients within 24 months.

PROPPR Timeline <i>Based on ROC fiscal year (January 1 – December 31) and budget.</i>					
Activities	Period 1 10/10-12/10	Period 2 1/11-12/11	Period 3 1/12-12/12	Period 4 1/13-12/13	Period 5 1/14-9-14
Planning	✓	✓			
Site Training		✓	✓		
IRB approval/Community Consultation		✓	✓		
Enrollment			✓	✓	✓
Follow-up to 30 days			✓	✓	✓
Trial Monitoring			✓	✓	✓
On-going Data Analysis			✓	✓	✓
Trial Close-out					✓
Sample Collection/Lab Analysis			✓	✓	✓

7.3 Sample Size for the Phase III trial

At the DSMB meeting, April 25, 2013, prior to any review of unblinded data the blinded members of the DSMB reviewed a prespecified adaptive analysis conducted by blinded ROC biostatisticians and recommended that the sample size be increased from 580 to 680 to maintain a power of >85%. NHLBI approved this modification.

Primary Outcomes:

24-hour mortality

For sample size estimation for the 24-hour mortality outcome, we chose a difference of 10% or greater increase in mortality from 11% at 24 hours to 21% when comparing 1:1:1 to 1:1:2. The trial is powered at 90%, with a two-sided alpha level of 0.05, adjusted for interim analyses to 0.044.⁷⁸ The required sample size is 580 subjects including subjects from the Vanguard stage.⁷⁹⁻⁸³ The 1:1:1 group mortality of 11% was selected based on a subset of published data available from a retrospective study showing 115 predicted MT patients had received 1:1:1 ratios and experienced an 11% mortality at 24 hours.⁴⁹ In contrast, 24 hour mortality was 41% in the 27 predicted MT patients receiving 1:1:2 ratios. We considered a between group difference in 24 hour mortality of 10% or greater to be clinically meaningful and of sufficient magnitude to influence clinical practice. Adjusting for site generally should increase power unless there is a lack of homogeneity of treatment effects across sites.

PROMMTT Effect Size Estimates for PROPPR

PROMMTT was a prospective observational study. To reduce survival bias as much as possible while allowing for individual variation in patients' cumulative blood product ratios over the 24 hour period following Emergency Department (ED) admission, we used Cox proportional hazards modeling with time-dependent covariates for the ratios (i.e., plasma:RBC and platelet:RBC ratios were treated separately). Cumulative ratios were re-computed for every half-hour interval through hour 6, and the cumulative ratios at hour 6 was re-applied to the last interval, >6-24 hours following ED admission. Vital status was recorded and survival time was computed for each patient over all the time intervals. Our analyses avoided subgroup analyses using the standard definition of massive transfusion (MT) due to concerns that the MT subgroup would 1) exclude many of the eligible and hemorrhaging patients expected to be enrolled into PROPPR (i.e., those who will die or receive interventions that control bleeding with no chance for a 10th RBC transfusion within 24 hours of ED admission), and 2) contribute to survival bias. We developed, *a priori*, an alternate subgroup definition free of survival bias to encompass the population of substantially bleeding (SB) trauma patients likely to be enrolled in PROPPR. The subgroup of SB patients was defined as follows: receipt of the first RBC transfusion within 2 hours of ED admission, either death or continuing RBC transfusions < 2 hours apart, and within 4 hours of ED admission, at least 5 RBC transfusions or death following 1-4 transfusions.

The hazard ratio (HR) estimates for the association of 24 hour mortality with plasma and platelet:RBC transfusion ratios in the SB subgroup of PROMMTT patients suggest an overall 0.60 relative risk estimate. This was computed from a 0.78 HR for the 1:1 vs 1:2 plasma:RBC ratios X a 0.77 HR for the 1:1 vs 1:2 platelet:RBC ratios = 0.6006, the HR for the joint association between 1:1:1 vs 1:1:2 plasma:platelet:RBC ratios and mortality within 24 hours of admission to the ED. These HRs were adjusted for potential confounding by center and patient characteristics including the number of units of RBCs received, age and Glasgow Coma scores.

Results from PROMMTT (Table 5) may not directly predict achievable effect sizes for the randomized PROPPR trial because PROPPR is testing blood product ratios that are fixed from the point of randomization, not varying over time. Nevertheless, a range of expected effect sizes has been estimated in the table below by applying adjusted⁸⁴ relative risk estimates (from the HR estimates) to the 24 hour mortality rate observed in the subgroup of PROMMTT patients with substantial bleeding, under 3 different assumptions. The adjustment provides more conservative estimates (relative risk estimates closer to the null of 1.0) than the HRs and a reasonable range of possible effect sizes for the 1:1:1 vs 1:1:2 transfusion ratio comparisons, to the extent that PROPPR can be expected to map onto PROMMTT.

Table 5. Estimated PROPPR 24 Hour Mortality Rates and Effect Size Estimates Applying Adjusted⁸⁴ Relative Risk Estimates from PROMMTT Hazard Ratios for the Subgroup of Substantially Bleeding Patients

Assumptions for PROMMTT Mortality Rate	1:1:2 Group	1:1:1 Group	Absolute Difference (Effect Size)	Estimated Statistical Power*
If PROMMTT rate applies to PROPPR 1:1:2 group Adjusted RR=0.6438	29.2%	18.8%	10.4%	82.2%
If combined groups sum to the PROMMTT rate, assuming a 50:50 split Adjusted RR = 0.6576	35.2%	23.1%	12.1%	88.5%
If PROMMTT rate applies to 1:1:1 group Adjusted RR=0.6791	43.0%	29.2%	13.8%	92.8%

*Assuming a 0.044 alpha level, two-sided Mantel Haenszel test, 580 total patients

30-day mortality

For the 30-day mortality, a 12% or greater difference in mortality from 23% in the 1:1:1 group is detectable given the same sample size (580), with 88% power, and a 10% or greater difference is detectable with 74% power assuming a 2-sided alpha of 0.044. The primary group of interest, 1:1:1, mortality was based on additional unpublished retrospective data as described for the primary outcome. Subjects in PROMMTT were followed only to hospital discharge, not 30 days. Adjusting for site should generally increase power unless there is a lack of homogeneity of treatment effects across sites.

Ancillary Clinical Outcomes

We will compare treatment groups on a variety of ancillary outcomes as listed in 6.2. For binary outcomes we can detect an absolute difference of 12% in outcomes from 50% (worst case scenario) with power of 80%, 2-sided alpha of 0.05, given a sample size of 290 per group. If some outcomes are rare as we expect, we can detect a difference from 0.03 of 0.029 with same power and alpha. For continuous outcomes, we can detect an effect size of as small as 0.233, a very small effect as defined by Cohen⁸⁵ for behavioral sciences at same alpha and power.

Laboratory Modeling

Because no previous prospective and comprehensive characterization of coagulopathy and inflammation after trauma currently exists, and the definitions of phenotypes of primary interest, while suggested by preliminary data (elevated INR, activation of anticoagulant pathways, dilution, hypothermia, etc.) are not codified, we expect to use the entire cohort of 580 for the systems biology (exploratory) analyses. This is predominantly a multivariate modeling approach

that is aimed at hypothesis generation rather than hypothesis testing. Due to the non-parametric nature of the corresponding analysis methodology (e.g., dynamic Bayesian networks, ensemble classification algorithms), it would be impossible to carry out a straightforward power analysis. Given the exploratory nature of this aim, we cannot determine the exact dimensionality and size of the models that may emerge. However, if we limit ourselves to the immediate Markov neighborhoods of the primary and secondary laboratory research outcome variables (i.e., perform automated variable selection), the dimensionality of the resulting sub-networks should be favorable for the purposes of model validation (using resampling techniques such as bootstrapping) and subsequent predictive modeling (Laboratory Aim 3).

Once phenotypes and relationships are identified, we will use more traditional statistical analyses to assess impact of the phenotypes and interactions among the phenotypes on outcomes. Based on work by Harrell with 580 subjects, depending on the final model chosen, we can build linear regression models that include up to 58 variables where outcome is continuous (amount of blood products, etc.), and logistic regression models that include up to six variables where the outcome is binary (mortality, MOF, etc).⁸⁶⁻⁸⁸ If the number of variables exceeds the number that can be included in a linear or logistic model we will prescreen using a p value of <0.25 to select the subset to include in the model. We may need to conduct separate analyses of the selected phenotypes depending on the number of baseline covariates of interest. This serious limitation of traditional statistical approaches emphasizes the need for the initial more complex approaches to understanding coagulopathy and inflammation as described in the analysis below.

8. ANALYSIS PLAN

8.1 Vanguard Stage

Assessment of Trial Feasibility

Once at least four sites are eligible to enroll subjects we will begin a Vanguard Phase to assess sites' abilities to recruit subjects and comply with the protocol. These early data will be used to assess trial procedures and feasibility. We will descriptively (graphically) compare the hypothesized timeline for recruitment to the observed time line for recruitment and to the NHLBI target range (ref). We will also collect the following site performance metrics of protocol compliance:

- Protocol deviations (both self-reported and study monitor evaluation)
- Time to blood product container delivery
- Time to complete enrollment
- Missed/unable to screen subjects
- Volume of data queries
- Evaluation of source documents and CRFs (study monitor site reports)
- Site response time (timely data entry, submission of regulatory documents)
- Adverse events management
- Site lab adherence to lab sampling process (processing/shipping errors)

We will complete analyses of data quality including missing data, error patterns, protocol violations, etc. to determine if modifications in the protocol or data collection procedures or trial manual of operations are needed or to determine if the protocol itself can be followed. The DSMB will review blinded data on recruitment, protocol deviations, laboratory data, data quality and adherence to study procedures, including a count of the number of instances when patients were not randomized, based on physician judgment in the presence of a positive ABC score (physician override). At the end of the Vanguard phase, the DSMB will develop recommendations for NHLBI to continue with the trial without modification, continue with modification including possible termination of a site or sites, or to discontinue the trial based on the inability to follow the protocol. The DSMB will determine if the Vanguard data can be included in final trial data set. This DSMB review will be in addition to the ongoing DSMB safety review completed each time the DSMB meets as described in Section 8.2.5 below. Regular blinded monitoring and quarterly reports will be submitted to the HCCC and Clinical Sites to maintain a constant focus on data quality.

8.2 Trial Analysis

8.2.1 Primary Clinical Outcomes

Analyses for each of the separate Phase III trial co-primary outcomes (24-hour and 30-day mortality) will be intent-to-treat. We will include all subjects in all primary analyses in the Phase III trial as randomized. We will compute mortality at both 24 hours and 30 days. For subjects who have not been reported as deceased by day 30 following ED admission from any of the sources queried we will use multiple imputation under the assumption that the missing data are not missing at random. The process for determining whether or not a subject is deceased at 30 days is described in detail in section 5.2.5. We will make extensive efforts to capture all data and anticipate less than a 10% of the subjects will be missing vital statistics at the 30 day co-primary outcome. The DSMB will be informed of the amount of missingness observed, will carefully monitor the amount of loss to follow-up throughout the trial and will call for further corrective actions or changes to the protocol in an effort to keep the value less than 10%.

We will analyze each of the 24-hour and 30-day mortality endpoints as a fixed point in time using a two-sided Mantel-Haenszel (M-H) test taking site, the stratifying variable, into account. This approach has more power than the survival analysis described below given the potential for crossing hazard functions.⁸⁹ We will also test homogeneity of the odds ratios across sites using the Breslow-Day test. The M-H test is robust to lack of homogeneity of odds ratio although power would be reduced. We will compute 95% confidence intervals on mortality by treatment group at 24 hours and 30 days. We will also conduct a sensitivity analysis of 30 day mortality to assess the effect of imputation as alive on the treatment group comparisons and confidence limits for the 30 day outcome.

To provide further insight we will compute 30-day Kaplan-Meier survival curves.⁹⁰ We will use Cox proportional hazards regression to take site (as a random effect) into account.^{91, 92} If the proportional hazards assumption is violated we will include a time treatment interaction in the model and choose the appropriate approach.⁹³ As an additional analysis, we will use the same Cox proportional hazards approach to adjust for baseline covariates such as age, gender, admission blood pressure and GCS, type and extent of injury, amount of pre-randomization blood products and other treatments received, time to randomization. Since site is a stratifying variable site will be included as a random effect. We will do pre-screening of covariates other than site at the 0.20 level before fitting the final model if our sample size is not sufficient to include all covariates in the model. We would follow the approach above to test for and take crossing hazards into account if applicable. As an additional exploratory analysis we will compare 30-day survival in the two groups adjusting for the covariates listed above and any additional baseline covariates that are imbalanced between treatment groups ($p < 0.10$) using the same screening approach to decrease the number of covariates included in the model, if necessary.

8.2.2 Analysis of Ancillary Clinical Outcomes

Unless there is sufficient power (predetermined before the analysis is begun) the approach to ancillary analysis will generally be the calculation of confidence limits on intervention group differences or model parameters rather than formal tests of significance at a specified critical level as the trial will not have high power to detect difference in all of these outcomes. However, these comparisons will add to the knowledge of the benefits and risks of the two interventions.

8.2.3 Analysis of Research Laboratory Data

A systems level framework is necessary to produce predictive models capable of diagnosing coagulopathic phenotypes and assessing the effectiveness of hemostatic resuscitation measures. Our goal is to develop an *in silico* model of coagulation to better understand the perturbations of this system after trauma. To accomplish this goal we will both expand our existing coagulation network model, and construct new network models of Protein C, complement, and coagulation in general from our PROMMTT and legacy data, as well as data from the measurements and clinical data in the PROPPR trial. Specifically, we will scrutinize the sub-networks representing structure/ function relationships of protein C and coagulation, and their interactions after injury.

Our analysis is divided into two overlapping modeling goals: A) building a network and functional model of coagulation (Laboratory Aims 1 and 2) and B) predictive modeling (Laboratory Aim 3), using predominantly machine learning methodology. Each is distinct but complimentary and serves to inform the other model (for

example, the latter would provide additional insights on the variable selection for the former). Ultimately our descriptive and predictive modeling efforts will involve the following steps and methods:

1. *Network Expansion and Construction.* We will begin by expanding our existing preliminary network model of coagulation to include all links to all known nodes up to 5 degrees away (i.e., in the extended Markov neighborhood) from protein C and proteins included in the classical coagulation cascade and complement system. Additional network proteins will be added to a network spreadsheet and imported into Matlab™ Pajek 1.8 (The Mathworks, Inc., Natick, MA) and Cytoscape 2.0 (Cytoscape Consortium, San Diego, CA) bioinformatic network software for visualization and analysis. This is a methodologically straightforward step that will lead to the creation of baseline networks (Laboratory Aim 1).

2. *Network Analysis.* Topological calculations of degree, degree exponent γ (where $P(k) \sim k^{-\gamma}$, path length, cluster coefficient of each node ($C_i = 2n_i/k(k-1)$), average cluster coefficient ($C(k) \sim k^{-1}$) and edge-betweenness (cluster decomposition) will be calculated with Cytoscape 2.0 and Guess.5 and Matlab™. Network topology will be mapped onto outcomes including coagulopathy, and infection. In this manner we will test the relation between perturbations in topology with the outcome of coagulopathy and infection. Again, this is a computationally straightforward step that will result in developing reference networks relevant to the Laboratory Aims 1 and 2.

3. *Dynamic and Data-driven Model(s) Construction.* We will next construct dynamic network (ordinary differential equation and dynamic Bayesian networks - based) models of the central coagulation system and its relationship to inflammation in general.⁹⁴⁻⁹⁶ These will serve as starting points (topology priors) for accomplishing Laboratory Aim 2 --- we will follow up by reverse-engineering (using our proprietary Bayesian network modeling software⁹⁷) data-driven network models from a subset of data from this project, the currently ongoing PROMMTT study, and protein C activation data from both steady-state (non-injured) and perturbed (injured) conditions. This cumulative model-refining process will continue as new experimental data are collected and new hypotheses are developed.

4. *Variable Importance Analysis.* In a parallel line of research to the biological network modeling above we will be creating statistical and computer science – based models (classifiers) to support treatment decision for optimal outcome given clinical observations. Due to the large number of clinical, physiological, and molecular variables we are proposing to collect, a necessary first step is determining which of these, by themselves or in concert, are most important to outcome, a task known as “variable selection”.^{98, 99} We will pursue various variable selection strategies that take into account variable interactions, including the Bayesian network Markov neighborhood analysis, ensemble decision tree classifiers and other (mostly machine learning) methods.^{100, 101} This analysis is directly relevant to the Laboratory Aim 3, but will also retrospectively influence our network modeling activities (Laboratory Aims 1 and 2)

5. *Clinical Prediction Analysis.* The next goal is to define statistical or computer science-based predictive models that can be used to identify subjects at high risk of a clinical outcome¹⁰². We will use machine learning techniques (ensemble decision tree classifiers, support vector machine classifiers and possibly naïve Bayesian classifiers) to find predictors with high specificity and sensitivity. From our experience, as well as from the recent literature, we expect these techniques to perform better (in terms of generalization classification accuracy, robustness and scalability) than the more traditional regression methods. We will finalize our analyses by using a Superlearning approach.¹⁰³ This approach expands the typical machine learning classification algorithms to construct final predictive models that are combinations of several machine learning classifiers, thus avoiding possible method-related biases, and guaranteeing substantially improved robustness. In addition to the complex systems analyses that would be conducted, we will also use more traditional statistical models for Laboratory Aim 3, incorporating phenotypes and interactions identified in Analysis #4 above. Linear models would be used for Laboratory Aim 3 to test the association between identified phenotypes and outcomes that are continuous (amount of blood products, etc) and logistic models to test associations with categorical outcomes (MOF, etc). These analyses will take appropriate baseline covariates and treatment group into account to assess the effect of the phenotypes beyond the effect of these covariates. This will accomplish Laboratory Aim 3.

6. *Model Validation.* We will use statistical resampling approaches (bootstrapping, dataset subdivision and cross-validation) to provide model validation. Taken together, our data and models will result in the first comprehensive natural history description of acute traumatic coagulopathy.

Our modeling goals are ambitious but both types of models lead to predictions that will be clinically tested. The results will themselves inform the second generation of models (thus going back from the Laboratory Aim 3 to Aims 1 and 2). This is one of the key strengths of this program—the tight coupling of clinician to analyst, sometimes in the same person. The project itself will adhere to a tight management scheme in which the teams meet around the continuously updated models to discuss their completeness and their descriptive and predictive accuracy. The models will, therefore, also serve as precise communication tools for the project scientists. Ultimately these models will identify a group of mediators that define coagulopathic phenotypes after trauma, and can be used to guide personalized medical and surgical treatment for wounded patients.

8.2.4 Missing Data

We expect no missing data for 24-hour mortality. For 30-day mortality, given the transient nature of many of the subjects, extensive efforts will be made to ascertain vital status (Data Collection section 5.2.5 above). Batch searches of the mortality databases will continue every quarter for subjects with unknown status, until trial closeout. For interim and final analyses, of subjects who have not been reported as alive or deceased by day 30 following ED admission from any of these sources we will use multiple imputation for the final value assuming missing not at random (MNAR). As sensitivity analyses we will report the data with and without imputation. We also report a secondary analysis consistent with that used in other trauma studies counting those missing as alive on day 30.

8.2.5 Monitoring for Effectiveness & Safety

Adaptive Design: At the time of the first interim analysis but before presentation of the interim analysis to the DSMB per FDA guidelines for adaptive designs (<http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm201790.pdf>) a blinded biostatistician from the ROC DCC will report on the power for the trial based on the observed 24 hour mortality rate in the 1:1:1 group (the comparator arm) only. If the mortality rate in the 1:1:1 arm is less than 11%, there is no need to consider an adjustment to the sample size as power to detect a 10% difference from 1:1:2 would be increased. If the mortality rate in the comparator arm is greater than 11% we will ask the DSMB to consider increasing the sample size to an amount to be determined by the difference between the comparator group rate and a clinically meaningful difference of 10%, two-sided $\alpha = 0.05$, power = 90%. The DSMB would not be provided and would not consider the observed difference between the two treatment arms at this time. The DSMB will then make a recommendation to NHLBI to maintain the sample size as planned, or to increase the sample size a specified amount based on the observed mortality in the 1:1:1 group. Final determination of the amount of the increase in sample size will be made by NHLBI based on availability of funds and based on recruitment progress to date, protocol adherence, and data quality but without any knowledge of the observed treatment group differences. Once this recommendation is made, the DSMB would then proceed with its regular meeting reviewing the interim analysis and safety analysis. This later discussion could, of course, change the recommendation if a decision was made to recommend trial termination for reasons of safety. No further consideration of a sample size increase would be made once the DSMB has seen the interim analysis.

Interim analyses for Effectiveness: There will be three formal effectiveness analyses. The two interim analyses for the DSMB will occur after the first 1/3 and 2/3 the projected 24-hour or 30-day mortality events are observed (whichever reaches its projected 1/3 and 2/3 first). The two co-primary outcomes will be separately monitored using a two-sided O'Brien-Fleming boundary with Lan-DeMets alpha spending function based on events for each of the two comparisons.³² The boundary is suggested as a guideline for the DSMB, and could be modified by the DSMB prior to the start of the trial. Other information could influence their decision to recommend continuing (or stopping) the trial in the face of a clear difference in either direction between treatment arms.

If the trial stops early because of interim analysis, we will report the adjusted p-values by using the stage wise ordering approach to account for the fact that an unadjusted p-value will tend to overstate the evidence against the null hypothesis in sequential trials.¹⁰⁴

We will not test for lack of a difference in effectiveness using a stochastic curtailment approach since the null hypothesis is also of clinical interest for both co-primary outcomes. If we cannot detect a difference between groups, we would want to use the full sample size to produce narrow and informative confidence intervals.

Safety analyses

At each DSMB meeting after the start of the trial we will present safety data by treatment group (labeled as A, B in the same manner proposed by the 2006 FDA Guidance for Clinical Trial Sponsors on the Establishment and Operation of Clinical Trial Data Monitoring Committees, unless the DSMB requires complete unblinding). This would include, but is not limited to, total counts of all serious adverse events, both unanticipated and anticipated, including a description of the event itself. Additional safety analyses will be developed as requested by the DSMB. We will report overall mortality but will only report mortality by treatment group (or A,B) at the formal interim analyses as these are the primary outcomes. After completion of the Vanguard phase, we will also continue to present process monitoring data to the DSMB (recruitment, data quality, etc.).

9. DATA MANAGEMENT

The subjects will be identified by a study number only. All hard copy source documentation will be kept in a secured, locked cabinet in the site's research coordinator's office. All study documents will be maintained in a secure location for two years following study completion unless superceded by participating site's requirements. The electronic data will be entered and maintained on a password protected web-based program designed for this trial.

The data entered for the PROPPR trial will be maintained at the HDCC in a relational database cluster. The cluster is composed of multiple servers, which provide redundant access to the data in the event of a hardware failure to one of the servers. This cluster is maintained behind a firewall, which is not accessible from the internet without a secure network connection. The data will be backed up nightly and copies of the data will be routinely stored off site in a secure vault. In addition to the data servers, the production web server will also be backed up routinely. The separate development web server will serve as a backup to the production server. Research laboratory results will also be downloaded to the study designated program.

9.1 Error Checking

Each item on the web forms will have validity checks performed to ensure that the data entered are accurate and that items are not skipped during entry by mistake. Checks will be developed by both clinical and laboratory investigators. Depending on the question, any item found that does not meet the respective edit criteria will have an appropriate error message displayed when the user tries to save the data. Errors will be classified as either "hard" errors meaning that a valid response is required before the data can be saved or as "soft" errors in which the entry operator can either correct the errors or override them to indicate that the data are correct although it does not meet the edit criteria. Examples of hard errors would be items such as identifiers and event dates. An example of a soft error would be values that are outside a pre-defined range. When the data record is saved, a form status field will be updated to indicate the current status of the form. There are currently four status states that the form can have. These statuses are: the form is incomplete, the form is complete, the form was saved with errors, and the form is complete with errors. For the first status, the entry user will have the option to save a record as "incomplete" for situations where they have partially entered a form and must stop because of an interruption. This will allow the user or the study coordinator to pull up the form at a later time and finish completing it. If the form was entered without any errors, then the record will be saved as complete. If the user overrides any soft errors found, the record will be saved as "saved with errors". Staff in the HDCC will have web-access to listings of subject specific errors needing correction by site. These errors can be corrected at the site or in the offices of the HDCC (given documentation of the change). All site investigators will be trained to follow regulatory procedures when making any changes in the paper forms or source documentation (no erasures, cross through error, write in correction, date, and initial). Once a follow-up about any errors has been done by the HDCC and the error has been corrected or certified as accurate, the status will be change to "complete with errors." Once a record has been saved by the site or HDCC as complete, they will no longer be allowed to make changes to the records. Any changes that result from obtaining new information would be made by the staff at the

HDCC. At the end of the trial after all possible corrections are made, the database will be locked and further changes will not be made.

9.2 Error Correction Follow-ups

Since there are times when data does not meet the required edit criteria such as out of range values, the sites still need to be able to save their data. However, such errors need to be followed up to ensure that the error was not by mistake. In this case, any soft error indicated will be logged to an error log data table through which the clinics can later generate a report of these errors that must be followed up on. This report will include the option for the clinic user to enter the correct value(s) if the record was saved by mistake or to indicate that the value saved was correct in which case they must provide an explanation as to why the error was overridden. These reports must be transmitted back to the HDCC where staff will process the corrections through an error log management system. This process is particularly important for clarifying missing data. Once these reports are received back by the HDCC staff and processed, the respective data record will be updated to the forth status of “complete with errors.” Since clinical staff must sign these reports, these reports will serve as audit records should the funding agency need to investigate the process.

9.3 Investigator Resources and Reporting

A secure website will be provided through which authorized study management personnel, study investigators and coordinators, and representatives of the funding agencies can log in to review trial recruitment status and other administrative reports about the trial conduct and data quality.

9.4 Archiving the Final Dataset for Public Use

Once the database is locked for analyses and primary study publications are completed, the HDCC will follow NHLBI guidelines related to archiving de-identified data and making it publically available when requested by the NHLBI.

10. QUALITY ASSURANCE

10.1 Training:

Training of research staff and nurses who will be responsible for recruitment and randomization of subjects is planned for the PROPPR study and in line with standard ROC procedures. A standard manual of operations developed by the HCCC and HDCC’s research teams will provide standard definitions of all study variables (i.e., data elements) and describe all data collection and data entry procedures in detail. Copies of the manual will be distributed to all Consortium sites to be used in training each site’s research team and will be available on the study website through the HDCC section of the ROC website. In addition to the planned training meetings, each site will be responsible for the complete education of their personnel in the conduct of the PROPPR study.

10.2 Laboratory Quality Assurance

All laboratory samples collected for research purposes for the PROPPR trial will be sent to the PROPPR Core Research Laboratory that is located on the 5th floor of the Medical School at UTHealth. Where certain assays require specific equipment or expertise, laboratory samples will be shipped from the core laboratory at UTHealth to specific research laboratories such as UCSF (Cohen) and the University of Vermont (Mann). A standard quality assurance process will be in place for every research laboratory test. Where the a research laboratory does not have a standard quality assurance process in place, a system for sending split samples for reanalysis (where possible) will be put into place. The strict quality control ethic of the core laboratory is a reflection of its personnel and has evolved from methodology that has been in place for many years and improved upon by the vast experience of the collaborators in large, inter-disciplinary studies. A list of quality control measures include: maintaining proper sample identification and storage, preventing contamination, inventory organization and database management and monitoring and maintenance of equipment and its performance. Importantly, standardized calibrated material will be used on a regular basis at all testing sites to validate both methods and performance.

10.3 Study Monitors

The study monitors will report to both the HCCC and HDCC. Monitors will be trained in trial procedures, trained to identify source documentation, to assess regulatory compliance, to review source documents for agreement with study records, to identify possible unreported adverse events, and to look for protocol violations. The study monitors will review subject medical records onsite only for the purpose of verifying research data as required by law. Each site will be visited by study monitors from the HDCC to certify that the site is ready to begin the trial and will be visited again a few months after the trial begins, if the site has been enrolling subjects. In addition, NHLBI and/or ROC DCC is expected to send representatives annually unless a problem is noted the study monitors.

10.4 ROC Processes for Generation, Evaluation, and Implementation of Protocols

ROC has developed a detailed process for generation, evaluation, implementation, and monitoring of protocols, which PROPPR is following. The PROPPR protocol is a ROC protocol, and once the protocol is approved by ROC, the NHLBI DSMB, the FDA, Health Canada, and site IRBs/REBs, the ROC process includes ongoing protocol review of trial progress by the NHLBI DSMB as well as by the ROC Management, Trauma and Executive Committees. In addition to the active site monitoring by the HDCC, site performance will be monitored by the ROC Study Monitoring Committee. Detailed functions of all the above can be found on the ROC website <https://roc.uwctc.org/tiki/tiki-index.php>. The FDA, Health Canada and local IRBs/REBs provide additional regulatory oversight. The FDA and Health Canada requires an investigational new drug application because the PROPPR protocol operates under exception from informed consent.

11. ADMINISTRATIVE STRUCTURE

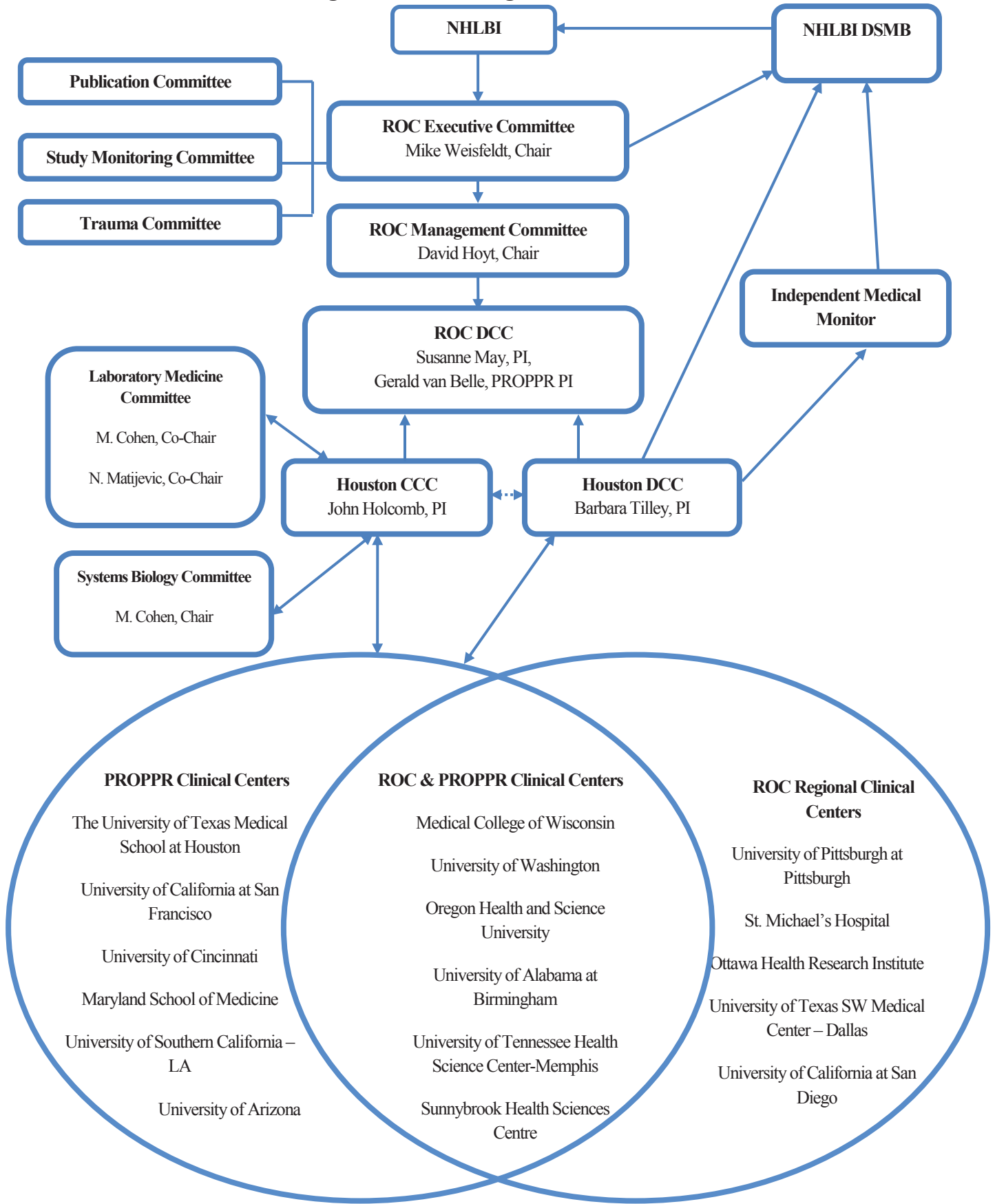
The HCCC and the HDCC are functioning as satellites in the ROC under separate sub-contracts. The HCCC and HDCC conduct the sub-contracts in accord with ROC governing procedures and NHLBI and FDA policies and guidelines. Figure 8 describes the organization structure for the PROPPR trial.

The PROPPR study is one of eight studies currently being conducted by the Resuscitation Outcomes Consortium (ROC). The ROC Data Coordinating Center (DCC) is responsible for clinical and data coordination for all ROC studies and is under the direction of Dr. Susanne May. Dr. van Belle is the Principal Investigator for the ROC PROPPR study. The UTHealth Clinical Coordinating Center (HCCC) and the UTHealth Data Coordinating Center (HDCC) are satellites of the ROC DCC for purposes of the PROPPR trial with the majority of the work being carried out by these satellites. The HCCC will oversee all clinical sites and the laboratory committee. The HDCC will perform data collection activities. Over the last six years, ROC has developed a detailed process for generation, evaluation, implementation, and monitoring of protocols, via several committees, all of which PROPPR is following. Once the protocol is approved by the NHLBI review committee, FDA, and local IRBs/REBs, the ROC quality assurance process includes ongoing protocol review of trial progress by the NHLBI DSMB as well as by the ROC Management, Trauma, Executive and Study Monitoring Committees.

To foster collaboration key personnel from the ROC DCC and HCCC and HDCC will meet on a regular basis. Two of the four meetings of the PROPPR investigators will overlap the semi-annual meetings for all ROC investigators. In addition, ROC DCC personnel communicate regularly with the HCCC and HDCC and will take part in site visits to satellite sites. PROPPR progress will be reported routinely at Trauma, Management and Executive Committee calls.

In addition, we have established the two committees (Laboratory and Systems Biology) and three subcommittees (Emergency Medicine, Anesthesiology, Transfusion Medicine) to assist with protocol administration and compliance.

Figure 8. PROPPR Organizational Structure



12. HUMAN SUBJECTS RESEARCH

12.1 Risks to Subjects

This study will randomize a total of 680 subjects in the Phase III trial who have sustained a major traumatic injury and are predicted to receive a MT. The Vanguard stage of this study will involve enrollment of subjects in at least four of the sites for up to a six month period of time. Based on past data, the majority of traumatic injuries occur in male subjects 45 years of age and younger. The majority of this population will have no significant pre-existing medical history. Children estimated to be less than 15 years of age, women who are known to be pregnant, and prisoners will be excluded from this trial. As all products used in this trial are approved by the AABB, FDA, and Health Canada and used in amounts currently in use across trauma centers, we anticipate no new risks to those seriously injured trauma patients. Subjects randomized will receive blood product ratios equivalent to ratios predominantly used at the Level I trauma centers in PROMMTT. See Figures 5 in Section 4.

12.2 Source of Data Collection

Data will be collected prospectively during the trial. This will include a daily review of the medical records and results of diagnostic studies. A description of the data collection process is detailed in section 5.2.5.

12.3 Potential Risks

Eligible subjects for this trial will have been identified as requiring multiple units of blood products due to their traumatic injury. There is a potential risk that products may be delayed due to the randomization process, however all participating sites will have plasma and RBCs rapidly available in the ED for use until the container with the randomized products is available. To monitor the potential risk, the clinical research staff will document relevant times including: time of ED admission, time of randomization, time MT called to the blood bank, and time study container delivered to bedside from the blood bank. If a delay or risk is identified, appropriate information/data will be sent to the DSMB to decide if further action needs to be taken.

Severely injured subjects who receive blood products will frequently incur complications such as death, multi-organ failure (MOF), respiratory complications, and infections. While there is no expectation of harm between groups, the risk of transfusion-related acute lung injury (TRALI) is increased as plasma and platelets use increases, however, most authors place this as a 1:10,000 rate, and this rate must be placed in the context of significantly decreased mortality reported in many recent publications.^{25, 66}

Subjects will have no additional costs for participating in the study. Subjects, or their 3rd party payer, will be responsible for all standard-of-care charges including the blood transfusions that are routinely given to trauma patients. Subjects will not be charged for lab tests specifically performed for research purposes.

12.4 Protection Against Risks

12.4.1 Protection of Human Subjects and Consent

This trial qualifies for the “Exception from informed consent required for emergency research” outlined in the FDA regulation 21CFR50.24 as follows:

1. Subjects are in a life-threatening situation and collection of valid scientific evidence is necessary to determine the safety and effectiveness of the particular interventions
2. Obtaining informed consent is not feasible because the subject cannot give reasonable consent due to medical condition, intervention must be given before consent can be obtained from a LAR, and cannot prospectively select subject
3. There is prospect of direct benefit to subject because they are in a life-threatening situation requiring intervention, risks associated with this study are reasonable compared to standard of care therapy
4. The research could not practically be carried out without a waiver
5. Diligent attempts will be made to contact the LAR or family member for them to object to subject’s continued study participation within the protocol-defined therapeutic window of the first 20 minutes and for the 24-hour study treatment duration

6. IRB has reviewed and approved the informed consent procedures and documents to be used with the subjects or LAR for this study.
7. Additional protection of rights will be provided which will include: community consultation and public notification, an established independent data safety monitoring committee, and efforts will be made to obtain informed consent from family members if the LAR is not available.

A detailed explanation of each criterion stipulated in the regulations for this exception and how our trial design applies to these criteria is outlined in Appendix 1. Once the subject is randomized, the site principal investigator or a designated member of the research team will make frequent attempts as soon as feasible, per local IRB/REB requirements, to contact a LAR and/or family member to provide information about the study and allow them the opportunity to withdraw the subject from continued participation in the study. A verbal withdrawal of the subject's further participation in the study will be considered binding. A log will be kept to document the attempts made to contact the LAR/family member. The log will be included in the paper data collection forms. Due to the severity of the injuries incurred, it is difficult to specify the time frame involved with obtaining the consent however all attempts will be made to obtain consent prior to completion of the study requested blood tests (72 hours after randomization). Attempts will continue to obtain consent from the LAR and/or patient throughout the hospitalization. Attempts to contact will include direct contact, telephone contact and written contact or any other contact options as approved by local IRB/REB policy. An assessment will be done at the time of approaching the LAR/subject for consent to assure the LAR and/or subject is competent to make a sound decision regarding the consent process. In the event that the subject does not survive following the traumatic injury, their information will be included in the data analysis. Written notification may be sent to the deceased's family regarding their participation in the study, per local IRB/REB policy.

Public notification and community consultation in accordance with local IRB and Canadian REB policies will be undertaken prior to IRB/REB approval. Because the population eligible for enrollment includes all citizens in the study regions, it will not be possible to target specific individuals although the local IRB/REB may recommend targeting specific groups. The community consultation plan for each trial site will be individualized to fit the IRB/REB requirements. The participating sites have considerable experience conducting community consultation. A variety of methods are employed including consultation with community leaders and targeted community groups, random telephone surveys,¹⁰⁵ and community meetings. Most sites provide an "opt out" process to individuals who do not want to be enrolled. The "opt out" process allows all members of the community to identify themselves if they choose to not be involved with the study. For this study, the "opt out" identifier (i.e., colored bracelet or identification card) will be determined by the local IRB/REB and will be made available through the community consultation programs. The identifier can be given to the individuals at time of meeting or mailed out to the individuals requesting the "opt out" process. Clinical research personnel will be trained to check for these patients prior to randomization.

A modified consent process will be conducted in the group of subjects who are screened, have initial blood drawn, and determined to be eligible (at the 0 hour blood draw) but are not randomized. The method of consent (i.e. waiver of consent, waiver of documentation, or full consent) will be dependent on the individual site's local IRB/REB policies and regulations.

The subject will be given the opportunity to continue or withdraw from the study when they become capable of providing informed consent. After all questions/concerns have been addressed, the subject will be given a consent form to sign, indicating whether he/she wants to continue or stop participating in the study. For those subjects considered as a minor, the one page consent form will be considered the assent form. If a LAR/family member previously signed the "Research Study Information and Consent Form," a copy of the signed form will be given to the subject.

12.4.2 Vulnerable Populations

While the NIH considers anyone under the age of 21 to be a part of the pediatric research group, there is wide variability in state laws defining the *adult* population. Taking this wide variability into consideration, all consent

related procedures, forms, and notification documents will be approved by the participating site's local IRBs or Canadian Research Ethics Boards (REBs) prior to the onset of the trial.

This trial may include subjects age 15 to 20. Subjects sixteen years of age and older are considered as adult trauma subjects in a large percent of the trauma centers. Sixteen and seventeen year olds are able to drive in most states and are at high risk for motor vehicle accidents resulting in blunt or penetrating injuries. Excluding this age group would significantly decrease our efforts to randomize 680 MT subjects in a two year period of time. Additionally, it is difficult to differentiate a 16 or 17 year old from one who is 21 or older at the time care is initiated in the ED until positive identification can be obtained. Children below the age of 15 or 50kg body weight will be excluded from this trial. Children's intravascular volume is different than the adult's, requiring adjustments to the standard adult treatment protocols. In addition, this trial will be conducted at Level I trauma centers which may or may not have affiliated pediatric programs.

Pregnant women will also be excluded from the PROPPR trial. Pregnant women have a significantly increased intravascular volume and physiologic reserve for bleeding which can require adjustments to the standard treatment protocols.

Prisoners admitted to the ED from a correctional facility will be excluded from enrollment. It is possible that subjects may be enrolled into the PROPPR trial who are under police observation as suspects. These subjects will remain in the study until discharge or incarcerated.

12.5 Roles/Responsibilities of Medical Monitor

An independent medical monitor will review all unexpected problems involving risk to subjects or others, SAEs and all transfusion-related deaths and provide an unbiased written report of the event. At a minimum, the medical monitor must comment on the outcomes of the event or problem and in case of a SAE or transfusion-related death, comment on the relationship to participation in the trial. Because a large number of deaths are expected (30-70% mortality)³⁹ due to the condition of the study population at entry to the trial, individual reports to the DSMB will be aggregated and reported on a timely schedule acceptable to the DSMB. If the death is considered unexpected and is either suspected or probably due to treatment, this event would be promptly reported to the medical monitor and the DSMB. The medical monitor must also indicate whether he/she concurs with the details of the report provided by the principal investigator. Reports for serious adverse events determined by either the investigator or medical monitor to be possibly or definitely related to participation must be promptly reported per FDA and/or Health Canada guidelines as described in Section 12.8.

12.6 Data Safety Monitoring Board (DSMB)

An independent DSMB has been established by the NHLBI. This committee will review and approve the protocol, and will develop a final plan for monitoring in collaboration with the HCCC, HDCC, ROC Management Committee and Trauma Committees, and NHLBI. The DSMB is governed by a charter that is designed for all ROC protocols.

The DSMB will help ensure the safety of the trial by monitoring adverse outcomes throughout the trial and by reviewing outcome data for possible harm. The DSMB will pay particular attention to missing 30-day mortality data. If the amount of missing 30-day mortality data approaches 10%, the DSMB will be asked to recommend specific changes to be made to the protocol in an effort to keep the value less than 10%. The committee reviews and approves the protocol and any amendments. In addition, the committee will review the results of the interim analyses. Although the DSMB and NHLBI will make the final decision about the interim monitoring plan, we anticipate that the DSMB will evaluate safety at intervals to be determined by the DSMB, expected to be approximately semi-annually but could occur more frequently if mandated by the DSMB. The DSMB will advise the investigators if a change in the protocol is warranted based on this interim monitoring. The DSMB will meet in person or by phone every six months or more often as decided by the DSMB.

12.7 Adverse Events

Expected Adverse Events

Common expected AE's/SAE's will include: trauma injury related infections, ventilator associated pneumonia (VAP), thrombotic complications (DVT, PE, MI, stroke), acute lung injury (ALI), acute respiratory distress syndrome (ARDS), acute kidney injury (AKI), MOF and intracranial operative interventions.

Adverse Events Reporting Procedure

All adverse events will be classified by: a) Severity (AE, SAE); b) Expected vs. Unexpected; and c) Related vs. Unrelated. Unrelated adverse events, not of study interest will not be recorded on the subject's Adverse Events Log or entered into the eCRF. Only study-related adverse events or events that are outcome measures of interest that occur during the study period (after randomization until study conclusion) will be recorded.

12.8 Serious Adverse Events

Expected Serious Adverse Events

The study population is expected to have a large number of unrelated, expected serious adverse events including death from trauma related injuries. The SAE will be recorded on the subject's AE/SAE log and follow local reporting requirements.

Unexpected, Serious Adverse Events:

Serious Adverse Events will include potential transfusion-related events such as possible transfusion-related death and/ or transfusion-related acute lung injury (TRALI), re-hospitalizations, or other unexpected SAEs. The site PI will classify the relatedness of the SAE to the study intervention.

Serious Adverse Events Reporting Procedure

SAE reporting for the PROPPR study will follow the FDA guidance on safety reporting requirements for IND and BA/BE studies dated September, 2010. In addition to following local reporting procedures, clinical sites will notify the HCCC/HDCC of a SAE or suspected transfusion-related death within three business days of discovery of the event and complete a MedWatch 3500 form and/or Health Canada's ADR form. The HDCC will report transfusion-related deaths to the DSMB, FDA, Health Canada, NHLBI, and IRBs/REBs within seven calendar days of receiving the site report. All other unexpected and possibly related SAE's will be reported within 15 calendar days of receiving the site report.

Adjudication Procedures for Cause of Death Classification:

An adjudication process will be incorporated to determine the cause of death for the subjects enrolled in PROPPR.

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APPENDIX 1

Exception from Consent for Emergency Research

We have outlined below each criteria stipulated in the regulations for this exception and how our trial design applies to these criteria.

CFR Sec. 50.24 Exception from informed consent requirements for emergency research

1. The human subjects are in a life-threatening situation, available treatments are unproven or unsatisfactory, and the collection of valid scientific evidence, which may include evidence obtained through randomized investigations, is necessary to determine the safety and effectiveness of particular interventions.

The proposed study is a randomized trial of ratios of blood products (plasma:platelets:RBCs) in trauma patients who present with massive bleeding requiring transfusion within hours of injury. These patients are in an immediate life threatening situation. Although only 3% of admissions to civilian trauma centers and 7-10% of combat support hospital admissions require MT (defined as ≥ 10 units of RBC's within 24 hours of admission), the majority of those patients receive MT in the first 3-6 hours after injury and have the highest incidence of death during that same time frame. Almost half of those civilian admissions suffer from truncal hemorrhage which is the leading cause of potentially preventable death with most deaths occurring within 6-12 hours of admission. In the combat support hospitals more than $\frac{3}{4}$ of all potentially preventable deaths are from truncal hemorrhage. Coagulopathy likely plays a significant role in preventable deaths due to hemorrhage as seriously injured patients in shock are the ones who most often present with coagulopathy in the ED. Trauma patients who are not coagulopathic rarely die. Trauma induced coagulopathy (TIC) is associated with *higher* transfusion requirements, a *greater* incidence of MOF, *longer* ICU and hospital stays, and a 4x risk of mortality compared to those with normal coagulation. Lack of a mechanistic understanding has led to wide variability in transfusion practice in seriously injured patients with wide variability in survival.

The deleterious impact of dilution-related abnormalities on coagulation and the impact of hypothermia, coagulopathy, and acidosis on survival have long been recognized. Although significant attention has been focused on preventing hypothermia and acidosis, little attention has been directed towards understanding the mechanisms involved with the early presentation of TIC. Indeed there is to date, no comprehensive and longitudinal characterization of the coagulopathic milieu after severe injury. Currently knowledge of the patterns of traumatic coagulopathy is extremely limited, and clinically useful diagnostic tools are essentially absent. Thus, therapeutic options are severely restricted

This proposed trial and these laboratory studies will define the understanding of the mechanisms of early coagulopathy associated with trauma, how best to mitigate and reverse the effects, and start describing optimal treatment regimens.

2. Obtaining informed consent is not feasible because:

- i. The subject will not be able to give their informed consent as a result of their medical condition**
- ii. The intervention under investigation must be administered before consent from the subjects' legally authorized representatives (LAR) is feasible; and**
- iii. There is no reasonable way to identify prospectively the individuals likely to become eligible for participation in the clinical intervention**

In order to perform this trial, the randomized intervention will be performed in the initial resuscitation period following patient arrival to the ED. As a result of the injuries, the patient is unable to provide consent for study enrollment. The patient will often be intubated and have altered mental status as a result of the injury. The legal next-of-kin are often not immediately available when the patient arrives to the ED. Because this trial involves

traumatic injury which is unpredictable, there is no way to prospectively identify individuals who are likely to become eligible for this trial. We will inform the family member or LAR at the earliest feasible opportunity of the subject's inclusion in the clinical investigation, the details of the investigation, other information contained in the informed consent document, and that he or she may discontinue the subject's participation at any time without penalty or loss of benefits to which the subject is otherwise entitled. Such notification is not usually feasible before or at the actual time of treatment and may be deferred until after resuscitation efforts have been completed. Such notification will be in person wherever possible and as soon as feasible (unless otherwise directed by an IRB).

3. Participation in the research holds out the prospect of direct benefit to the subjects because:

- i. Subjects are facing a life-threatening situation that necessitates intervention;**
- ii. Appropriate animal and other preclinical studies have been conducted, and the information derived from those studies and related evidence support the potential for the intervention to provide a direct benefit to the individual subjects; and**
- iii. Risks associated with the investigation are reasonable in relation to what is known about the medical condition of the potential class of subjects, the risks and benefits of standard therapy, if any, and what is known about the risks and benefits of the proposed intervention or activity.**
 - a. Subjects who are eligible for this trial are facing a life-threatening situation which will necessitate intervention including multiple units of blood products. There is currently no single standard of care for the ratio of blood products to be chosen and conflicting information from published research.
 - b. Previous observational studies have been done to evaluate the impact of product ratios associated with clinical outcomes
 - c. The subjects eligible for this trial have been determined to need a MT therefore the risks associated with this trial are risks associated with transfusion of blood products.

Risk/Benefit Assessment:

Eligible subjects for this trial will have been identified as requiring multiple units of blood products due to their traumatic injury. The risks associate with transfusion of any blood products include the chance of transmission of viral diseases, hypotension, allergic reactions, shortness of breath, blood clotting complications, hypoventilation and fever. These risk factors are minimized through the local blood center's protocols for infectious disease testing. Severely injured subjects who receive blood products will frequently incur complications such as death, multi-organ failure (MOF), respiratory complications, and infections. While there is no expectation of harm between groups, the risk of transfusion-related acute lung injury (TRALI) is increased as plasma and platelets use increases, however, most authors place this as a 1:10,000 rate, and this rate must be placed in the context of significantly decreased mortality reported in many recent publications.^{25, 66}

There is a potential risk that products may be delayed due to the randomization process, however all participating sites will have plasma and RBCs rapidly available in the ED for use until the container with the randomized products is available. To monitor the potential risk, the clinical research staff will document relevant times including: time of ED admission, time of randomization, time MT called to the blood bank, and time study container delivered to bedside from the blood bank. If a delay or risk is identified, appropriate information/data will be sent to the DSMB to decide if further action needs to be taken.

This trial intends to benefit all subjects with the use of the MT algorithm to predict which subjects will require a massive transfusion. With the utilization of the algorithm we hope to predict earlier who will need blood products as well as those who will not require blood products. Other possible benefits for the treatment groups include decreased mortality, multi-organ failure, hospital length of stay and need for blood products. Additional benefits for the general population include 1) updated data regarding coagulopathy complications in trauma subjects and potential treatment regimens, and 2) the availability of a more precise algorithm to assist the trauma surgeons and emergency medicine physicians to predict the need for blood product transfusions.

4. The clinical investigation could not practicably be carried out without the waiver.

This trial could not be conducted without the waiver of consent, due to the need to administer blood products rapidly upon recognition that a patient requires a MT.

5. The proposed investigational plan defines the length of the potential therapeutic window based on scientific evidence, and the investigator has committed to attempting to contact a LAR for each subject within that window of time and, if feasible, to asking the LAR contacted for consent within that window rather than proceeding without consent. The investigator will summarize efforts made to contact LAR and make this information available to the IRB at the time of continuing review.

The initial resuscitation time period for this trial begins at the time the patient arrives in the ED. MT protocols are often initiated within the first 10 minutes of ED arrival. Due to the nature of the injury and the intensity of the resuscitation efforts, it is not often feasible to obtain consent prior to the MT protocol being initiated and prior to randomization into the study. The legal next of kin are often not immediately available when the patient arrives to the ED. We will, however, make a reasonable attempt to contact a LAR for each subject at the earliest feasible opportunity to obtain consent rather than proceeding without consent. The LAR or family will be informed of the subject's inclusion in the clinical trial, the details of the trial, other information contained in the informed consent document, and that he or she may discontinue the subject's participation at any time without penalty or loss of benefit to which the subject is otherwise entitled. Such notification is not usually feasible before or at the time of treatment and must be deferred until after resuscitation efforts have been completed. Such notification will be in person wherever possible and as soon as feasible (unless otherwise directed by a local IRB/REB).

Where allowed or mandated by the local IRB/REB, a script will be available which can be used to inform patients or their LAR of the study and obtain verbal consent where feasible. In addition, due to the continuation of the intervention into the hospital, repeated attempts will be made to contact that patient or LAR at the earliest feasible opportunity after hospital arrival to notify them of study participation and seek consent for ongoing participation. Efforts to contact LARs will be tracked and reported to the local IRB/REB. Attempts will continue to obtain consent from the LAR and/or patient throughout the hospitalization. Attempts to contact will include direct contact, telephone contact and written contact of any other contact options as approved by local IRB/REB policy. An assessment will be done at the time of approaching the LAR/subject for consent to assure the LAR and/or subject is competent to make a sound decision regarding the consent process.

When approached for notification of study participation following enrollment, the patient or their LAR will have the option of withdrawing from the study. During the notification process, the details of the trial will be reviewed along with potential risks and benefits, the endpoints of interest and the process by which these endpoints are evaluated. When notified of trial enrollment, the patient or their legal representative will be given the opportunity to withdraw from further data and sample collection. If the patient or LAR withdraws, all further data collection and blood sampling will cease. A verbal withdrawal of the subject's further participation in the study will be considered binding. Data collected prior to the point of withdrawal or until subject is discharged from the hospital will be reviewed for study purposes. All research laboratory samples collected up to the point of withdrawal will be obtained and analyzed. In this circumstance, we will be limited to a description of baseline data and data collected up to the point of patient withdrawal and survival to hospital discharge to ensure that subjects who withdraw are comparable among the groups. Our previous experience suggests that refusals of this nature are rare. It will be up to local IRBs/REBs to determine if and when a written consent form is required for continued participation.

As this is an emergency research study we will be seeking an emergency waiver of consent. We will contact the LAR for continued trial participation, at the earliest feasible time for the LAR to provide informed consent. All

study procedures already performed and yet to be completed will be explained, and the legal representative's consent for continued participation will be requested. If the subject becomes competent to provide consent during their admission, he/she will be approached by the research coordinator for approval for all study procedures including the 30-day follow-up interviews.

Taken together with the lack of current satisfactory treatment, the life-threatening nature of these trauma types, and the prospect of benefit to participants, these factors provide sufficient support for an emergency exception from informed consent in order to evaluate an intervention that may have significant outcome benefits to this patient population.

6. The IRB has reviewed and approved informed consent procedures and an informed consent document consistent with FDA and HHS regulations. These procedures and the informed consent document are to be used with subjects or their LAR in situations where use of such procedures and documents is feasible. The IRB has reviewed and approved procedures and information to be used when providing an opportunity for a family member to object to a subject's participation in the clinical investigation consistent with paragraph (a)(7)(v) of this section.

All procedures, consent forms and notification documents will be approved by the participating site's local IRBs or Canadian Research Ethics Boards (REBs) prior to the onset of the trial.

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

Public notification and community consultation in accordance with local IRB and Canadian REB policies will be undertaken prior to IRB/REB approval. Because the population eligible for enrollment includes all citizens in the study regions, it will not be possible to target specific individuals although the local IRB/REB may recommend targeting specific groups. The community consultation plan for each trial site will be individualized to fit the local IRB/REB requirements. The participating sites have considerable experience conducting community consultation. A variety of methods are employed including consultation with community leaders and targeted community groups, random telephone surveys,¹⁰⁵ and community meetings. Most sites provide an "opt out" process to individuals who do not want to be enrolled. The "opt out" process allows all members of the community to identify themselves if they choose to not be involved with the study. For this study, the "opt out" identifier (i.e., colored bracelet or identification card) will be determined by the local IRB and will be made available through the community consultation programs. The identifier can be given to the individuals at time of meeting or mailed out to the individuals requesting the "opt out" process. Clinical research personnel will be trained to check for these patients prior to randomization. Clinical research personnel will be trained to check for these patients prior to randomization.

ii. Public disclosure to the communities in which the clinical investigation will be conducted and from which the subjects will be drawn, prior to initiation of the clinical investigation, of plans for the investigation and its risks and expected benefits;

Our suggested approach to public disclosure/community consultation will follow techniques previously approved by local IRBs and employed at individual centers, such as random-digit dialing, open-forums, public announcements via newspaper or radio, and other locally approved methods of contact with the public. Visual aides, such as power point, flyers or posters can be used in the presentations, and all material will be in lay terminology. Each communication will include information as to the purpose of the trial, the consent process, the risk and benefits to the community/patient, and the time commitment required. As each community is unique and may require specific or special needs, the local IRBs/REBs will approve the methods for their community and ensure that community consultation practices are both appropriate and complete before consent is given to begin the trial.

During the course of public notification/community consultation, including public advertising of the study, individuals in the community not wishing to be enrolled in the trial will be provided opportunity to “opt out” in advance for treatment. Those contacting a published address and /or telephone number for the investigators will be given a bracelet or its equivalent without cost which, when displayed, indicates ineligibility for the study. A letter will accompany the bracelet/item indicating that it must be displayed on person in a recognizable manner in order to be identified by providers. Providers will be trained to recognize such bracelets or their equivalent, and that the identification of such an item would exclude the patient from trial enrollment.

iii. Public disclosure of sufficient information following completion of the clinical investigation to apprise the community and researchers of the study, including the demographic characteristics of the research population, and its results;

Public disclosures will be performed both prior to trial enrollment (with opportunity and a mechanism for the community to contact the investigators with their response) and at the completion of the trial in the form of multimedia press releases organized by the ROC and by local sites at the direction of the IRB/REB. These will include plans for the trial, including potential risks and benefits, and a summary of the results of the trial upon completion. In the event that the press releases are not widely circulated, advertisements will also be placed in local papers describing the trial. Information regarding the trial will also be available on the ROC website.

iv. Establishment of an independent data monitoring committee to exercise oversight of the clinical investigation;

An independent data and safety monitoring committee will oversee the trial. Please see section 12.5 of the Protocol.

v. If obtaining informed consent is not feasible and a LAR is not reasonably available, the investigator has committed, if feasible, to attempting to contact within the therapeutic window the subject's family member who is not a LAR, and asking whether he or she objects to the subject's participation in the clinical investigation. The investigator will summarize efforts made to contact family members and make this information available to the IRB at the time of continuing review.

We expect that the majority of subjects who meet the enrollment criteria will either be unconscious or have an altered mental status secondary to acute blood loss, traumatic brain injury or intoxicating substances, and thus will not be in a position to provide informed consent in the ED setting. Accordingly, it may not be feasible to attempt to obtain informed consent during the therapeutic window. We will inform the family member or LAR at the earliest feasible opportunity of the subject's inclusion in the clinical trial, the details of the trial, other information contained in the informed consent document, and that he or she may discontinue the subject's participation at any time without penalty or loss of benefits to which the subject is otherwise entitled. (See more details in item 5 above.) Such notification is not usually feasible before or at the actual time of treatment and must be deferred until after resuscitation efforts have been completed. Such notification will be in person wherever possible and as soon as feasible (unless otherwise directed by a local IRB). A log will be kept to document the attempts made to contact the LAR/family member. The log will be included in the paper data collection forms.

APPENDIX 2

DATA COLLECTION FLOWSHEET

ASSESSMENTS	Pre ED	ED	OR	IR	Inpatient 1st 24 hrs	Inpatient Daily Assess.	Discharge Info	30 Days
Eligibility Criteria	x	x						
Demographics		x					x	
Trauma Activation	x							
EMS Care	x							
Unit arrival information		x	x	x	x			
Informed consent process		x	x	x	x	x		
Vital Signs	x	x	x	x	x	x		
Glasgow Coma Scale	x	x	x	x	x	x	x	
Extended Glasgow Outcome Score							x	
Mortality		x	x	x	x	x	x	x
Life Saving Interventions	x	x	x	x	x	x		
Injury Information	x	x						
Blood Products (including age of product)	x	x	x	x	x	x		
Non-blood Fluids	x	x	x	x	x	x		
Medications	x	x	x	x	x	x		
Surgical Procedures			x		x	x		
Interventional Radiology Procedures				x		x		
Angiogram				x				
Lab Results		x	x	x	x	x		
Hemostasis Obtained		x	x	x	x			
*Research Lab Sample Collection		x	x	x	x	x		
Multi-Organ Failure Assessment						x		
Complications					x	x	x	
Injury Severity Score (ISS)							x	
Subject Disposition							x	
Past Medical History							x	

* Research lab samples time points:
 For all subjects (screened, eligible, or randomized): 0 hour
 For all randomized subjects: 2, 4, 6, 12, 24, 48, and 72 hours

STATISTICAL ANALYSIS PLAN

Pragmatic, Randomized Optimal Platelet and Plasma Ratios (PROPPR)

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1. STUDY OVERVIEW

1.1 Objective and Study Design

The objective of this study is to conduct a Phase III multi-site, randomized trial in 580 subjects comparing the effectiveness and safety of 1:1:1 transfusion ratios of plasma and platelets to red blood cells (RBCs), the closest approximation to reconstituted whole blood; with the 1:1:2 ratio. The co-primary outcomes will be 24-hour and 30-day mortality. The PROPPR Trial will be conducted under exception from informed consent (EFIC). The trial will begin with a Vanguard stage (lasting up to six months) conducted in at least four sites to assess feasibility of trial recruitment and protocol adherence. Laboratory data from the trial will add to the understanding of trauma induced coagulopathy (TIC) and inflammation.

2. DEFINITION OF TARGET POPULATION AND STUDY SAMPLES

2.1 Target Population

The target population for this study is composed of patients who are admitted to a Level 1 trauma center, meet the inclusion and exclusion criteria detailed in the protocol, and are predicted to require a massive transfusion.

2.2 Study Outcomes

2.2.1 Primary Outcomes

Absolute percent (rather than relative percent) group difference in 24-hour and 30-day mortality (Co-Primary outcomes).

2.2.2 Ancillary Clinical Outcomes

Time to hemostasis (defined as no further blood product transfusions required for two hours), hospital-free days, ventilator-free days, ICU-free days within the first 30 days or hospital discharge, whichever comes first); incidence of major surgical procedures (e.g., thoracotomy, craniotomy, laparotomy, major amputation), complications (transfusion-related acute lung injury, acute lung injury, acute kidney infection, multiple organ failure, acute respiratory distress syndrome, sepsis, open abdominal complications, infections, thromboembolic complications, re-bleeding requiring an arteriogram or unscheduled return to the OR after PROPPR transfusion protocol discontinuation, hyperkalemia, hypocalcaemia during hospitalization; the number and type of blood products used from randomization until hemostasis is achieved, the number and type of blood products used after hemostasis is achieved to 24 hours post-admission, and functional status at hospital discharge or 30 days, whichever comes first, as measured by discharge destination and GOSE.

2.2.3 Primary Laboratory Outcomes

Models will be developed to identify drivers and sequelae of TIC and inflammation, and to characterize the natural history of the coagulation milieu. The principal modeling approach will be reverse-engineering of the biological networks from the research laboratory data augmented by the existing expert knowledge. Both baseline (Laboratory Aim 1) and dynamic (Laboratory Aim 2) models will be developed. When interpreting the resulting models (Laboratory Aim 3), special emphasis will be put on the primary and ancillary clinical outcome measures for laboratory analyses, including mortality, incidence of coagulation abnormalities, time to hemostasis, total blood product transfusions, incidence of organ injury (i.e., acute lung injury and acute renal failure) and ventilator associated pneumonia, 30-day mortality, ventilator-free, ICU-free and hospital-free days and incidence of nosocomial infections.

3. GENERAL STATISTICAL CONSIDERATIONS

3.1 Randomization (The process is described in detail in the protocol)

A stratified, permuted blocked randomization scheme will be used to assure balance over time in the intervention groups. Block sizes will be randomly chosen to avoid revealing a treatment assignment in this unblinded trial. Randomization will be stratified by site. For consistency in all sites, randomization of blood products will be completed at the blood bank. Randomization code lists will be prepared by the HDCC and sent to the contact person at the blood bank at each site who will keep the codes in a secure location. In the rare event that the blood bank mis-randomizes a patient (for example: takes the wrong entry on the list) we will include the patient in all analyses and analyze the patient “as randomized so analyzed”. The next patient will be randomized using a new assignment from a new list. To further limit the potential frequency and impact of this type of protocol deviation we intend to give the blood bank a minimum number of treatment assignments (no more than 10 numbers). A mis-randomization is considered a type of protocol non-adherence and the site will be issued emergency treatment assignments until the new list arrives and the old list is returned. Every effort will be made to keep this number small. Depending on the frequency of mis-randomizations, we will do a sensitivity analysis to study the impact of this type of protocol non-adherence.

3.2 Blinding

Although it will be impossible to mask intervention assignment at the bedside in a double- or single-blinded manner, concealing the blood products in a sealed container until the moment of actual transfusion will maintain rigor and prevent bias as much as possible, while maintaining the ability to care for these critically ill subjects. To promote blinding, a “sham” platelet bag will be attached to the outside of each container that does not contain platelets. Adherence to the treatment protocol will be carefully monitored and protocol deviations will be identified through the data collected or reported to the Houston Data Coordinating Center (HDCC) by study coordinators. The co-primary outcomes, 24-hour and 30-day mortality, are endpoints making blinding less of a concern in terms of outcome assessment. The HDCC PI, Co-investigator, and Program Manager will have access to unblinded data. The HDCC and ROC DCC will have access to only blinded data.

3.3 Multiplicity

Group sequential methods will be used to control for interim analysis of effectiveness.¹ For exploratory outcomes and safety analyses, no adjustment of Type I error probability is planned.

4. SAMPLE SIZE DETERMINATION

4.1 Sample Size for the Phase III trial

4.1.1 Primary Outcomes

For sample size estimation for the 24-hour mortality outcome, we chose a difference of 10% or greater increase in mortality from 11% at 24 hours to 21% when comparing 1:1:1 to 1:1:2. We considered a difference of 10% from the 1:1:1 referent group to be clinically meaningful and of sufficient magnitude to influence clinical practice. This pragmatic trial is powered at 90% to show superiority with a two-sided alpha level of 0.05, adjusted for interim analyses to 0.044.² The required sample size is 580 subjects.³⁻⁷ The 1:1:1 group mortality of 11% was selected based on a subset of published data available from a retrospective study showing 115 predicted MT patients had received 1:1:1 ratios and experienced an 11% mortality at 24 hours.⁸ In contrast, 24-hour mortality was 41% in the 27 predicted MT patients receiving 1:1:2 ratios. Adjusting for site generally should increase power unless there is lack of homogeneity of treatment effects across sites.

The PROPPR trial differs from an application for product approval that would require a significant difference on both outcomes. The PROPPR trial is testing two separate questions relating to short term (24

hour) and long term (30 day) outcomes. Consistent with the approach described by O'Brien we will not adjust the alpha level for multiple testing and will be using a per comparison error rate.

For the 30-day mortality, a 12% or greater difference in mortality from 23% in the 1:1:1 group is detectable given the same sample size (580), with 88% power, and a 10% or greater difference is detectable with 74% power assuming a 2-sided alpha of 0.044. The primary group of interest, 1:1:1, mortality was based on additional unpublished retrospective data as described for the 24-hour mortality. Power analysis for 24-hour and 30-day mortality is shown in the Figure below. Adjusting for site should generally increase power unless there is lack of homogeneity of treatment effects across sites.

Because no data from randomized trials exists to use in developing this sample size we present a power analysis of a variety of scenarios for 24-hour and 30-day mortality.

Figure 1: Power Curve Analysis

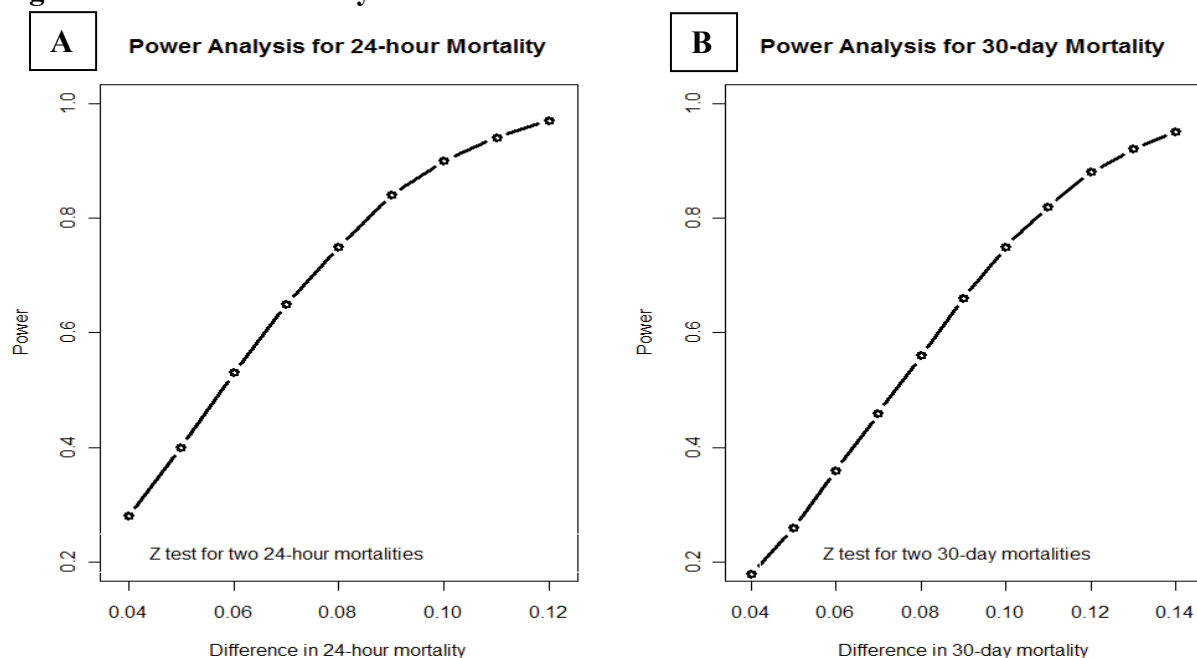


Figure 1a: A two-sided Z test at a **0.044** significance level is used. The 24-hour mortality in Group 1 (1:1:1 ratio) is fixed at **0.11**. The overall sample size is fixed at **580** (290 per group). The absolute difference in 24-hour mortality proportion between the two groups is varied from 0.04 to 0.15.

Figure 1b: A two-sided Z test at a **0.044** significance level is used. The 30-day mortality in Group 1 (1:1:1 ratio) is fixed at **0.23**. The overall sample size is fixed at **580** (290 per group) with no losses to follow-up. The absolute difference in 30-day mortality is varied from 0.04 to 0.20.

4.1.2 Ancillary Clinical Outcomes

We will compare treatment groups on a variety of ancillary outcomes as listed in 6.2. For binary outcomes (incidence of major surgical procedures or complications during initial hospitalization) we can detect absolute difference of 12% in outcomes from 50% (worst case scenario) with power of 80%, 2-sided alpha of 0.05, given a sample size of 290 per group. If some outcomes are rare, as we expect, we can detect a difference from 0.03 of 0.029 with same power and alpha. For outcomes involving counts (number of complications,) we have enough power with 580 to detect rate ratio above 1.⁹ For ordinal outcomes such as ventilator free days, ICU free days, hospital-free days and time to hemostasis we will use the Wilcoxon rank sum test. With a sample size of 290 in each group we will have greater than 80% power to detect a

probability of 0.430 that ventilator free, ICU free, and hospital free days differ between the 1:1:1 group and 1:1:2 group with a 0.050 two-sided significance level.¹⁰ We are considering time to hemostasis as an ordinal outcome for the reasons discussed in the analysis plan. For a time to event analysis of time to hemostasis 580 patients (290 in each group) will give us over 90 percent power in this study to detect a treatment difference with a 2-sided alpha of 0.05, of a hazard ratio over 1.4.¹¹

4.2 Sample Size for Laboratory Modeling

Because no previous prospective and comprehensive characterization of coagulopathy and inflammation after trauma currently exists, and the definitions of phenotypes, while suggested by *preliminary data (elevated INR, activation of anticoagulant pathways, dilution, hypothermia, etc.) are not codified, we expect to use the entire cohort of 580 for the systems biology (exploratory) analyses. This is predominantly a multivariate modeling approach, aimed at hypothesis generation rather than hypothesis testing.* Due to the nature of the corresponding analysis methodology (e.g., dynamic Bayesian networks, ensemble classification algorithms), it would be impossible to carry out a straightforward power analysis. Given the exploratory nature of this aim, we cannot determine the exact dimensionality and size of the models that may emerge. However, if we limit ourselves to the immediate Markov neighborhoods of the primary and secondary laboratory research outcome variables (i.e., perform automated variable selection), the dimensionality of the resulting sub-networks should be favorable for the purposes of model validation (using resampling techniques such as bootstrapping) and subsequent predictive modeling (Laboratory Aim 3).

Once phenotypes and relationships are identified, we will use more traditional statistical analyses to assess impact of the phenotypes and interactions among the phenotypes on outcomes. Based on work by Harrell, with 580 subjects, depending on the final model chosen we can build linear regression models that include up to 58 variables where outcome is continuous (amount of blood products, etc.), and logistic models that include up to six variables where the outcome is binary (mortality, MOF, etc).¹²⁻¹⁶ If the number of variables exceeds the number that can be included in a linear or logistic model we will prescreen using a p value of <0.25 to select the subset to include in the model. We may need to conduct separate analyses of the selected phenotypes depending on the number of baseline covariates of interest. This serious limitation of traditional statistical approaches emphasizes the need for the initial more complex approaches to understanding coagulopathy and inflammation as described in the analysis below.

5. ANALYSIS PLAN

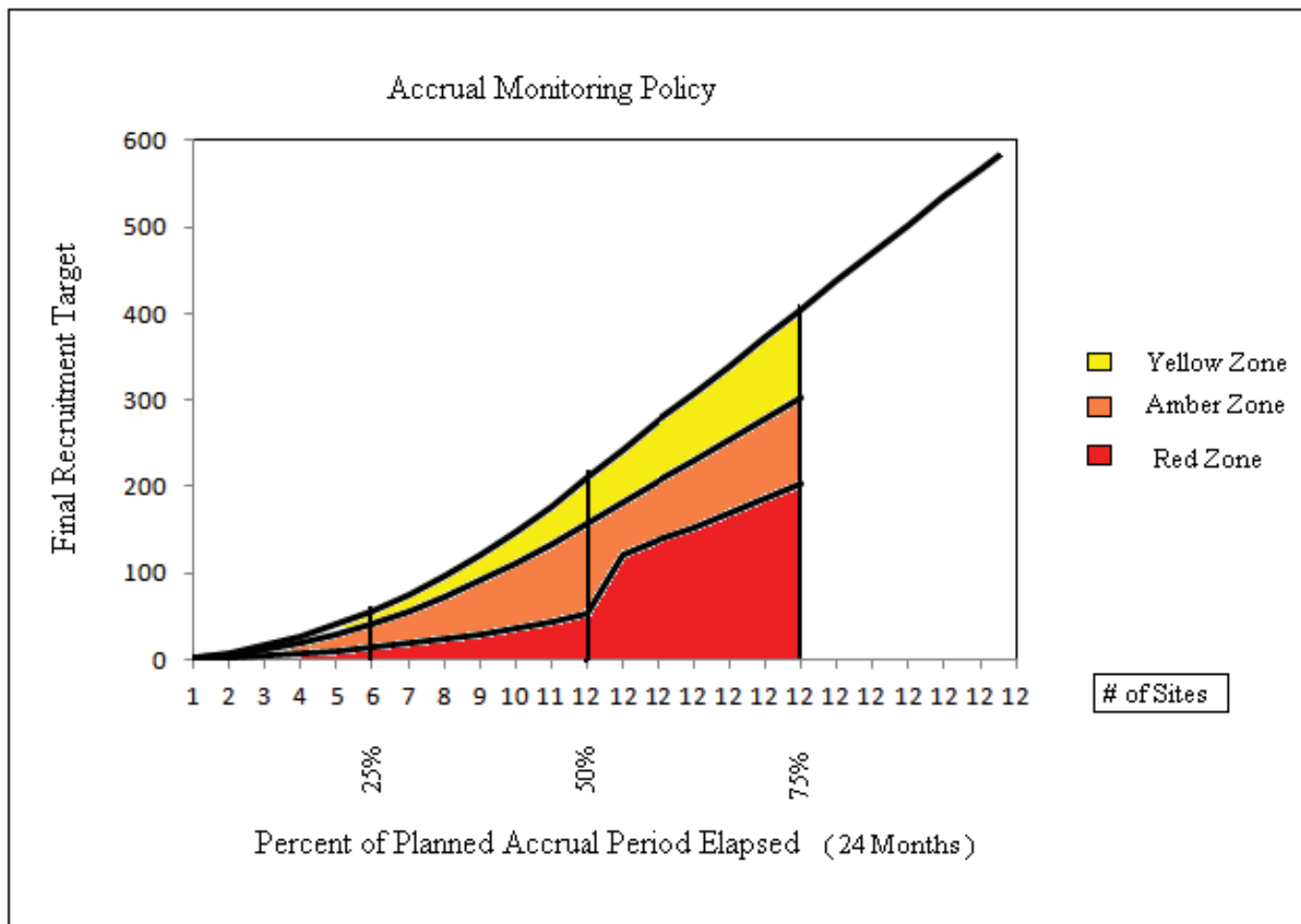
5.1 Vanguard Stage of Trial

The Vanguard stage of the trial begins when at least 4 centers are enrolling and lasts for up to 6-months or to the time 25% of projected enrollment, whichever occurs earlier. The DSMB and ROC will review blinded data on recruitment, protocol deviations, laboratory data collection, data quality and adherence to study procedures. The DSMB will develop recommendations for NHLBI to continue with the trial without modification, continue with modification, or to discontinue the trial and re-design. The DSMB will determine if the Vanguard data can be included in final trial data. We will analyze the following benchmarks as priority considerations for determining feasibility:

5.1.1 Recruitment

We will descriptively (graphically) compare the hypothesized timeline for recruitment to the observed time line for recruitment. Based on unpublished site selection data and PROMMTT there is expected to be an average of 2.7 patients/month/site over 24 months. The following projected enrollment graph takes into account the sequential nature of the addition of sites throughout recruitment (assuming 1 site per month until 12 sites are reached). In addition we will use as a guide for determination of suboptimal recruitment as suggested by the NHLBI accrual guidelines (link to reference: http://www.nhlbi.nih.gov/funding/policies/accrual_guidelines.htm). This is demonstrated in Figure 2 below.

Figure 2: Projected PROPPR Accrual: The delineation of Yellow (accrual is $<100\%$ and $\geq 75\%$ of benchmark), Amber ($<75\%$ of the benchmark, but above the minimally acceptable levels (as defined by the Red Zone)), and Red Zone ($<25\%$ at the 25% time point or $<25\%$ at the 50% time point or $<50\%$ at the 75% time point).



5.1.2 Protocol Compliance/Adherence

We will analyze data quality including missing data, error patterns, protocol violations to determine if modifications in the protocol or data collection procedures or trial manual of procedures are needed or to determine if the protocol itself can be followed.

5.1.2 (a) Site Performance Metrics

The following metrics will be used to evaluate site performance:

- Time to Complete Community Consultation
- Time to IRB Approval
- Time to 1st Enrollment

All sites are encouraged to begin the community consultation and IRB approval processes within their own institutions as soon as FDA approval is obtained.

5.1.2 (b) Site Performance Metrics of Protocol Compliance

- Protocol deviations (both self-reported and study monitor evaluation)
- Time to blood product container delivery

- Time from admission to randomization
- Missed/unable to screen subjects
- Volume of Data Queries
- Evaluation of Source Documents and CRF's (study monitor site reports)
- Site Response Time (timely data entry, submission of regulatory documents)
- Adverse Events Management
- Site lab adherence to lab sampling process (processing/shipping errors)

5.1.2 (c) Monitoring of Sites

Each site will receive monthly reports of each of the above metrics. Depending on the number, severity (or quality) of the non-compliance metrics a site will come under review for participation in the study. These metrics will be considered in relation to the other participating sites and a final decision of corrective action plan will be made by the HCCC. The sites will also receive reports on site visits by the HDCC, and reports on data monitoring as will be described further in the Manual of Operations.

5.2 Phase III Trial Analysis

5.2.1 Treatment Group Comparability at Baseline

Summary statistics for the following baseline variables will be computed and compared between treatment groups. The statistical tests for comparison will be the Wilcoxon rank sum test for continuous scale variables and Fisher exact tests for categorical variables. Summary statistics for the baseline variables listed will be presented by treatment group for all randomized. Also completers will be compared to non-completers on these baseline variables.

Baseline Variables

- Demographics:
 - *Age*
 - *Gender*
 - *Race/ethnicity*
- Glasgow Coma Scale
- Blood Pressure
- Pulse
- Severity of injury (described by several different measures,¹⁷ ex. AIS, RTS, ISS, TRISS)

EMS/Pre-Hospital Care:

- Mechanism of Injury
- Pre-Hospital Care
- Pre-Hospital Life Saving Interventions
- Pre-Hospital Fluids and Blood Products

In addition we will include summary statistics on the following variables:

- ABC Score
- TASH Score
- Anticoagulant Medications Prior to Injury
- Procoagulant Medications Given After Injury
- Fluids and Blood Products Given Prior to Randomization
- Clinical Lab Results from Pre-randomization Blood Sample
- Life Saving Interventions Prior to Randomization

5.2.2 Primary Clinical Analysis

Primary Clinical Aim: To separately compare as co-primary outcomes, 24-hour mortality and 30-day mortality between 1:1:1 and 1:1:2 groups adjusting for clinical site.

Primary Clinical Hypothesis 1: A greater proportion of subjects who are predicted to have a massive transfusion and randomized to the 1:1:1 ratio group will survive to 24 hours after Emergency Department (ED) admission compared with subjects randomized to the 1:1:2 ratio.

Primary Clinical Hypothesis 2: A greater proportion of subjects who are predicted to have a massive transfusion and randomized to the 1:1:1 ratio group will survive to 30 days after ED admission compared with subjects randomized to the 1:1:2 ratio.

Analyses for the Phase III trial co-primary outcomes (24-hour and 30-day mortality) will be intent-to-treat. As stated in section 6 of the Protocol, the two outcomes will be considered as separate study questions and both outcomes will be reported in the initial report on the PROPPR trial. We will compute mortality at both 24 hours and 30 days. The process for determining whether or not a subject is deceased at 30 days is described in detail in the protocol. We will analyze 24-hour and 30-day mortality as a fixed point in time using a two-sided Mantel-Haenszel (M-H) test taking site, the stratifying variable, into account. This approach has more power than the survival analyses described below given the potential for crossing hazard functions.¹⁸ We will also test homogeneity of the odds ratios across sites using the Breslow-Day test. The M-H test is robust to lack of homogeneity of odds ratio although power would be reduced. We will compute 95% confidence intervals on mortality by treatment group at 24 hours and 30 days. We have extensive efforts (described in the last paragraph of Section 5.2.5 in the protocol) developed to capture all data and anticipate less than a 10% of the subjects will be missing vital status at the 30 day outcome. The DSMB will be informed of the amount of missingness observed and will carefully monitor the amount of loss to follow-up throughout the trial and will call for further corrective actions if deemed necessary.

To provide further insight we will compute 30-day Kaplan-Meier survival curves.¹⁹ To compare survival between treatment groups, we will use Cox proportional hazards regression to take site (as a random effect) into account.²⁰⁻²¹ As an ancillary analysis, we will adjust for other demographic variables and for type and severity of injury. We will use a similar approach to explore the effect of pre-randomized blood products on survival. Before interpreting the Cox analyses, we will test for proportional hazards (see discussion below).

Some subjects who are predicted to receive a MT and randomized may not have an MT either because this represents a treatment effect, or because they died before having a chance to receive an MT, or because they were misclassified. We will include all subjects in all primary and secondary analyses in the Phase III trial as randomized.

Adjusting for baseline covariates:

We will compare hazard rates for mortality between treatment groups adjusting for age, gender, type and severity of injury, amount of pre-randomization blood products received, time to randomization and site (as a random effect). We will follow the approach below to test for and take crossing hazards into account if applicable. As an additional analysis, we will compare mortality in the two groups adjusting for the covariates listed above and any additional baseline covariates that are imbalanced between treatment groups. If there are too many covariates to include in the model we will use a prescreening approach, testing covariates at the 0.20 level and including those that meet the latter criteria for significance.

Testing for proportional hazards:

The validity of Cox regression model relies heavily on the assumption of proportionality of hazards. There are certain types of non-proportionality that will not be detected by the tests of non-proportionality alone but that might become obvious when looking at the graphs. We will use both graphical methods²² and statistical tests to check the proportional hazards assumption.

We first use the graphic methods for detecting violations of the proportional hazards assumption. The plot of survival curves are based on the Cox Model and Kaplan-Meier estimates for each subgroup decided by covariates. Clear departures of two estimates indicate evidence against the assumption of proportional hazards. Another plot to be used is the plot of difference of the log cumulative baseline hazards versus time. Under proportional hazards, this plot is constant over time and centered on the estimated log-hazard ratio. Any time trend of the difference will suggest the violation of the proportionality assumption. Note that both plots only inform us if baseline hazards are proportional or not, and do not give detailed information about the type of departure from the proportionality.

The plot of martingale residuals could be further applied to determine the functional form to be used for a given covariate to best explain its effect on survival through a Cox proportional hazards model. The best functional form could be a transformation of the covariates (Z), such as $\log Z$, or it may be a discretized version of the covariate. Under this situation, the martingale residuals are useful for determining cut points for the covariates. For example, we assume that Z_1 is a single covariate of the covariate vector Z for which we are unsure of what functional form of Z_1 to use. Let $f(Z_1)$ be the best function of the covariate Z_1 to explain on survival. To find the form of the function f , we will fit the data based on Z and compute the martingale residuals. Then we plot these residuals against the values of Z_1 . The smoothed-fitted curve then gives an indication of the best function. For example, if the plot is linear, no transformation of Z_1 is needed. If the plot is a piece-wise constant, then a discretized version of Z_1 is suggested.

To formally test the assumption of the proportional hazards for the treatment effect, we will generate a time treatment interaction and refit the model to include the time treatment interaction. If the effect of the time treatment interaction is significantly different from zero, then the proportionality assumption is violated, and we will include a time treatment interaction in the model and choose the appropriate non-parametric approach.²³

5.2.3 Analyses of Ancillary Clinical Outcomes

It is important to note that we consider the ancillary analyses described here as exploratory. We are aware that the trial was not designed with power to detect any specific differences we may see in these outcomes, but the ancillary outcomes are considered important enough to investigate to provide further insight into the overall aims of the trial. Given the many analyses we plan to perform, we will use confidence levels as the primary approach to interpretation and will not adjust for multiple comparisons.

Ancillary Clinical Aim:

To compare subjects predicted to have a massive transfusion and randomized to the 1:1:1 or 1:1:2 ratio groups on a variety of ancillary clinical outcomes measured from randomization to initial hospital discharge after adjusting for site.

Ancillary Clinical Hypotheses 1:

Subjects predicted to have a massive transfusion and randomized to 1:1:1 will differ in number of hospital-free, ventilator-free, and ICU-free days from the 1:1:2 ratio group.

Ancillary Clinical Hypothesis 2:

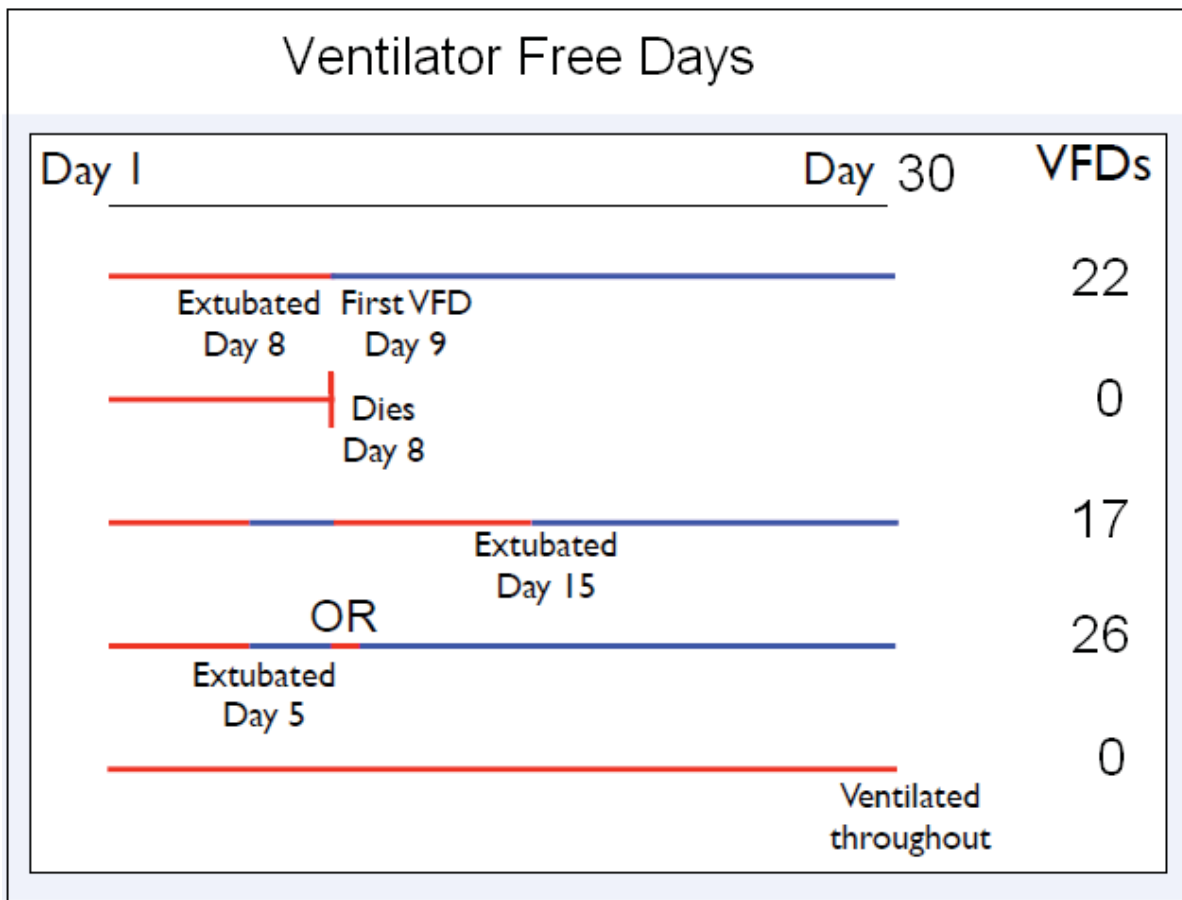
Subjects predicted to have a massive transfusion and randomized to the 1:1:1 and 1:1:2 ratio groups will differ in time to hemostasis, major surgical procedures, and in the incidence of transfusion-related serious adverse events during initial hospitalization; will differ in the amount of study blood products given to hemostasis and in the amount of blood products given from hemostasis to 24 hours; and will differ in functional status at initial hospital discharge, and in initial hospital discharge status.

Hospital-free days, Ventilator-free days, ICU-free days:

Statistical comparison of two groups treating “free days” as a continuous variable will be performed.²⁴ Free days will be defined as the number of days an individual was not in the hospital or on the ventilator or in the ICU within the 30-day follow-up period. Individuals dying during 2 hour treatment window will be scored as 0 days.

The figure below illustrates how ventilator free days would be calculated in different scenarios.

Figure 3: Ventilator Free Days



Let $\Delta_i = 1$ if subject is alive at 30 days and 0 if subject is dead at 30 days and let X_i = number of days (up to 30) out of hospital (or off ventilator, or not in ICU). Then Δ_i is survival at 30 days and the $X_i | \Delta_i = 1$ indicates the duration of time out of hospital (or off ventilator or not in ICU) if the individual survives to day 30. In this way we can treat the “free days” as a composite outcome of free days if a person survives and for individuals who die the free days are set to 0. Boxplots showing mean, median and quartile values for the number of “free days” will be given separately for each treatment group and we will use a Wilcoxon rank sum test to test for effects on both outcomes.

We have chosen the construction of the composite outcome instead of using the classic time to event data

analysis (a log rank test of time to event of interest) to allow for treating the “bad outcome” of death and extended stays in hospital, ICU and on ventilation equally. Patient death or patients ventilated throughout the study period (never having ventilator free days) are both considered clinically bad outcomes. In this way, assigning both of these extreme scenarios equally (both are given the assignment of 0) allows for us to look at the other cases of patients that were well enough to survive to be in the hospital, ICU, on ventilation, and reach hemostasis as an event and test for a difference in these events by treatment group.

The composite outcomes are limited. For example, those who are off a ventilator for a long period of time and later die are counted as having zero time off ventilator. More complex analytical approaches will be explored to deal with this limitation.

Incidence of Major Surgical Procedures:

The group difference, with its confidence interval for incidence of major surgical procedures, will be calculated by using normal approximation. Major surgical procedures are defined in the Manual of Operations. Logistic regression will be used to compute a confidence interval for the odds ratio for the incidence of each class major surgical procedures adjusting for baseline covariates. If the incidence of major surgical procedures is very low, the Clopper-Pearson method will be performed to find the exact confidence interval of the incidence for each treatment group.²⁵

A subject could experience multiple major surgical procedures during hospitalization. Assuming the recurrent event comes from a Poisson process²⁶, we can calculate a confidence interval for the group difference in terms of frequency of multiple major surgical procedures during hospitalization. The Poisson regression model for recurrent event data will be used to compute a confidence interval of the group difference for the means of major surgical procedures adjusting for baseline covariates.

Complications during initial hospitalization:

Again a subject may have multiple complications of the same subtype during hospitalization. We will also treat the occurrence of complications during hospitalization as total count of the number of events a subject may experience. Statistical comparison of two groups will parallel those for major surgical procedures during hospitalization described above.

Type and amount of study blood products used from randomization until hemostasis is achieved, type and amount of blood products used after hemostasis is achieved to 24 hours post admission:

These variables could include for example crystalloid and artificial colloid use which is not standardized or randomized, but will be recorded throughout the trial. In addition the use of pharmacological adjuncts (rFVIIa, amicar, tranexamic acid, PCCs, fibrinogen concentrates, etc) and cryoprecipitate for hemorrhage control while not standardized or randomized will be recorded throughout the trial.

Functional status at hospital discharge or 30 days, whichever comes first, as measured by discharge destination and GOSE:

Discharge destination (eg. home, rehabilitation, nursing home) is a categorical variable. These data will be analyzed using a multinomial logistic regression, again adjusting for baseline covariates. The Glasgow Outcome Scale Exam (GOSE) is an ordered categorical variable and will be analyzed using the Proportional Odds model (which gives us the ability to adjust for clinically important covariates) will be considered and also a multinomial logistic regression model (baseline logit model) with an appropriate base category selected.

Time to Hemostasis:

Kaplan-Meier curve with confidence intervals for time to hemostasis will be given separately for each treatment group. Those who die before hemostasis is attained will be censored at the time of death. Cox

proportional hazards model or other appropriate semi-parametric survival regression models will be applied to take baseline covariates into account for treatment comparison. If there are crossing hazards we would follow the approach described above for mortality. Hemostasis is defined in the protocol.

Time to hemostasis is one of the ancillary outcomes (as are the hospital-free, ICU-free and ventilator-free days outcome) that we acknowledge are susceptible to the informative censoring/missingness bias. For time to hemostasis, censoring could be informative. Those who are censored have died and may have died because they could not achieve hemostasis, i.e. they bled to death. In light of this we will also analyze time to hemostasis using a composite endpoint similar to that discussed above. However for time to hemostasis, a shorter time to hemostasis is considered a good outcome, so 24 hours will be used for those who die.

In general many of the ancillary analyses may require further development prior to the final analysis of the outcomes. If a new methodology is developed or identified prior to the end of the trial that allows us to redefine the constructs to provide additional clinical utility then, with the approval of the DSMB, the Statistical Analysis plan will be revised.

5.2.4 Laboratory Hypotheses and Aims

Overall Laboratory Hypothesis:

Subjects predicted to have a massive transfusion will differ in their coagulation and inflammatory phenotypes at admission and over time which will be affected by resuscitation and affect outcome.

Laboratory Aim 1:

To develop models characterizing TIC in enrolled patients at ED admission.

Hypothesis 1:

Severely injured trauma patients enrolled into PROPPR will differ in their coagulation and inflammatory phenotypes at admission by subjects' demographic and baseline injury characteristics.

Laboratory Aim 2:

To develop models characterizing the dynamics of TIC in order to identify mechanistic drivers and sequelae of coagulation and inflammation, and to characterize the natural history of the coagulation/inflammatory milieu in enrolled subjects.

Hypothesis 2:

Coagulation and inflammatory phenotypes identified at admission will display dynamic changes. These phenotype changes will be driven by injury demographics and resuscitation.

Laboratory Aim 3:

To assess the effect of key components of coagulation and inflammatory models on primary and ancillary outcomes.

Hypothesis 3:

Coagulation and inflammatory profiles identified in *Laboratory Aims 1 and 2* will be associated with primary and ancillary clinical outcomes.

Models will be developed to identify drivers and sequelae of TIC and inflammation, and to characterize the natural history of the coagulation milieu. The principal modeling approach will be reverse-engineering of the biological networks from the research laboratory data augmented by the existing expert knowledge. Both baseline (Laboratory Aim 1) and dynamic (Laboratory Aim 2) models will be developed. When interpreting the resulting models (Laboratory Aim 3), special emphasis will be put on the primary and ancillary clinical outcome measures for laboratory analyses, including mortality, time to hemostasis, incidence of coagulation abnormalities, total blood product transfusions, incidence of organ injury (i.e., acute lung injury and acute renal failure) and ventilator

associated pneumonia, 30-day mortality, ventilator-free, ICU-free and hospital-free days and incidence of nosocomial infections.

5.2.5 Analysis of Laboratory Data

A systems level framework is necessary to produce predictive models capable of diagnosing coagulopathic phenotypes and assessing the effectiveness of hemostatic resuscitation measures. Our goal is to develop an *in silico* model of coagulation to better understand the perturbations of this system after trauma. To accomplish this goal we will both expand our existing coagulation network model, and construct new network models of Protein C, complement, and coagulation in general from our PROMMTT and legacy data, as well as data from the measurements and clinical data in the PROPPR trial. Specifically, we will scrutinize the sub-networks representing structure/ function relationships of protein C and coagulation, and their interactions after injury.

Our analysis is divided into two overlapping modeling goals: A) building a network and functional model of coagulation (Laboratory Aims 1 and 2) and B) predictive modeling (Laboratory Aim 3), using predominantly machine learning methodology. Each is distinct but complimentary and serves to inform the other model (for example, the latter would provide additional insights on the variable selection for the former). Ultimately our descriptive and predictive modeling efforts will involve the following steps and methods:

1. *Network Expansion and Construction.* We will begin by expanding our existing preliminary network model of coagulation to include all links to all known nodes up to 5 degrees away (i.e., in the extended Markov neighborhood) from protein C and proteins included in the classical coagulation cascade and complement system. Additional network proteins will be added to a network spreadsheet and imported into MatlabTM Pajek 1.8 (The Mathworks, Inc., Natick, MA) and Cytoscape 2.0 (Cytoscape Consortium, San Diego, CA) bioinformatics network software for visualization and analysis. This is a methodologically straightforward step that will lead to the creation of baseline networks (Laboratory Aim 1).

2. *Network Analysis.* Topological calculations of degree, degree exponent γ (where $P(k) \sim k^{-\gamma}$, path length, cluster coefficient of each node ($C_i = 2n_i/k(k-1)$), average cluster coefficient ($C(k) \sim k^{-1}$) and edge-betweenness (cluster decomposition) will be calculated with Cytoscape 2.0 and Guess.5 and MatlabTM. Network topology will be mapped onto outcomes including coagulopathy, and infection. In this manner we will test the relation between perturbations in topology with the outcome of coagulopathy and infection. Again, this is a computationally straightforward step that will result in developing reference networks relevant to the Laboratory Aims 1 and 2.

3. *Dynamic and Data-driven Model(s) Construction.* We will next construct dynamic network (ordinary differential equation - and dynamic Bayesian networks - based) models of the central coagulation system and its relationship to inflammation in general.²⁷⁻²⁹ These will serve as starting points (topology priors) for accomplishing Laboratory Aim 2 --- we will follow up by reverse-engineering (using our proprietary Bayesian network modeling software³⁰) data-driven network models from a subset of data from this project, the currently ongoing PROMMTT study, and protein C activation data from both steady-state (non-injured) and perturbed (injured) conditions. This cumulative model-refining process will continue as new experimental data are collected and new hypotheses are developed.

4. *Variable Importance Analysis.* In a parallel line of research to the biological network modeling above we will be creating statistical and computer science – based models (classifiers) to support treatment decision for optimal outcome given clinical observations. Due to the large number of clinical, physiological, and molecular variables we are proposing to collect, a necessary first step is determining which of these, by themselves or in concert, are most important to outcome, a task known as “variable selection”.³¹⁻³² We will pursue various variable selection strategies that take into account variable interactions, including the Bayesian network Markov neighborhood analysis, ensemble decision tree classifiers and other (mostly machine learning) methods.³³⁻³⁴ This analysis is directly relevant to the Laboratory Aim 3, but will also retrospectively influence our network modeling activities (Laboratory Aims 1 and 2).

5. *Clinical Prediction Analysis.* The next goal is to define statistical or computer science-based predictive models that can be used to identify subjects at high risk of a clinical outcome.³⁵ We will use machine learning techniques (ensemble decision tree classifiers, support vector machine classifiers and possibly naïve Bayesian

classifiers) to find predictors with high specificity and sensitivity. From our experience, as well as from the recent literature, we expect these techniques to perform better (in terms of generalization classification accuracy, robustness and scalability) than the more traditional regression methods. We will finalize our analyses by using a Superlearning approach.³⁶ This approach expands the typical machine learning classification algorithms to construct final predictive models that are combinations of several machine learning classifiers, thus avoiding possible method-related biases, and guaranteeing substantially improved robustness. In addition to the complex systems analyses that would be conducted we will also use more traditional statistical models for Laboratory Aim 3 incorporating phenotypes and interactions identified in Analysis #4 above. Linear models would be used for Laboratory Aim 3 to test the association between identified phenotypes and outcomes that are continuous (amount of blood products, etc) and logistic models to test associations with categorical outcomes (MOF, etc). These analyses will take appropriate baseline covariates and treatment group into account to assess the effect of the phenotypes beyond the effect of these covariates. This will accomplish Laboratory Aim 3.

6. *Model Validation.* We will use statistical resampling approaches (bootstrapping, dataset subdivision and cross-validation) to provide model validation. Taken together, our data and models will result in the first comprehensive natural history description of acute traumatic coagulopathy.

Our modeling goals are ambitious but both types of models lead to predictions that will be clinically tested. The results will themselves inform the second generation of models (thus going back from the Laboratory Aim 3 to Aims 1 and 2). This is one of the key strengths of this program—the tight coupling of clinician to analyst, sometimes in the same person. The project itself will adhere to a tight management scheme in which the teams meet around the continuously updated models to discuss their completeness and their descriptive and predictive accuracy. The models will, therefore, also serve as precise communication tools for the project scientists. Ultimately these models will identify a group of mediators that define coagulopathic phenotypes after trauma, and can be used to guide personalized medical and surgical treatment for wounded patients.

5.2.6 Economic Analysis

We will perform a detailed “piggy-back” economic study on the clinical trial. This includes the collection of ICD-9 code data that will allow estimation of severity-specific cost of an admission. We will use decision analysis modeling to estimate lifetime economic cost and consequences expected for the outcomes subgroups in the clinical study. A more detailed outline of the analysis plan can be found in the Appendix.

5.3 Cause of Death

For subjects not reported as deceased by day 30 following ED admission, batch searches of the mortality databases will continue every quarter until trial close-out. Date (and cause of death when available) for out-of-hospital deaths will be documented; however, underlying and contributing causes of death will most likely be unavailable from these sources. Selected elements from the medical records (OR clinical notes, ED History & Physical, initial CT scans, etc.) will be collected in a HIPPA compliant manner and presented to a death adjudication committee for all in-hospital deaths (within 30 days of ED admission), for subjects enrolled in this study. Cause of death data will be displayed descriptively by treatment group. The adjudication of cause of death is outlined in the Data Safety Monitoring Plan.

5.4 Subgroup Analysis

Before proceeding to the test of treatment effects within sub-groups we would ask for a clinical rationale. We would test for a sub-group treatment interaction at a 0.2 critical level to reduce the potential for spurious results. Any subgroup analyses would be considered post hoc and reported as requiring confirmation in future studies.

Analyses of post-randomization sub-groups are subject to many biases. For example, the presence/need for massive transfusion can be considered an outcome. We could test for a difference in this outcome between treatment groups since it is possible that MT post-randomization is influenced by treatment. However, a sub-

group analysis of treatment effect within MT (yes, NO) would be an example of post-randomization analyses the would be subject to confounding or effect modification and misinterpretation if we concluded that treatment was effective in those with MT but NOT effective in those without MT. Thus any analyses of post-randomization sub-groups would be considered on a case by case basis requiring tailored use of advanced statistical methods³⁷ and careful interpretation.

Site Effects

We will stratify on site in randomization and will include site as a stratification variable in the primary analysis using Mantel Haenszel and as a random effect in other analyses as described above.

Pre-treatment scoring systems to Predict Massive Transfusion and Substantial Bleeding

At screening, in addition to collecting ABC scores³⁸, we are collecting data for the Trauma Associated Severe Hemorrhage (TASH) Score³⁹ to allow comparisons between the two scores. This will only be possible if the data can be collected prior to randomization. If a subject is randomized and treated, it is possible that the treatment can reduce the number of MTs and interfere with any assessment by instrument, particularly if the score is based on post-randomization data. If a newly validated scale becomes available prior to the start of the trial, we will also collect data on that scale. We will use MT status on all screened subjects (whether or not a subject is randomized into the trial), to assess the screening instruments.

By ascertaining MT status on all screened subjects (whether or not a subject is randomized), we will assess the performance characteristics (positive predictive value and sensitivity) of the MT predictive algorithm and the combination of the MT predictive algorithm with attending trauma surgeon's over-ride (if algorithm score is negative, but trauma surgeon's judgment is positive for a predicted MT patient). Due to concerns that subgroup analyses using the traditional definition of MT introduces survival bias by excluding the patients who die early with no chance to receive 10 units of RBC, the PROMMTT study investigators developed an alternate definition (substantial bleeding) for their subgroup analyses. They defined substantial bleeding as receipt of the first RBC unit within 2 hours of ED admission and, by hour 4, either at least 5 consecutive RBC transfusions < 2 hours apart or death within 2 hours of the last RBC transfusion. Survival bias in subgroup analyses will be avoided by assignment of predictive algorithms. Use of the substantial bleeding definition as the dependent variable (gold standard) to assess the performance characteristics (sensitivity, positive predict value, area under the ROC curve) of the combined ABC score and physician judgment in PROMMTT patients gave higher values than use of the traditional MT definition. PROPPR will proceed similarly and compare the use of MT and substantial bleeding definitions in assessing the performance of the combined ABC score and physician judgment in identifying the true MT or substantially bleeding patients among all screened patients.

We will compare scores on sensitivity and positive predictive values using a binomial test of proportions. We will also compute 95% confidence limits on each parameter for each score.

5.5 Missing Data

Under the ITT principle, all patients who are randomized are included in the analyses. We do not expect missing data for 24-hour mortality; however, given the transient nature of many of the subjects, extensive efforts will be made to ascertain 30-day mortality (see section 5.2.6 in the Protocol on Data Collection). For interim and final analyses, subjects who have not been reported as deceased by day 30 following ED admission from any of the searched sources, we will use multiple imputation for the final value. We will also include an analysis consistent with that used in other trauma studies counting those missing as alive on day 30. An analyses with and without imputation will be carried out.

For multiple imputation we will use a general location model^{40,41} for participants with missing information on mortality using the covariates specified in the Statistical Analysis plan for the Cox Proportional hazards model (age, gender, type and severity of injury, amount of pre-randomization blood products received, time to randomization, site and the treatment assignment). This model will be used to generate several pseudo-complete records for each of the individuals with missingness. We will then conduct the primary data analysis for each of the imputed data sets and the results of these analyses will be combined using Rubin's rules.⁴²

In the event that the missingness mechanism is MNAR we will conduct sensitivity analyses under a selection model approach.

Current follow up experience with ROC suggests that for the non-MT patients that survived to hospital discharge (including those known to survive to 28 days) only about 6% were of unknown status. For the MT patients there were only 1% of unknown status. Efforts to keep loss to follow-up for mortality below 10% are described in last paragraph of section 5.2.5 of the Protocol. For other exploratory outcomes, we will also use a multiple imputation approach for missing data where an ITT analysis is required. We will also perform a sensitivity analysis of those patients who are completers vs non-completers within each treatment arm as well as for the overall study.

In addition, some of the ancillary outcomes proposed in this study (hospital-free days, ICU-free days, ventilator-free days, time to hemostasis) are at risk for informative censoring/missingness.⁴³ This occurs when a baseline covariate is associated with both the outcome and the chance that that outcome is censored/missing. As described above, we will analyze these outcomes using both traditional and other methods that adjust for the informative censoring patterns within the model.⁴⁴⁻⁴⁵

Some of the analytical approaches we are using are in a field that is under development. Prior to the analysis of the co-primary outcomes, if new methodology becomes available that will enhance the methods proposed then they may be adopted prior to the time of the primary trial analysis. If a revision occurs then, with the permission of the DSMB, this Statistical Analysis plan will be revised.

6. MONITORING FOR EFFECTIVENESS AND SAFETY

6.1 Adaptive Design

A subcommittee of the DSMB, consisting of a statistician, a trauma expert, and a hematologist, will review the early mortality data (before the formal 1/3 mortality analysis) by treatment group without a statistical analysis. This would be done at each meeting until the adaptive design analysis is conducted.

If they believe these data raise major safety concerns regarding trial continuation, they would alert the full DSMB, who would then have the option to see and request formal analysis of these data by treatment group. If these early data do not create cause for concern by the DSMB subcommittee, the other DSMB members will see only the aggregate deaths until the adaptive design analysis occurs just before the first interim look (when about 1/3 of anticipated deaths have occurred). Per FDA guidelines for adaptive designs,⁴⁶ we will report on the power for the trial based on the observed 24-hour mortality rate in the 1:1:1 group (the comparator arm) only. This analysis will be conducted by the blinded ROC DCC biostatistician. This biostatistician, as described below in Section 6.2, will remain blinded during the trial. This subcommittee would not be able to participate in the adaptive analysis discussion of sample size, if a change is recommended to the DSMB, since they will have seen the data by treatment arm.

If the mortality rate in the 1:1:1 arm is less than 11%, there is no need to consider an adjustment to the sample size as power to detect a 10% difference from 1:1:2 would be increased. If the mortality rate in the 1:1:1 comparator arm is greater than 11%, we will ask the DSMB to consider increasing the sample size to an amount to be determined by the difference between the comparator group rate and a clinically meaningful difference of 10%, two-sided alpha = 0.05, power = 90%.

The DSMB will not be provided with data on the 1:1:2 group and will not consider the observed difference between the two treatment arms if the subcommittee did not express concern after evaluating the mortality data by treatment group. The DSMB will then make a recommendation to NHLBI to maintain the sample size as planned, or to increase the sample size a specified amount based on the observed mortality in the 1:1:1 group. Final determination of the amount of the increase in sample size will be made by NHLBI based on availability of funds and based on recruitment progress to date, protocol adherence, and data quality but without any knowledge of the observed treatment group differences. If funds are not available to increase the sample size, we will continue with the rest of the trial as originally planned but acknowledging the reduction in power. Once this recommendation is made, the DSMB would then proceed with its regular meeting reviewing the interim analysis and safety analysis. This later discussion could, of course, change the recommendation if a decision was made to recommend trial termination for reasons of effectiveness or safety. No further consideration of a sample size increase would be made once the DSMB has seen the interim analysis.

6.2 Interim Analyses for Effectiveness

There will be three formal effectiveness analyses. The two interim analyses for the DSMB will occur after the first 1/3 and 2/3 of the projected 24-hour or 30-day mortality events are observed (whichever reaches its projected 1/3 and 2/3 first). The two co-primary outcomes will be separately monitored using a two-sided O'Brien-Fleming boundary with Lan-DeMets alpha spending function based on events for each of the two comparisons.⁴⁷ The boundary is suggested as a guideline for the DSMB and could be modified by the DSMB prior to the start of the trial. Other information could influence their decision to continue (or stop) the trial in the face of a clear difference in either direction between treatment arms. Only the DSMB and biostatisticians associated with the HDCC would see unblinded data by treatment group, possibly by A/B in the same manner proposed by the 2006 FDA Guidance for Clinical Trial Sponsors on the Establishment and Operation of Clinical Trial Data Monitoring Committees, initially depending on the preference of the DSMB and whether or not there was an analysis to consider an adaption of the design (Section 6.1). The biostatisticians in the ROC DCC would remain blinded and would be able to participate in discussions of future changes in the trial design if necessary. The unblinded biostatisticians (HDCC) would not participate in these trial design change discussions.

If the trial stops early because of interim analysis, we will report the adjusted p-values by using the stage wise ordering approach to account for the fact that an unadjusted p-value will tend to overstate the evidence against the null hypothesis in sequential trials.⁴⁸

We will not test for lack of a difference in effectiveness using a stochastic curtailment approach since the null hypothesis is also of clinical interest for both co-primary outcomes. If we cannot detect a difference between groups, we would want to use the full sample size to produce narrow and informative confidence intervals.

6.3 Safety Analyses

The DSMB will review unblinded SAEs, expected complications and other AEs reported by the sites, and clinical laboratory data, if reported, by treatment group coded as A/B in the same manner proposed by the 2006 FDA Guidance for Clinical Trial Sponsors on the Establishment and Operation of Clinical Trial Data Monitoring Committees. If there are obvious complications that would require unblinding, we will use different coded values for safety and effectiveness and the order will not necessarily be the same. We will use a uniform

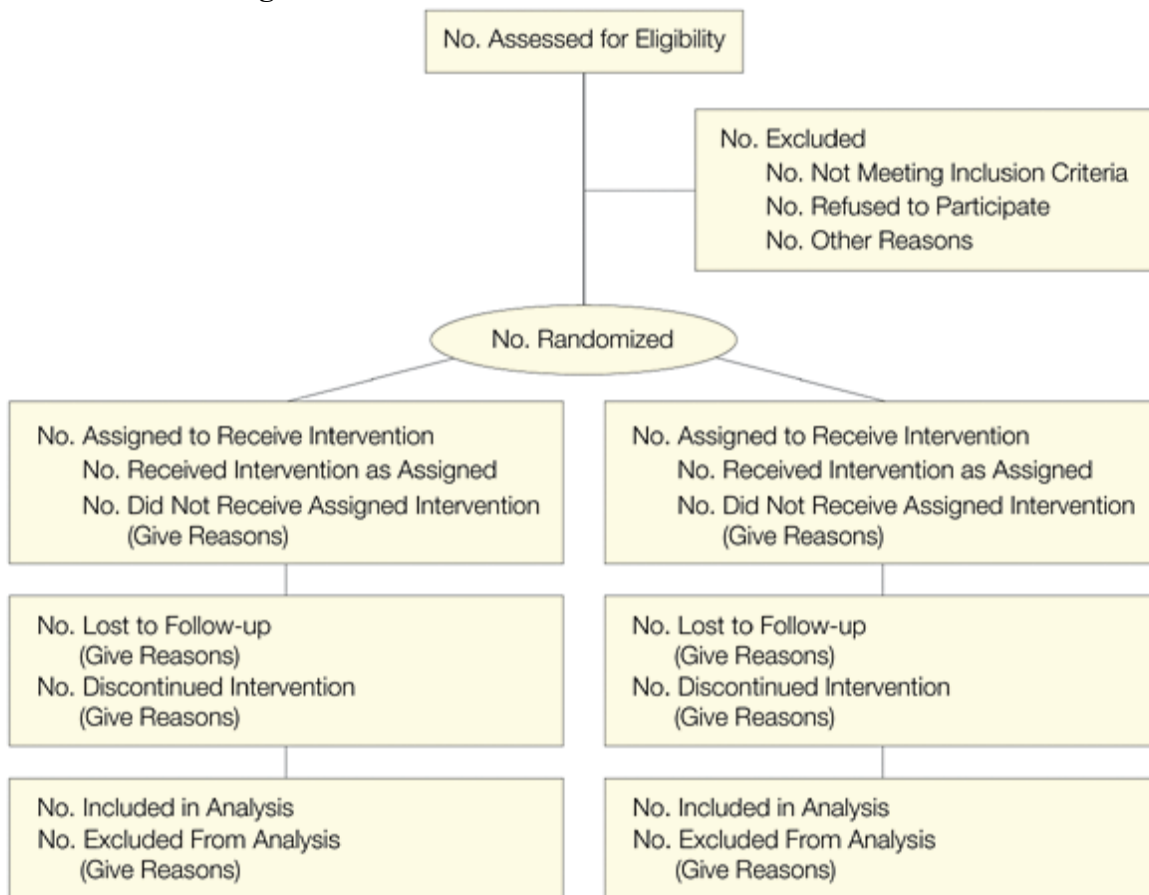
boundary of 0.001. We will also report overall mortality (combined across treatment groups) at each of their regular DSMB meetings regarding PROPPR. If a concern arises regarding the distribution of adverse events or the overall mortality rate the DSMB could become unblinded. However, prior to seeing mortality by treatment group the DSMB would consider whether the adaptive approach to trial design as described above should be considered. Additional safety analyses will be developed as requested by the DSMB.

7. REPORTING PROCEDURES

7.1 CONSORT Diagram

We will account for every subject randomized into the study using a CONSORT diagram.

Figure 4: CONSORT Diagram



Profile of a Randomized Controlled Trial (from <http://jama.ama-assn.org/site/misc/ifora.xhtml#fig>)

7.2 Primary Reporting for the PROPPR Trial

Primary reporting for the PROPPR trial will follow the classic CONSORT Checklist items (see appendix). http://jama.ama-assn.org/site/misc/auinst_chk.pdf.

7.3 DSMB Reports

Standard format for DSMB reports will be developed and sent to the DSMB for review before the initial safety analyses are presented, and the format will be added as an appendix to this report.

7.4 Publications

Before the HDCC begins an analysis for a manuscript or presentation, the first author or writing group will have their hypotheses and analysis plan reviewed and approved by a designated team at the PROPPR HDCC and HCCC.

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Appendix A: CONSORT Checklist

CONSORT CHECKLIST

Table. CONSORT 2010 Checklist of Information to Include When Reporting a Randomized Trial^a

Section and Topic	Item No.	Checklist Item	Reported on Page No.
Title and abstract	1a	Identification as a randomized trial in the title	
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	
Introduction Background and objectives	2a	Scientific background and explanation of rationale	
	2b	Specific objectives or hypotheses	
Methods Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	
Participants	4a	Eligibility criteria for participants	
	4b	Settings and locations where the data were collected	
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	
Outcomes	6a	Completely defined prespecified primary and secondary outcome measures, including how and when they were assessed	
	6b	Any changes to trial outcomes after the trial commenced, with reasons	
Sample size	7a	How sample size was determined	
	7b	When applicable, explanation of any interim analyses and stopping guidelines	
Randomization Sequence generation	8a	Method used to generate the random allocation sequence	
	8b	Type of randomization; details of any restriction (such as blocking and block size)	
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	
Results Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analyzed for the primary outcome	
	13b	For each group, losses and exclusions after randomization, together with reasons	
Recruitment	14a	Dates defining the periods of recruitment and follow-up	
	14b	Why the trial ended or was stopped	
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	
Numbers analyzed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing prespecified from exploratory	
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	
Comment Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	
Generalizability	21	Generalizability (external validity, applicability) of the trial findings	
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	
Other information Registration	23	Registration number and name of trial registry	
Protocol	24	Where the full trial protocol can be accessed, if available	
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	

^aWe strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomized trials, noninferiority and equivalence trials, nonpharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up-to-date references relevant to this checklist, see <http://www.consort-statement.org>.