Henry Gill Cryer, MD, PhD

Clinical Research Protocol

TRANSFUSION OF WHOLE BLOOD IN A CIVILIAN TRAUMA CENTER: A PROSPECTIVE EVALUATION OF FEASIBILITY AND OUTCOMES

Version Date:	04/17/2017
Investigational Product:	Cold stored whole blood
IND Number:	exempt
Development Phase:	Phase 2
Sponsor/Investigator:	Henry Gill Cryer, MD, PhD 757 Westwood Plaza Suite 8501 Los Angeles, CA 90095
Funding Organization:	National Trauma Institute
Principal Investigator:	Name: Henry Gill Cryer, MD, PhD Telephone: (310) 267-9594 E-mail: hcryer@mednet.ucla.edu
Medical Monitor:	Name: William Mower, MD Telephone: (310) 825-2111 E-mail: wmower@mednet.ucla.edu
Coordinating Center:	n/a

Protocol Title: TRANSFUSION OF WHOLE BLOOD IN A CIVILIAN TRAUMA CENTER: A PROSPECTIVE EVALUATION OF FEASIBILITY AND OUTCOMES

Protocol Date: December 5, 2016, revised April 17, 2017

Table of Contents

LIST OF ABBREVIATIONS

AABB	American Association of Blood Banks
AE	adverse event
ALT	alanine aminotransferase
ARDS	acute respiratory distress syndrome
AST	aspartate aminotransferase
BUN	blood urea nitrogen
CAT	Calibrated Automated Thromboelastogram
CFR	Code of Federal Regulations
CRF	case report form
FWB	Fresh whole blood
FDA	Food and Drug Administration
GCS	Glasgow coma scale
HIPAA	Health Insurance Portability and Accountability Act of 1996
IRB	Institutional Review Board
IV	intravenous
MA	Maximum amplitude
mL	milliliter
OHRPP	Office of Human Research Protection Program
PI	Principal Investigator
PPE	Personal Protective Equipment
PRBC	Packed Red Blood Cells
R	Reaction time
RBC	Red blood cell
SAE	serious adverse event
TACO	Transfusion-associated circulatory overload
TBSA	Total body surface area
TEG	thromboelastography
TRALI	Transfusion-associated lung injury
UCLA	University of California Los Angeles
WB	Whole blood

PROTOCOL SYNOPSIS

TITLE	**TRANSFUSION OF WHOLE BLOOD IN A CIVILIAN TRAUMA CENTER: A PROSPECTIVE EVALUATION OF FEASIBILITY AND OUTCOMES**
SPONSOR/INVESTIGATOR	Henry Magill Cryer, MD, PhD
FUNDING ORGANIZATION	National Trauma Institute (Department of Defense/Army Medical Research Acquisition Activity)
NUMBER OF SITES	1
RATIONALE	Our study will test the feasibility of providing whole blood (WB) for resuscitation of adult male trauma patients in hemorrhagic shock and determine the effects of WB on clinical outcomes as well as the effects on coagulation, fibrinolysis, and inflammation, and hemolytic transfusion reactions compared to standard blood component therapy.
STUDY DESIGN	Prospective cohort study
PRIMARY OBJECTIVE	1. Compare volume of blood transfusion within 24 hours of admission between patients receiving whole blood and standard component therapy.
SECONDARY OBJECTIVES	The study will also compare: 1. Functional coagulopathy as measured by thromboelastography;

	2. 30-day mortality; 3. Number of units of blood products transfused by component/product; 4. Complications; 5. Hemolysis; 6. Wastage of blood units.
NUMBER OF PATIENTS	Whole blood arm - 49 male patients over apparent age 18. Component therapy arm –49 male and female patients over apparent age 18.
PATIENT SELECTION CRITERIA	Inclusion Criteria: • Whole blood arm: Male trauma patients over apparent age 18 with massive transfusion activation and presenting systolic blood pressure <100, who are receiving or will receive whole blood for initial resuscitation • Component therapy arm: Male and female trauma patients over apparent age 18 with massive transfusion activation and presenting systolic blood pressure <100, who have received, are receiving or will receive component therapy for initial resuscitation. Exclusion Criteria: • Patients with burns over >20% total body surface area
TEST PRODUCT, DOSE, AND ROUTE OF ADMINISTRATION	Group O+ whole blood stored at 1-6 °C for up to 10 days. Patients may receive up to 4 units during the initial phase of the proposed study, to be increased to up to 6 units (see 5.2 Safety Evaluations) Product is administered to replace ongoing blood loss and restore/maintain hemodynamic stability and hemostasis. Product will be transfused intravenously as needed during initial traumatic resuscitation only. Following administration of whole blood if further transfusion is needed during initial trauma resuscitation, or after resuscitation is completed, the patient will receive standard

	component therapy (packed red blood cells, plasma and platelets in a 1:1:1 ratio).
CONTROL PRODUCT, DOSE AND ROUTE OF ADMINISTRATION	Component therapy consisting of packed red blood cells, thawed fresh frozen plasma and platelets in a 1:1:1 ratio. Product will be transfused intravenously as needed to replace ongoing blood loss and restore/maintain hemodynamic stability and hemostasis.
DURATION OF PATIENT PARTICIPATION AND DURATION OF STUDY	Patient participation includes up to 24 hours of transfusion, up to 5 days of laboratory testing, and up to one year of follow up by chart review. Follow up by chart review will continue throughout the patient's hospital stay or up to one year, whichever is shorter. Most hospital stays are anticipated to be approximately 14 days. **Screening:** not applicable **Treatment:** 1 day / 24 hours. Patients will receive whole blood only during initial resuscitation for traumatic hemorrhage, which is generally complete within a few hours. **Follow-up:** 5 days (laboratory testing) + chart review for up to 1 year if the patient remains in the hospital The total duration of study enrollment and completion of follow up is expected to be two years.
CONCOMMITANT MEDICATIONS	Allowed: all Prohibited: none

EFFICACY EVALUATIONS:	
PRIMARY ENDPOINT	1. Primary analysis will compare volume of blood transfusion within 24 hours of admission for patients with traumatic hemorrhagic shock without severe TBI (GCS ≤8 and evidence of brain injury on imaging) who have received WB versus component therapy for initial resuscitation.
SECONDARY ENDPOINTS	1. Volume of blood transfusion within 24 hours of admission for patients with severe TBI (GCS ≤8 and evidence of brain injury on imaging) and for entire cohort of patients. 2. Thromboelastography parameters including maximum amplitude, reaction time, alpha angle, fibrinolysis, and platelet function at 3 and 6 hours of resuscitation, after every 6 units of whole blood equivalent (WB or PRBCs), at the time of completion of resuscitation (time of last unit of blood product given), and on ICU days 1, 3 and 5. 3. 30-day mortality; 4. Duration of need for renal replacement therapy, mechanical ventilation, ICU admission, and hospital stay; 5. Number of units of blood products transfused by component/product during first 24 hours of admission and total for hospitalization; 6. Complications which include 30-day mortality, clinical coagulopathy, infection, venous thromboembolism, cerebrovascular accident, acute coronary syndrome, transfusion-related lung injury, transfusion-associated cardiac overload; 7. Hemolysis as measured by haptoglobin, bilirubin, lactate dehydrogenase, and direct antiglobulin 8. Wastage of blood units
SAFETY EVALUATIONS	Complications which include death, clinical coagulopathy, infection, venous thromboembolism, cerebrovascular accident, acute coronary syndrome, transfusion-related lung injury, transfusion-associated cardiac overload; Hemolysis as measured by haptoglobin, bilirubin, lactate

	dehydrogenase, and direct antiglobulin.
PLANNED INTERIM ANALYSES	When approximately 50% of patients have been enrolled in the study, an interim analysis for safety will be conducted by the statistician and independent medical monitor. Serious adverse events will be monitored by the medical monitor on an ongoing basis throughout the study.
STATISTICS **Primary Analysis Plan**	For continuous variables including the primary endpoint (volume of blood products transfused during the first 24 hours of admission), the statistical method will depend on the distribution of the data. If the data appears to follow a normal distribution, a t-test will be used, otherwise, a nonparametric Wilcoxian rank sum test will be used. To analyze coagulopathy over time a mixed effects linear regression model will be used. A mixed effects linear regression model will also be used to adjust for confounding variables such as sex, age, comorbidities, severity of injury, and physiologic parameters of shock including pH and temperature , and use of a rapid transfuser (which more effectively warms blood).
Rationale for Number of Patients	A sample size of 49 whole blood group (study drug) and 49 component therapy group (control) patients (98 patients total) is estimated. 18 of these per group are expected to have severe TBI and will be excluded from primary analysis, leaving 31 patients per group for the primary analysis. Using a Type I error rate of 0.05, and effect size based on reduction of PRBC and plasma transfusions described in the sensitivity analysis in a study by Cotton et al, 31 patients per group is expected to have 80% power to detect a significant difference in volume of transfusion in 24 hours. [1]

1 BACKGROUND

Massive hemorrhage is a major cause of potentially preventable death following trauma. [2-6] A common consequence of hemorrhagic shock is uncontrollable bleeding from coagulopathy, leading to death from exsanguination. Even when bleeding is controlled, patients are at increased risk of complications and mortality. [7,8] Reconstituted whole blood, or component therapy with PRBCs, plasma, and platelets was introduced by the military in recent conflicts in Iraq and Afghanistan with remarkable results and has been adopted by most civilian trauma centers. [9] Despite improving coagulopathy, it is apparent that transfusion of blood components is not equivalent to whole blood transfusion. [10-12] Transfusion of high plasma volumes may be associated with increased risk of allergic reaction, transfusion associated acute lung injury (TRALI), hypervolemic cardiac failure/transfusion-associated circulatory overload (TACO), and ARDS. [13]

Military services that have recently reintroduced fresh whole blood (FWB) for standard resuscitation of massive hemorrhage have found that FWB offers a survival advantage over component therapy, and that risks of transfusion reactions are similar for FWB and component therapy with PRBCs. [6] On the civilian side, whole blood is an FDA-licensed product that has been in use in pediatric open heart surgery and autologous transfusion but is no longer commonly available for other indications. However, the military results are renewing interest in whole blood for trauma resuscitation. The use of low-titer (anti-A/anti-B) group O-positive whole blood that is leukoreduced with a platelet-sparing filter was recently approved by the UCLA Blood and Blood Derivatives Committee (Transfusion Committee) for male trauma patients.

To provide effective treatment for traumatic hemorrhage, blood product transfusion must provide the oxygen-carrying capacity provided by red blood cells and the hemostatic capacity provided by platelets and clotting factors. Whole blood offers the advantages of more precisely approximating shed blood; decreased volume of additives per transfusion episode; and exposure to a decreased number of donors for a patient undergoing massive transfusion. As described below, the military experience, initial civilian studies, and preclinical studies indicate that whole blood may be superior to component therapy particularly with regard to hemostatic capacity, though it remains to be seen whether this will translate into differences in coagulopathy, inflammation, transfusion requirements, and mortality. Additional considerations include optimal storage conditions that maintain the function of red cells, platelets, and clotting factors; as well as careful consideration and mitigation of the risk of transfusion reaction when the group O plasma in whole blood units is administered to non-group O recipients. Evidence of the safety of this product is provided in the following sections.

1.1 Overview of Non-Clinical Studies

Laboratory studies indicate that cold-stored whole blood retains hemostatic ability over at least 10 days at 2-6°C, and likely longer. Spinella et al [14] and Pidkoke et al [15] have extensively reviewed the hemostatic capacity of cold-stored WB and the viability of platelets in this product. In those reviews, there is convincing evidence that cold-stored platelets not only retain function but that they perform better than room temperature-stored platelets in aggregation studies. Most of the studies in these reviews evaluated storage up 7 days. However, several studies have specifically addressed platelet function in WB stored for 21 days or longer with similar findings. Jobes et al demonstrated that in unfiltered WB stored at 1-6°C, platelet counts decrease starting at day 4 and then level off and stabilize by day 7.[16] Additionally, platelet function in cold stored WB as measured by platelet aggregometry showed no change in in the aggregation responses to ADP and epinephrine stimulation from day 1 to day 21 of storage but did have a decline in response to collagen by day 7 and ristocetin by day 17. However, despite a gradual decline in platelet count and response to some agonists, global platelet function in the stored unit as measured by thromboelastography (TEG) was maintained within the normal range until day 14 of storage [16].

Pidkoke et al. found similar results showing that refrigerated (1-6°C) WB improved impedance platelet aggregation and TEG results compared to WB units stored at 22°C for 21 days. In this study, prolongation of prothrombin time was also attenuated by refrigeration. [17] Functional clotting capacity as measured by thromboelastography remained relatively stable in refrigerated units over 21 days. In studies performed at UCLA, we found similar results with TEG values maintained within the normal range for up to 35 days of storage at 1-6°C in unfiltered WB (manuscript accepted, *Journal of Trauma and Acute Care Surgery*).

Standard leukoreduction techniques for blood processing remove platelets as well as white blood cells. Our study proposes to use a platelet-sparing leukoreduction filter which has previously been evaluated for leukoreduction of whole blood (Imuflex WB-SP, Terumo). [18]. The filter works with a polyurethane membrane that permits passage of RBCs and platelets but impedes white blood cells. Theoretically passage through the filter could activate RBCs and platelets and target them for destruction, but this was not the case based on *in vivo* recovery of radiolabeled red cells and platelets after autologous transfusion. PRBCs, platelets, and plasma produced by the filter were tested to and confirmed to meet FDA requirements, including leukoreduction standards. [18]

Additionally, Strandenes et al. recently demonstrated that global platelet function as measured by ROTEM is maintained in WB that has been stored at 2-6°C for up to 21 days after filtration with this platelet-sparing filter. In that study, maximum clot firmness began to decrease by day 10-14 but remained within the reference range through day 21. [19] These data provide convincing evidence that platelet function in a 10-day-old unit of filtered WB will be adequate to support hemostasis.

Optimal storage conditions for refrigerated whole blood have also been investigated. Using the platelet-sparing filter, Yazer et al studied whether whole blood should be agitated to enhance platelet function, or whether this would damage the red cells. [20] In this study, whole blood was maintained between 20-24°C before leukoreduction occurred within 8 hours; blood was then immediately placed in a 1-6°C refrigerator. Units then underwent infectious disease testing over 3 days, and were then delivered to the transfusion service. This timeline and storage plan is the same as that used in the proposed study. After processing, units were stored for 21 days under different rocking conditions. Based on thromboelastography and markers of hemolysis, the recommended storage condition for cold stored whole blood is stationary for up to 10 days. Importantly, platelet function did not appear to be affected by either leukoreduction or cold storage.

Platelets were routinely stored at 4°C until the 1980s, and appeared to be safe during this time. These storage conditions were phased out because of the decreased circulation time of cold-stored platelets compared to platelets stored at room temperature, in order to best serve patients requiring prophylactic transfusions. [21] It was assumed, but not proven, that these would be as effective as cold-stored platelets for actively hemorrhaging patients. Taken together, the above evidence indicates that in actively bleeding patients, cold-stored platelets are likely to achieve faster hemostasis than room temperature-stored platelets, and are more likely to prevent re-bleeding in patients requiring high mean arterial pressure, such as spinal cord injury patients.

1.2 Overview of Clinical Studies

The change in standard practice from whole blood transfusion, to fractionation and component transfusion, occurred after the 1970s because of concerns over resource utilization and safety. However, there was no evidence documenting equivalence between whole blood and components for trauma patients, and new data is now suggesting that whole blood may be superior for obtaining hemostasis and oxygen delivery for the acutely bleeding trauma patient. [22]

The first evidence that whole blood may be superior to component therapy for trauma patients comes from military studies in Iraq and Afghanistan using fresh whole blood (FWB). FWB was initially used as a source of platelets, in addition to PRBCs and

plasma, when platelets were not available in far-forward military settings. [23] A retrospective analysis of prospectively collected data from all transfused patients at 6 forward surgical teams comparing standard component therapy to component therapy with whole blood substituted for platelets found that when adjusted for confounding variables, patients who received FWB had a significant survival advantage. [23] Of note, a subgroup analysis of patients who received "universal-donor" group O FWB (49%) versus type-specific product found there was no difference in mortality between these groups, and that there were no transfusion reactions.

Similarly, a retrospective analysis of 354 combat casualties transfused at least one unit of PRBCs compared standard component therapy with PRBCs, plasma, and warm FWB. [24] Patients who received whole blood had significantly lower transfusion volume requirements, and received significantly less preservative and anticoagulant additive volume, compared to patients receiving standard component therapy. 24 hour and 30 day survival was significantly higher in the whole blood group.

On the civilian side, a recent randomized controlled pilot trial compared whole blood leukoreduced with a non-platelet-sparing filter and stored for up to 5 days at 1-6°C, supplemented with warm stored platelets, to regular component therapy in severely injured patients predicted to require large volume transfusion. [1] There was a trend towards decreased plasma and platelet requirements in the whole blood group in the on-protocol analysis. In post hoc analysis use of whole blood was associated with a reduction in the amount of transfusion when traumatic brain injury was excluded.

Current FDA regulations stipulate that whole blood must be type-specific. However, as reviewed by Spinella et al (7) and Berseus et al [25], there is convincing evidence that the risk of a hemolytic transfusion reaction from transfusion of low titer group O WB is minimal. As these authors point out, the US Army used low titer group O WB as a universal donor blood in the Vietnam conflict. Per their report, there was only one reported hemolytic transfusion reaction from transfusion of group O WB where a group A patient incorrectly received a unit that was labeled high titer. There were no reported hemolytic transfusion reactions following transfusion of low titer group O WB to non-group O patients. Berseus et al have reviewed the known literature on hemolytic transfusion reactions after transfusion of group O products to non-group O recipients and found that virtually no such reactions occur in association with low titer O products. [25] The authors concluded that "there is an overwhelming experience, particularly from the transfusion of low titer universal group O whole blood in the military service, showing that the frequency of severe hemolytic transfusion reactions is nearly negligible". [25] Finally, Yazer et al have shown that it is safe to initiate resuscitation from hemorrhagic shock in male patients with low titer group O+ WB in a civilian trauma center. In this study, patients were transfused up to 2 units uncrossmatched group O positive WB

leukoreduced using a platelet-sparing filter and stored at 1-6°C for up to 10 days for male trauma patients with hypotension due to hemorrhage. Outcomes were compared to historical controls. No adverse reactions and no laboratory evidence of increased hemolysis in the whole blood group was found.[26] That institution has now increased their initial transfusion of low titer group O+ WB to 4 units with similar results (personal communication). Our study seeks to build on this work by establishing efficacy of cold stored whole blood leukoreduced with a platelet sparing filter. Only low anti-A, anti-B group O-positive whole blood will be used as a universal donor in the proposed study. Platelets, which are not typically crossmatched, have occasionally resulted in hemolytic reactions after transfusion. In the 25 publications reviewed, all but one reaction resulted from a unit with a titer of >1:100 (saline) or >1:400 (antiglobulin); often, titers were >1:1000. Our study proposes to use a cutoff of <1:100.

There have been reports of hemolytic reactions when a patient initially transfused with unmatched group O whole blood later received type-specific transfusion. A similar situation is encountered in current massive transfusion protocols and hemolytic reactions are prevented using the following precautions. Currently when the patient's blood type is unknown during a massive transfusion activation at UCLA, sets of 6 group O PRBC units, 6 pre-thawed group A plasma units and 1 apheresis platelet unit are provided. Once the blood type of the patient is determined, the blood bank switches to providing type-specific products unless the patient's blood type is group B or AB (i.e. incompatible with the group A plasma); under this circumstance, the blood bank provides another set of 6 group O PRBC units and compatible plasma before switching to type specific PRBCs. We have not identified any hemolytic transfusion reactions using this approach. We intend to follow this same practice with the transfusion of low titer group O+ WB. If after receiving low titer group O+ WB the patient's blood type is found to be a type other than group O, the blood bank will provide a set of group O PRBC units and compatible plasma units, before switching to type specific PRBC units.

Providing further evidence of the safety of this approach, our institution participated in a multi-center study evaluating the safety of the use of thawed group A plasma in trauma patients. [27] This study evaluated trauma patients that received group A plasma as part of the initial resuscitation (354 B and AB patients and 809 A patients). The two study groups were comparable in terms of age, gender, TRISS probability of survival, and total number of blood products transfused. The study found that the use of group A thawed plasma during the initial resuscitation of injured patients of unknown ABO group was not associated with increased in-hospital mortality, early mortality or hospital length of stay for group B and AB patients compared to group A patients. Additionally, of the 17 participating centers, only 4 (24%) provide low-titer group A plasma; the other 13 centers do not perform titers of the plasma components. This

experience indicates that transfusion of group A thawed plasma, regardless of anti-B titer, is safe. A manuscript reporting these findings was recently accepted by *Transfusion* (accepted March 15, 2017).

2 STUDY RATIONALE

The evidence described above indicates that low titer group O cold-stored whole blood stored for up to 10 days may be a superior product for obtaining hemostasis and maintaining oxygen delivery in hemorrhaging trauma patients in the emergent setting, compared with component therapy. A prior study has demonstrated the safety of this universal donor product in adult male civilian trauma patients [28]; however, efficacy must be determined in a prospective civilian context.

The advantage of cold-stored whole blood may be due to several factors, or a combination of factors, including superior platelet function, lack of storage lesion, and decreased volume of preservative and anticoagulant additives. These factors will be evaluated in our study.

2.1 Risk / Benefit Assessment

As described above (See sections 1.1 and 1.2, Overview of Non-Clinical and Clinical Studies), low titer group O cold-stored whole blood stored for up to 10 days has been shown to be safe as a universal donor product. There is substantial evidence from literature spanning over 40 years, described above, that low-titer group O whole blood can safely be used as a universal donor product and retains hemostatic capacity. The risk of hemolytic reaction when a patient initially transfused with unmatched group O whole blood later receives type-specific transfusion is mitigated by using low titer whole blood and by requiring that patients who have received uncrossmatched group O whole blood must receive a further 6 units of type-specific plasma transfused prior to receipt of type-specific PRBCs to prevent a reaction. In the interim the patient will receive group O PRBCs. There is also evidence that whole blood may be a superior hemostatic product compared to component therapy, and that it may reduce transfusion requirements, development of coagulopathy, and ultimately mortality. The risk-benefit profile of whole blood transfusion is therefore quite favorable. The evidence for this analysis is described extensively in sections 1.1 and 1.2, Overview of Non-Clinical and Clinical Studies.

3 STUDY OBJECTIVES

3.1 Primary Objective

The primary objective is to assess the clinical efficacy of whole blood compared to component therapy for trauma patients requiring massive transfusion, as measured by volume of blood products transfused within the first 24 hours of admission.

3.2 Secondary Objectives

The secondary objectives are to evaluate:

1. Functional coagulopathy as measured by thromboelastography;
2. 30-day mortality;
3. Number of units of blood products transfused by component/product;
4. Complications;
5. Hemolysis;
6. Wastage of blood units

See Section 5 for definitions of primary and secondary endpoints.

4 STUDY DESIGN

4.1 Study Overview

This is a single center, prospective cohort study. Enrollment of approximately 98 patients is planned, 49 receiving whole blood and 49 receiving component therapy. This is an observational study planned during initiation of a change in practice at our institution initially allowing up to 4 units of whole blood transfusion for adult male trauma patients. If initial patients receiving whole blood show no evidence of hemolysis and thrombocytopenia compared to patients receiving component therapy, up to 6 units of whole blood transfusion will be allowed (see below, and section 5.2, Safety Evaluations). Patients in the whole blood, or index, group are adult male trauma patients presenting in hemorrhagic shock (systolic blood pressure < 100) requiring massive transfusion activation who receive whole blood for resuscitation. Two control groups will be utilized. The prospective control group consists of adult male patients who present during times when whole blood is not available, as well as adult female patients, presenting in hemorrhagic shock (systolic blood pressure <100) requiring massive transfusion activation. Additionally, a second control group consisting of historical

controls receiving component therapy will be analyzed (see Section 6, Patient Selection, for details of index and control groups).

All patients will have, in addition to standard labs, additional labs drawn for thromboelastography, platelet function determination, and coagulation factor levels (see Section 9.1.6, Other Clinical Procedures, for details of specific lab tests and timing).

Patients who meet all inclusion criteria and none of the exclusion criteria will be enrolled into the study.

Total duration of patient participation will be approximately 1 year (365 days), or the duration of the hospital stay, whichever is shorter. This includes up to 5 days of laboratory testing during initial resuscitation and subsequent admission, and up to one year of follow up via chart review if the patient remains in the hospital. Total duration of the study including recruitment and follow up is expected to be 2 years.

5 CRITERIA FOR EVALUATION

5.1 Primary Efficacy Endpoint

The primary objective is to assess the clinical efficacy of whole blood compared to component therapy for trauma patients requiring massive transfusion. Primary analysis will compare volume of blood transfusion within 24 hours of admission for patients without severe TBI (defined as GCS ≤8 and imaging evidence of brain injury) for patients who received WB versus component therapy for initial resuscitation.

Secondary Efficacy Endpoints

The secondary objectives will evaluate the effect on survival; the development of multiple complications related to survival; and wastage data. The full list of secondary endpoints is as follows:

1. Volume of blood transfusion within 24 hours of admission for patients with severe TBI (GCS ≤8 and imaging evidence of brain injury) and for entire cohort of patients.
2. Thromboelastography parameters including maximum amplitude, reaction time, alpha angle, fibrinolysis, and platelet function at 3 and 6 hours of resuscitation, after every 6 units of whole blood equivalent (WB or PRBCs) given, at the time of completion of resuscitation (time of last unit of blood product given), and on ICU days 1, 3 and 5.
3. 30-day mortality;

4. Duration of need for renal replacement therapy, mechanical ventilation, ICU admission, and hospital stay;
5. Number of units of blood products transfused by component/product during first 24 hours of admission and total hospitalization;
6. Complications which include clinical coagulopathy, infection, venous thromboembolism, cerebrovascular accident, acute coronary syndrome, transfusion-related lung injury, transfusion-associated cardiac overload during total hospitalization;
7. Hemolysis as measured by haptoglobin, bilirubin, lactate dehydrogenase, and direct antiglobulin on ICU day 1;
8. Wastage of blood units

5.2 Safety Evaluations

- Hemolysis will be measured by haptoglobin, bilirubin, lactate dehydrogenase, and direct antiglobulin tests on ICU day 1. Clinical surveillance for evidence of hemolysis will also be ongoing. The Blood and Blood Derivatives committee will review data for the first 10 patients to receive whole blood for evidence of hemolysis and thrombocytopenia. If the risks of these conditions are judged by the Committee to be equivalent to risks seen following treatment with similar amounts of component therapy, the Committee will allow an increase from up to 4 units to up to 5 units of whole blood to be transfused. After 10 patients have received whole blood under this protocol, the Committee will again evaluate for hemolysis and thrombocytopenia and increase to a maximum of 6 units of whole blood if risks are found to be equivalent to component therapy.

- Alternatively, should the safety of up to 4 units of whole blood be published before 10 patients have received whole blood, the Committee will allow an increase to a maximum of 5 units of whole blood. The Committee will assess the first 10 patients to receive whole blood under this protocol and if the risks of hemolysis and thrombocytopenia are judged by the Committee to be equivalent to risks seen following treatment with similar amounts of component therapy, the Committee will allow up to 6 units of whole blood to be transfused.

- The Blood and Blood Derivatives Committee at UCLA, also known as the Transfusion Committee, is composed of Transfusion Medicine faculty members. The Committee performs routine monthly monitoring of all transfusion-related safety

data, including evidence of hemolysis and other transfusion reactions. In addition, the Committee has agreed to perform a separate evaluation of the cohort of patients receiving whole blood and compare this to equivalent patients receiving component therapy (as defined in the inclusion and exclusion criteria for the study), for the purposes of evaluating the safety of whole blood. Drs. Ziman and Ward, who are co-investigators in this study, are members of the Blood and Blood Derivatives Committee but will be recused from safety analysis related to this study to prevent conflict of interest. No other member of the Committee has a relationship to the study.

- The incidence of adverse events will be evaluated including: death, clinical coagulopathy, hemolysis, infection, venous thromboembolism, cerebrovascular accident, acute coronary syndrome, transfusion-related lung injury, transfusion-associated cardiac overload.
- The occurrence of any whole blood transfusion given to patients other than adult male trauma patients will also be recorded
- In addition to ongoing monitoring and final analysis, an interim safety analysis when approximately 50% of patients have been enrolled will determine if there is a difference in adverse events as defined above between groups.
- The interim analysis will be performed by the independent medical monitor. The independent medical monitor, Dr. William Mower, is an emergency medicine physician with extensive experience in clinical investigation and has no relation to the study other than to monitor safety. The independent medical monitor has the authority to stop the study if safety concerns arise, including but not limited to evidence for the pre-specified stopping criteria (see Section 12.5, Stopping Criteria). Dr. Mower is also independent of the Blood and Blood Derivatives Committee.

6 PATIENT SELECTION

6.1 Study Population

All trauma patients who present with SBP<100 who require activation of the massive transfusion protocol and who do not have severe burns (>20% total body surface area) will be eligible for enrollment.

Enrollment of male and female patients:

At UCLA and at least two other institutions [28] the standard of care for traumatic resuscitation for adult males is either:

1) Low anti-A anti-B titer (<1:100) group O+ whole blood

or, when whole blood is not available,

2) Component therapy, consisting of group O+ PRBCs, group AB or A plasma, and untyped platelets

The standard of care for adult females is component therapy, consisting of group O- PRBCs, group AB or A plasma, and untyped platelets. Group O- whole blood is not available at our institution at this time. During a critical traumatic resuscitation, it is often impossible to determine precise age and menopausal status before initiation of transfusion. In order to avoid any possibility of alloimmunization of reproductive-age females, the UCLA Blood and Blood Derivatives Committee has approved group O+ whole blood only for use in males.

The index and control groups in the study therefore consist of:

1) Index group: male trauma patients presenting with SBP<100 who require activation of the massive transfusion protocol and who do not have severe burns >20% TBSA who receive whole blood;

2) Prospective control group: Prospectively-identified cohort of patients who present during the study with SBP<100 who require activation of the massive transfusion protocol and who do not have severe burns >20% TBSA but who are either not eligible or not able to receive whole blood. This category consists of patients who are female (who are not eligible to receive whole blood) and male patients who present when whole blood is out of stock;

3) Historical control group: male trauma patients who presented with SBP<100 who required activation of the massive transfusion protocol and who did not have severe burns >20% TBSA who received component therapy for traumatic hemorrhage requiring massive transfusion protocol activation, during the 5 years prior to initiation of the study.

A mixed effects linear regression model will be used to adjust for confounding variables, including sex.

Enrollment of patients of unknown precise age:

Due to the emergent nature of a critical code trauma activation, it is often not possible to determine exact age of patients prior to initiating treatment, including transfusion, for

hemorrhaging patients. Patients who appear to be adults, ie over age 18, will be eligible for inclusion in the study. Every effort will be made to ensure that patients enrolled are at least 18 years old, while respecting the necessities of clinical care. However, we acknowledge it is possible that there may be an instance where a patient is included who is not over age 18.

6.2 Inclusion Criteria

All trauma patients who present with SBP<100 who require activation of the massive transfusion protocol and who do not have severe burns (>20% total body surface area) will be eligible for enrollment. The study plans to include all such patients who present during the study period of approximately 2 years.

Within this group of patients, all adult male patients who received or will receive whole blood are eligible for inclusion in the whole blood group. All adult male patients who received or will receive component therapy initially are eligible for inclusion in the component therapy group. All adult female patients are eligible for inclusion in the component therapy group.

6.3 Exclusion Criteria

1. Patients with burns over >20% total body surface area
2. Patients who appear to be under age 18

7 CONCURRENT MEDICATIONS

All patients should be maintained on the same medications throughout the entire study period, as medically feasible, with no introduction of new chronic therapies related to the study.

7.1 Allowed Medications and Treatments

There are no prohibited medications or treatments.

8 STUDY TREATMENTS

8.1 Method of Assigning Patients to Treatment Groups

Treatment groups are not assigned as this is an observational study. Treatment is determined by the eligibility of the patient (male or female) and the availability of whole blood when the patient presents. Adult male patients meeting eligibility who have

received or will receive whole blood for traumatic resuscitation will be enrolled in the whole blood group. Enrollment of 49 patients receiving whole blood is planned. Adult male patients meeting eligibility criteria who present when whole blood is not available will receive component therapy and will be enrolled in the component therapy group. Enrollment of 49 control (component therapy group) patients is planned. Female patients meeting eligibility criteria will also be included in the component therapy group (see Section 6.1, Study Population, for rationale).

8.2 Blinding

The study is not blinded.

8.3 Formulation of Test and Control Products

1. Test Product: Low-antibody (anti-A/anti-B) titer (<1:100) Group O+ banked whole blood stored at 1-6 °C for up to 10 days. After collection, product undergoes infectious disease and immunohematology testing per regulatory requirements and leukocyte-reduction with a platelet-sparing filter.

2. Control Product: Component therapy manufactured according to standard practices, consisting of packed red blood cells, thawed fresh frozen plasma and platelets in a 1:1:1 ratio.

8.3.1 Packaging and Labeling

Whole blood is supplied by the UCLA Blood Bank in bags containing approximately 250 milliliters of product. It is labeled with a large-print, brightly-colored label identifying it as whole blood.

8.4 Supply of Study Drug at the Site

The UCLA Blood & Platelet Center will identify repeat volunteer male whole blood donors to maintain a predetermined inventory of 12 units of low-titer (<1:100) O-positive whole blood. These donors will be identified by titering the retention samples from a prior donation. The study will be explained to potential donors as a recruitment tool.

All donors of whole blood units will be tested for transfusion-transmitted infectious diseases, identical to the AABB and FDA regulations for all blood donors. Donor infectious disease testing is performed at CTS (Creative Testing Solutions) in Tempe, Arizona; the average tum-around time for completion of testing and blood availability in inventory is 48 hours. Whole blood units will undergo pre-storage leukoreduction with a platelet-sparing filter (Imuflex WB-SA, Terumo, Tokyo, Japan) and then stored at 1-6°C

for 10 days. If not used by day 10, units will be fractionated into PRBC units to be released into general inventory for transfusion, and plasma which will be discarded.

The goal is to maintain up to 12 units of low-titer type O-positive whole blood weekly. 6 units will be stored in the Trauma Bay blood refrigerator, and 6 units will be stored in the blood bank.

8.4.1 Dosage/Dosage Regimen

Adult male patients may receive up to 4 units of whole blood if needed during initial traumatic resuscitation in the initial phase of the study, and up to 6 units if deemed to be safe (see section 5.2, Safety Evaluations).

8.4.2 Dispensing

The blood bank dispenses all blood products.

8.4.3 Administration Instructions

Blood products are transfused intravenously.

8.4.4 Storage

Whole blood will be stored by the study site in a refrigerator designated for the storage of blood products maintained at 1-6ºC. If the temperature of study drug storage in the clinic/pharmacy exceeds or falls below or above this range, this will be reported to the Sponsor/Investigator or designee and captured as a deviation.

8.5 Study Drug Accountability

An accurate and current accounting of the dispensing and return of study drug for each patient will be maintained on an ongoing basis by a member of the study site staff at the blood bank. The amount of study drug dispensed and returned will be recorded in blood bank accountability records. The study monitor will verify these documents throughout the course of the study.

9 STUDY PROCEDURES AND GUIDELINES

A Schedule of Events representing the required testing procedures to be performed for the duration of the study is diagrammed in Appendix 1.

Due to the emergent nature of treatment, and the fact that both whole blood and component therapy are considered standard of care for traumatic resuscitation of adult males, the UCLA Institutional Review Board has waived the requirement for informed

consent and Health Insurance Portability and Accountability Act (HIPAA) authorization. As an FDA approved product, whole blood is not considered an investigational treatment by the Institutional Review Board and therefore these requirements have been waived for the entire study. Patients will be informed of which product they received and of the study by their clinical providers, but not directly by study personnel.

9.1 Clinical Assessments

9.1.1 Concomitant Medications

All concomitant medication and concurrent therapies will be documented on admission or as soon as possible thereafter. Dose, route, unit frequency of administration, and indication for administration and dates of medication will be captured.

9.1.2 Demographics

Demographic information (date of birth, gender, race) will be recorded on admission.

9.1.3 Medical History

Relevant medical history, including history of current disease, other pertinent respiratory history, and information regarding underlying diseases will be recorded on admission.

9.1.4 Vital Signs

Body temperature, blood pressure, pulse and respirations will be recorded on admission and at least every 4 hours thereafter while the patient is in the ICU.

9.1.5 Oximetry

Oximetry will be measured on room air on admission.

9.1.6 Other Clinical Procedures

All patients will have, in addition to standard labs, additional labs drawn for thromboelastography, platelet function determination, and coagulation factor levels, on admission, after each 6 units of whole blood or PRBCs transfused, at 3 and 6 hours of resuscitation, at the time of hemostasis, and on ICU days 1,3 and 5. Tests for hemolysis will be performed along with regular clinical labs on ICU day 1. These labs will be drawn during clinical lab collection so no extra needle stick is required.

9.1.7 Adverse Events

Information regarding occurrence of adverse events will be captured throughout the study. Duration (start and stop dates and times), severity/grade, outcome, treatment and relation to study drug will be recorded on the case report form (CRF).

Morbidity and mortality in this study population (patients requiring massive transfusion for trauma) is high. [29] Therefore we expect a relatively large number of adverse events, and resulting SAE reports, for reasons unrelated to the study.

9.2 Clinical Laboratory Measurements

9.2.1 Hematology

Patients will receive standard hematology tests including complete blood counts as standard of care no less than daily (see also Schedule of Events).

9.2.2 Blood Chemistry Profile

Patients will receive this no less than daily as standard of care.

9.2.3 Pregnancy Test

Female patients of childbearing age will receive this on admission or shortly thereafter as standard of care.

9.3 Research Laboratory Measurements

9.3.1 Thromboelastography (TEG)/platelet mapping

For TEG testing, 1 mL of patient blood sample will be added to a plastic vial containing kaolin. The vial will be capped securely and the contents will be mixed by gentle inversion. 340 microliters of the resultant mixture will be pipetted into a disposable TEG cup containing 20 microliters of calcium chloride preloaded in the TEG. To minimize any risk of splashing, personnel will use standard personal protective equipment. The cup will be raised into the TEG chamber and testing will begin. Platelet mapping assesses platelet function by repeating TEG parameters in the presence of platelet activators and inhibitors. After approximately 30 minutes, testing is complete. The TEG apparatus is completely enclosed. All syringes, pipettes, and cups used for this procedure will be placed in a biohazard container.

9.3.2 Calibrated Automated Thromboelastogram (CAT) testing

For CAT testing, blood not used for TEG will be transferred to 5 mL plastic centrifuge tubes. The tubes will be capped securely and centrifuged. The resulting plasma will be transferred to plastic cryotubes, which are enclosed, and frozen until testing. All syringes, pipettes, and centrifuge tubes will be placed in a biohazard container.80 microliters of thawed plasma will be pipetted to each well of a 96-well plate (less wells used for controls). The plate is then placed in the CAT for testing, which is enclosed. At the end of the CAT testing cycle, the plate and pipettes will be placed in a biohazard container. All transfers of potentially biohazardous material will take place in a biosafety cabinet to minimize the risk of splashes. At all times during laboratory testing, proper PPE will be worn and, at the end of each testing day, laboratory surfaces will be disinfected according to protocol.

10 EVALUATIONS BY VISIT

10.1 Visit 1 (Days 1-365)

Hospital Stay visit: typically 1-14 days, but can be as long as one year.

1. Assign the patient a unique study number. This number is planned to be listed on a bracelet in a packet present in the ED. The bracelet will be placed on the patient's wrist during initial admission procedures to identify the patient as a participant in the study.
2. All demographic data, past medical history, and concurrent medications are recorded by hospital staff as part of standard admission procedures. This information will be reviewed by study personnel during analysis.
3. Physical examination, vital signs, and standard clinical labs are performed and recorded by hospital staff.
4. Additional blood samples will be collected for TEG/platelet mapping and CAT (one 4 mL sodium citrate and one 4 mL heparinized tube per draw). This will be performed at the following time points: on admission, after each 6 units of whole blood equivalent (WB or PRBCs) transfused, at 3 and 6 hours of resuscitation, at the time of hemostasis determined by the trauma surgeon, and on ICU days 1,3 and 5.
5. Standard clinical labs including complete blood counts (white blood cell count, hemoglobin, hematocrit, and platelet count), blood chemistries, and standard coagulation labs (prothrombin time, international normalized ratio, partial thromboplastin time) will be performed at least daily as standard of care. This data will also be collected as part of the study. Of note, serial platelet counts obtained

as standard of care will be monitored no less frequently than daily at noon (see also Schedule of Events).

6. In addition to standard clinical labs, hemolysis labs will be performed as listed above on ICU day 1 which will be billed to the study.
7. Follow up by chart review will continue for the duration of the hospital stay (typically roughly 14 days but can be up to one year).
8. Chart review during the hospital stay will collect the following data:

Data collected on initial 24 hours of hospital stay:

Medical record number

Date of birth /age

Sex

Race

Weight

Height

Injury date/time

Arrival date/time

Emergency Department exit date/time

Operating room arrival time

Tier (defines levels of critical condition for trauma patients)

Injury description

Mechanism of injury

Injury Severity Score

Glasgow Coma Scale and vitals

Intubation status and location of intubation

Whether patient received cardiopulmonary resuscitation

Total fluids received in the first 24 hours

Data collected on initial 5 days of hospitalization:

Vital signs: systolic and diastolic blood pressure, heart rate, respiratory rate, oxygen saturation, temperature

Hemoglobin

Hematocrit

White blood cell count
Platelet count
Sodium
Potassium
Chloride
Bicarbonate
Blood urea nitrogen
Creatinine
Glucose
Aspartate transaminase (AST)
Alanine transaminase (ALT)
Total bilirubin
Direct bilirubin
Alkaline phosphatase
pH
Lactate
PaCO2 (arterial CO2 partial pressure)
PaO2 (arterial oxygen partial pressure)
Base excess
Prothrombin time (PT)
INR
Partial thromboplastin time (PTT)
Fibrinogen
Thrombin
Antithrombin Complex
Factor V
Factor VII
Factor VIII
Factor X
D-dimer
Lactate dehydrogenase (LDH)

Haptoglobin

Data collected on entire hospitalization:
Procedures performed during hospitalization
Operations performed during hospitalization
Discharge date
Disposition
Discharge capacity
Date and cause of death if applicable
Comorbidities
Admission medications
Total hospital and intensive care unit length of stay
Number of days on ventilator
Number of days on dialysis
Number of days on vasopressor medications
Number and nature of operative procedures
Development of venous thromboembolism
Development of acute kidney injury
Development of acute respiratory distress syndrome
Development of liver failure
Suspected or confirmed transfusion reaction
Hemolytic transfusion reaction
Transfusion associated lung injury
Development of cardiac failure
Development of clinical coagulopathy

11 ADVERSE EXPERIENCE REPORTING AND DOCUMENTATION

11.1 Adverse Events

An adverse event (AE) is any untoward medical occurrence in a clinical investigation of a patient administered a pharmaceutical product and that does not necessarily have a causal

relationship with the treatment. An AE is therefore any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the administration of an investigational product, whether or not related to that investigational product.

The Investigator and Independent Medical Monitor will probe, via discussion with the patient's clinical providers, for the occurrence of AEs during the patient visit and record the information in the site's source documents. Adverse events will be recorded in the patient CRF. Adverse events will be described by duration (start and stop dates and times), severity, outcome, treatment and relation to study drug, or if unrelated, the cause.

AE Severity

The National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0 will be used to assess and grade AE severity, including laboratory abnormalities judged to be clinically significant. The guidelines shown in Table 1 below will be used to grade severity. It should be pointed out that the term "severe" is a measure of intensity and that a severe AE is not necessarily serious.

Table 1. AE Severity Grading

Severity (Toxicity Grade)	Description
Mild (1)	Transient or mild discomfort; no limitation in activity; no medical intervention or therapy required. The patient may be aware of the sign or symptom but tolerates it reasonably well.
Moderate (2)	Mild to moderate limitation in activity, no or minimal medical intervention/therapy required.
Severe (3)	Marked limitation in activity, medical intervention/therapy required, hospitalizations possible.
Life-threatening (4)	The patient is at risk of death due to the adverse experience as it occurred. This does not refer to an experience that hypothetically might have caused death if it were more severe.

AE Relationship to Study Drug

The relationship of an AE to the study drug should be assessed using the following the guidelines in Table 2.

Table 2. AE Relationship to Study Drug

Relationship to Drug	Comment
Definitely	Previously known toxicity of agent; or an event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is not explained by any other reasonable hypothesis.
Probably	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is unlikely to be explained by the known characteristics of the patient's clinical state or by other interventions.
Possibly	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to that suspected drug; but that could readily have been produced by a number of other factors.
Unrelated	An event that can be determined with certainty to have no relationship to the study drug.

11.2 Serious Adverse Experiences (SAE)

An SAE is defined as any AE occurring at any dose that results in any of the following outcomes:

- death
- a life-threatening adverse experience
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity
- a congenital anomaly/birth defect

Other important medical events may also be considered an SAE when, based on appropriate medical judgment, they jeopardize the patient or require intervention to prevent one of the outcomes listed.

11.2.1 Serious Adverse Experience Reporting

Study site will document all SAEs that occur (whether or not related to study drug) per UCLA OHRPP Guidelines. The collection period for all SAEs will begin after admission and end after discharge.

In accordance with the standard operating procedures and policies of the Institutional Review Board, the site investigator will report SAEs to the IRB.

11.3 Protocol Defined Important Medical Findings Requiring Real Time Reporting

The following additional event is to be reported in real time to the IRB and FDA:

Hemolytic transfusion reactions: Defined as a clinical syndrome within 24 hours of blood transfusion including one or more symptoms (back pain, shortness of breath, hematuria, chills, dizziness, fever, urticaria) with clinical or laboratory evidence of hemolysis (hematuria, decreased haptoglobin, increased lactate dehydrogenase or bilirubin, or positive direct antigen test). Requires immediate cessation of ongoing blood transfusion and reporting as SAE.

11.4 Medical Monitoring

The Sponsor/Investigator, as well as the Independent Medical Monitor, will monitor for adverse effects and should be contacted directly to report medical concerns or questions regarding safety: Medical monitor (Dr. William Mower) phone: (310) 825-2111 e-mail: wmower@mednet.ucla.edu

12 DISCONTINUATION AND REPLACEMENT OF PATIENTS

12.1 Early Discontinuation of Study Drug

A patient may be discontinued from study treatment at any time if the patient, or Sponsor/Investigator feels that it is not in the patient's best interest to continue. The following is a list of possible reasons for study treatment discontinuation:

- Hemolytic transfusion reaction
- Adverse event that in the opinion of the Sponsor/Investigator would be in the best interest of the patient to discontinue study treatment
- Protocol violation requiring discontinuation of study treatment

If a patient is withdrawn from treatment due to an adverse event, the patient will be followed and treated by the Sponsor/Investigator until the abnormal parameter or symptom has resolved or stabilized.

12.3 Withdrawal of Patients from the Study

A patient may be withdrawn from the study at any time if the patient or the Sponsor/Investigator feels that it is not in the patient's best interest to continue.

All patients are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the Sponsor/Investigator to provide a reason for patient withdrawals. The reason for the patient's withdrawal from the study will be specified in the patient's source documents.

12.4 Replacement of Patients

Patients who withdraw from the study will be replaced.

12.5 Stopping Criteria

Pre-specified stopping criteria are as follows:

1. Hemolytic transfusion reaction: Defined as a clinical syndrome within 24 hours of blood transfusion including one or more symptoms (back pain, shortness of breath, hematuria, chills, dizziness, fever, urticaria) with clinical or laboratory evidence of hemolysis (hematuria, decreased haptoglobin, increased lactate dehydrogenase or bilirubin, or positive direct antigen test). Requires immediate cessation of ongoing blood transfusion and reporting as SAE. As this is an unexpected and serious event, any hemolytic transfusion reaction following the administration of whole blood will prompt temporary cessation of the study while the event is evaluated. If the event indeed occurred after administration of low titer group O-positive whole blood, the study will be stopped.

2. Additionally, interim analysis will seek evidence for increased incidence of clinical coagulopathy, increased transfusion requirements, and increased incidence of complications as defined above in Secondary Endpoints, or increased mortality, all of which will prompt stopping the study.

13 PROTOCOL VIOLATIONS

A protocol violation occurs when the patient or Sponsor/Investigator fails to adhere to significant protocol requirements affecting the inclusion, exclusion, patient safety and primary endpoint criteria. Protocol violations for this study include, but are not limited to, the following:

- Failure to meet inclusion/exclusion criteria
- Switching from component therapy to whole blood

Failure to comply with Good Clinical Practice (GCP) guidelines will also result in a protocol violation. The Sponsor/Investigator will determine if a protocol violation will result in withdrawal of a patient.

When a protocol violation occurs, it will be discussed with the Sponsor/Investigator and a Protocol Violation Form detailing the violation will be generated. This form will be signed by a Sponsor/Investigator or representative. A copy of the form will be filed in the site's regulatory binder and in the Sponsor/Investigator's files.

14 DATA SAFETY MONITORING

The UCLA Institutional Review Board has approved an Independent Medical Monitor, Dr William Mower. The Sponsor/Investigator and Independent Medical Monitor will review clinical data and probe for evidence of adverse events via discussion with the patient's clinical providers as described above. An interim analysis for safety is planned when approximately 50% of patients have been enrolled. The monitor will also ensure that records are maintained appropriately throughout the study.

15 STATISTICAL METHODS AND CONSIDERATIONS

Prior to the analysis of the final study data, a detailed Statistical Analysis Plan (SAP) will be written describing all analyses that will be performed. The SAP will contain any modifications to the analysis plan described in this protocol.

15.1 Data Sets Analyzed

All eligible patients who are enrolled in the study and receive at least one unit of blood, and who undergo TEG testing as described above, will be included in analysis.

Data sets include:

1. Prospectively identified patients who received whole blood or component therapy and did not have severe TBI

2. Prospectively identified patients who received whole blood or component therapy and who had severe TBI (GCS 8 or less with evidence of brain injury on imaging)

3. Prospectively identified patients who received whole blood or component therapy, all patients regardless of TBI status

4. Prospectively identified male patients who received whole blood and who did not have severe TBI, compared to historical control male patients who received component therapy and who did not have severe TBI

15.2 Demographic and Baseline Characteristics

The following demographic variables will be summarized: age, sex, race, height and weight, comorbidities, and home medications.

15.3 Analysis of Primary Endpoint

Primary analysis compares volume of blood products transfused in the first 24 hours of admission in patients without severe TBI (severe TBI is defined as GCS ≤8 and evidence of brain injury on imaging) receiving whole blood versus component therapy during initial resuscitation.

For continuous variables including the primary endpoint, the statistical method will depend on the distribution of the data. If the data appears to follow a normal distribution, a t-test will be used, otherwise, a nonparametric Wilcoxian rank sum test will be used.

15.4 Analysis of Secondary Endpoints

Continuous variables and categorical variables will be analyzed as described above (section 15.3). Mixed effects linear regression models will be used to evaluate coagulopathy over time and to adjust for confounding variables.

To analyze coagulopathy over time we will use a mixed effects linear regression model.

A mixed effects linear regression model will also be used to adjust for confounding variables such as sex, age, comorbidities, severity of injury, and physiologic parameters of shock including pH and temperature, and use of a rapid transfuser (which more effectively warms blood).

Quarterly safety monitoring will compare categorical variables including mortality and development of complications (duration of need for renal replacement therapy,

mechanical ventilation, ICU admission, and hospital stay; clinical coagulopathy, infection, venous thromboembolism, cerebrovascular accident, acute coronary syndrome, transfusion-related lung injury, transfusion-associated cardiac overload; transfusion reactions including clinical transfusion reactions and rates of hemolysis as measured by haptoglobin, bilirubin, lactate dehydrogenase, and direct antiglobulin). Categorical variables will be compared using the chi-squared test.

15.5 Interim Analysis

When approximately 50% of patients have been enrolled in the study, an interim analysis for safety is planned to be conducted by the independent medical monitor. (See Section 5.2, Safety Evaluations).

Sample Size

Number of patients to be enrolled was calculated using a Type I error rate of 0.05, and effect size based on reduction of PRBC and plasma transfusions described in the sensitivity analysis in a study by Cotton et al. [1]

As in the sensitivity analysis by Cotton et al, patients with severe TBI (defined as TBI on imaging with GCS of 8 or less) will be excluded from primary analysis. Approximately 35-40% of patients meeting inclusion criteria are expected to fulfill criteria for severe TBI. It is not possible to identify patients with severe TBI prior to beginning resuscitation for hemorrhagic shock, so all patients meeting inclusion criteria will be enrolled. In the primary analysis, only patients without severe TBI will be compared. Secondary analyses will compare 1) patients with severe TBI receiving WB versus component therapy and 2) the entire cohort of enrolled patients, with and without TBI, receiving WB versus component therapy. Using this method, a total sample size of 49 whole blood arm (study drug) and 49 component therapy arm (control) patients (98 patients total) is planned. 35-40% (approximately 18 of the total 49) of eligible patients are expected to have severe TBI and to be excluded from primary analysis based on typical presentations of trauma patients. 49 total patients per group will achieve a sample size of approximately 31 patients per arm without TBI to complete the primary analysis. The FDA will be notified in advance if sample size estimates change.

16 DATA COLLECTION, RETENTION AND MONITORING

16.1 Data Collection Instruments

The Sponsor/Investigator will prepare and maintain adequate and accurate source documents designed to record all observations and other pertinent data for each patient treated with the study drug.

Study personnel will enter data from source documents corresponding to a patient's visit into the protocol-specific electronic database containing the electronic Case Report Form (eCRF) when the information corresponding to that visit is available. Patients will not be identified by name in the study database or on any study documents to be collected by the Sponsor/Investigator (or designee), but will be identified by a patient number.

If a correction is required for an eCRF, the time and date stamps track the person entering or updating eCRF data and creates an electronic audit trail.

The Sponsor/Investigator is responsible for all information collected on patients enrolled in this study. All data collected during the course of this study must be reviewed and verified for completeness and accuracy by the Sponsor/Investigator. A copy of the CRF will remain at the Sponsor/Investigator's site at the completion of the study.

16.2 Data Management Procedures

The data will be entered into a validated database. The Data Management group will be responsible for data processing, in accordance with procedural documentation. Database lock will occur once quality assurance procedures have been completed.

All procedures for the handling and analysis of data will be conducted using good computing practices meeting FDA guidelines for the handling and analysis of data for clinical studies.

16.3 Data Quality Control and Reporting

The study database utilizes REDCap, a secure platform for electronic data capture. After data have been entered into the study database, a system of computerized data validation checks will be implemented and applied to the database on a regular basis. Queries are entered, tracked, and resolved through the system directly.

16.4 Archival of Data

The database is safeguarded against unauthorized access by established security procedures; appropriate backup copies of the database and related software files will be

maintained. Databases are backed up by the database administrator in conjunction with any updates or changes to the database.

At critical junctures of the protocol (e.g., production of interim reports and final reports), data for analysis is locked and cleaned per established procedures.

16.5 Availability and Retention of Investigational Records

The Sponsor/Investigator must make study data accessible to the IRB, and Regulatory Agency (e.g., FDA) inspectors upon request. The Sponsor/Investigator must ensure the reliability and availability of source documents from which the information on the CRF was derived.

16.6 Monitoring

Monitoring will be conducted the Independent Medical Monitor, the Sponsor/Investigator and associated staff according to the U.S. CFR Title 21 Parts 50, 56, and 312 and ICH Guidelines for GCP (E6).

16.7 Patient Confidentiality

In order to maintain patient confidentiality, only a patient number and date of birth will identify all study patients in the electronic database/eCRF and other documentation.

17 ADMINISTRATIVE, ETHICAL, REGULATORY CONSIDERATIONS

The study will be conducted according to the Declaration of Helsinki, Protection of Human Volunteers (21 CFR 50), Institutional Review Boards (21 CFR 56), and Obligations of Clinical Investigators (21 CFR 312).

To maintain confidentiality, all study records will be kept in a secured database. Clinical information will not be released without written permission of the patient, except as necessary for monitoring by the FDA. The Sponsor/Investigator must also comply with all applicable privacy regulations (e.g., Health Insurance Portability and Accountability Act of 1996, EU Data Protection Directive 95/46/EC). De-identified data will be made available to other investigators.

17.1 Protocol Amendments

Any amendment to the protocol will be written by the Sponsor/Investigator. Protocol amendments cannot be implemented without prior written IRB approval except as necessary to eliminate immediate safety hazards to patients. A protocol amendment

intended to eliminate an apparent immediate hazard to patients may be implemented immediately, provided the IRBs are notified within five working days.

17.2 Institutional Review Board

The protocol has been reviewed and approved by the IRB. Serious adverse experiences regardless of causality will be reported to the IRB in accordance with the standard operating procedures and policies of the IRB, and the Sponsor/Investigator will keep the IRB informed as to the progress of the study. The Sponsor/Investigator will obtain assurance of IRB compliance with regulations.

Any documents that the IRB may need to fulfill its responsibilities (such as protocol, protocol amendments, payment or compensation procedures, or other pertinent information) have been submitted to the IRB. The IRB's written unconditional approval of the study protocol and waiver of the informed consent form are confirmed to be in the possession of the Sponsor/Investigator prior to study initiation. The IRB unconditional approval statement has been transmitted to the Sponsor/Investigator or designee prior to the shipment of study supplies to the site. This approval refers to the study by exact protocol title and number and identifies the documents reviewed and the date of review.

Protocol and/or informed consent modifications or changes may not be initiated without prior written IRB/ approval except when necessary to eliminate immediate hazards to the patients or when the change(s) involves only logistical or administrative aspects of the study. Such modifications will be submitted to the IRB and written verification that the modification was submitted and subsequently approved should be obtained.

The IRB must be informed of revisions to other documents originally submitted for review; serious and/or unexpected adverse experiences occurring during the study in accordance with the standard operating procedures and policies of the IRB; new information that may affect adversely the safety of the patients of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

17.3 Waiver of Informed Consent

Informed consent and HIPAA authorization have been waived by the Institutional Review Board because 1) both whole blood and component therapy are considered standard of care for adult male trauma patients, and availability of whole blood (as well as patient gender) determines which therapy a patient will receive; and 2) due to the emergent nature of traumatic resuscitation, it is not possible to inform patients of the

study prior to initiating study-related testing (additional blood draw for TEG) or prior to initiation of therapy by the clinical team.

18 PUBLICATIONS

The preparation and submittal for publication of manuscripts containing the study results shall be in accordance with a process determined by mutual written agreement among the study Sponsor/Investigator and participating institution. The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

19 Investigator Responsibilities

By signing the Agreement of Investigator form, the Sponsor/Investigator agrees to:

1. Conduct the study in accordance with the protocol and only make changes after notifying the Sponsor/Investigator (or designee), except when to protect the safety, rights or welfare of patients.
2. Personally conduct or supervise the study (or investigation).
3. Ensure that the requirements relating to obtaining informed consent and IRB review and approval meet federal guidelines, as stated in § 21 CFR, parts 50 and 56.
4. Report any AEs that occur in the course of the study, in accordance with §21 CFR 312.64.
5. Ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.
6. Maintain adequate and accurate records in accordance with §21 CFR 312.62 and to make those records available for inspection
7. Ensure that an IRB that complies with the requirements of §21 CFR part 56 will be responsible for initial and continuing review and approval of the clinical study.
8. Promptly report to the IRB all changes in the research activity and all unanticipated problems involving risks to patients or others (to include amendments and IND safety reports).
9. Seek IRB approval before any changes are made in the research study, except when necessary to eliminate hazards to the patients/patients.
10. Comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements listed in § 21 CFR part 312.

20 Appendix I: Schedule of Events

Assessment	Baseline, Enrollment, Initial Resuscitation: Visit 1 (Day 0)	Ongoing assessment: Visit 1 (Daily monitoring throughout hospital stay)	Laboratory assessment: Visit 1 (Noon on ICU day 1)	Laboratory assessment: Visit 1 (Daily at noon, ICU days 1,3,5)	Follow up by Chart Review: Visit 1 (Day 0 - 365)
Informed Consent Form	N/A				
Demographics	X				
Trauma characteristics	X				X
Medical History	X				X
Current Medications	X				
Blood Chemistries	X	X			
Hematology/complete blood counts including serial platelet count monitoring	X	X			
TEG/platelet mapping and CAT testing	X			X	
Hemolysis testing			X		
Standard coagulation labs	X	X			
Fluid and blood product resuscitation volume	X				X
Vital Signs	X	X			
Inclusion/Exclusion Criteria	X				
Enrollment	X				
Concomitant Medications	X				X
Clinical outcomes					X
Discharge information					X

Adverse Events	**X**	**X**			**X**
Operations/Procedures	**X**	**X**			**X**

21 REFERENCES

REFERENCES

1. Cotton BA, Podbielski J, Camp E, et al. A randomized controlled pilot trial of modified whole blood versus component therapy in severely injured patients requiring large volume transfusions. *Annals of surgery.* 2013;258(4):527-532; discussion 532-523.
2. Frith D, Brohi K. The acute coagulopathy of trauma shock: clinical relevance. *The surgeon : journal of the Royal Colleges of Surgeons of Edinburgh and Ireland.* 2010;8(3):159-163.
3. Nunez TC, Young PP, Holcomb JB, Cotton BA. Creation, implementation, and maturation of a massive transfusion protocol for the exsanguinating trauma patient. *The Journal of trauma.* 2010;68(6):1498-1505.
4. Sambasivan CN, Schreiber MA. Emerging therapies in traumatic hemorrhage control. *Current opinion in critical care.* 2009;15(6):560-568.
5. Shaz BH, Dente CJ, Harris RS, MacLeod JB, Hillyer CD. Transfusion management of trauma patients. *Anesthesia and analgesia.* 2009;108(6):1760-1768.
6. Spinella PC. Warm fresh whole blood transfusion for severe hemorrhage: U.S. military and potential civilian applications. *Critical care medicine.* 2008;36(7 Suppl):S340-345.
7. Brohi K, Singh J, Heron M, Coats T. Acute traumatic coagulopathy. *The Journal of trauma.* 2003;54(6):1127-1130.
8. Burruss S TA, Hiatt J, Cryer H. . Factors associated with mortality in massively transfused trauma patients. . *AAST 2010. Boston MA. Poster Presentation.* 2010.
9. Borgman MA, Spinella PC, Perkins JG, et al. The ratio of blood products transfused affects mortality in patients receiving massive transfusions at a combat support hospital. *The Journal of trauma.* 2007;63(4):805-813.
10. Armand R, Hess JR. Treating coagulopathy in trauma patients. *Transfusion medicine reviews.* 2003;17(3):223-231.

11. Ciavarella D, Reed RL, Counts RB, et al. Clotting factor levels and the risk of diffuse microvascular bleeding in the massively transfused patient. *British journal of haematology.* 1987;67(3):365-368.
12. Spahn DR, Rossaint R. Coagulopathy and blood component transfusion in trauma. *British journal of anaesthesia.* 2005;95(2):130-139.
13. Watson GA, Sperry JL, Rosengart MR, et al. Fresh frozen plasma is independently associated with a higher risk of multiple organ failure and acute respiratory distress syndrome. *The Journal of trauma.* 2009;67(2):221-227; discussion 228-230.
14. Spinella PC, Pidcoke HF, Strandenes G, et al. Whole blood for hemostatic resuscitation of major bleeding. *Transfusion.* 2016;56 Suppl 2:S190-202.
15. Pidcoke HF, Spinella PC, Ramasubramanian AK, et al. Refrigerated platelets for the treatment of acute bleeding: a review of the literature and reexamination of current standards. *Shock (Augusta, Ga.).* 2014;41 Suppl 1:51-53.
16. Jobes D, Wolfe Y, O'Neill D, et al. Toward a definition of "fresh" whole blood: an in vitro characterization of coagulation properties in refrigerated whole blood for transfusion. *Transfusion.* 2011;51(1):43-51.
17. Pidcoke HF, McFaul SJ, Ramasubramanian AK, et al. Primary hemostatic capacity of whole blood: a comprehensive analysis of pathogen reduction and refrigeration effects over time. *Transfusion.* 2013;53 Suppl 1:137s-149s.
18. Snyder EL, Whitley P, Kingsbury T, Miripol J, Tormey CA. In vitro and in vivo evaluation of a whole blood platelet-sparing leukoreduction filtration system. *Transfusion.* 2010;50(10):2145-2151.
19. Strandenes G, Austlid I, Apelseth TO, et al. Coagulation function of stored whole blood is preserved for 14 days in austere conditions: A ROTEM feasibility study during a Norwegian antipiracy mission and comparison to equal ratio reconstituted blood. *The journal of trauma and acute care surgery.* 2015;78(6 Suppl 1):S31-38.
20. Yazer MH, Glackin EM, Triulzi DJ, Alarcon LH, Murdock A, Sperry J. The effect of stationary versus rocked storage of whole blood on red blood cell damage and platelet function. *Transfusion.* 2016;56(3):596-604.
21. Nair PM, Pidcoke HF, Cap AP, Ramasubramanian AK. Effect of cold storage on shear-induced platelet aggregation and clot strength. *The journal of trauma and acute care surgery.* 2014;77(3 Suppl 2):S88-93.
22. Spinella PC, Holcomb JB. Resuscitation and transfusion principles for traumatic hemorrhagic shock. *Blood reviews.* 2009;23(6):231-240.

23. Nessen SC, Eastridge BJ, Cronk D, et al. Fresh whole blood use by forward surgical teams in Afghanistan is associated with improved survival compared to component therapy without platelets. *Transfusion.* 2013;53 Suppl 1:107s-113s.
24. Spinella PC, Perkins JG, Grathwohl KW, Beekley AC, Holcomb JB. Warm fresh whole blood is independently associated with improved survival for patients with combat-related traumatic injuries. *The Journal of trauma.* 2009;66(4 Suppl):S69-76.
25. Berseus O, Boman K, Nessen SC, Westerberg LA. Risks of hemolysis due to anti-A and anti-B caused by the transfusion of blood or blood components containing ABO-incompatible plasma. *Transfusion.* 2013;53 Suppl 1:114s-123s.
26. Yazer MH, Jackson B, Sperry JL, Alarcon L, Triulzi DJ, Murdock AD. Initial safety and feasibility of cold-stored uncrossmatched whole blood transfusion in civilian trauma patients. *The journal of trauma and acute care surgery.* 2016;81(1):21-26.
27. Dunbar Nea. Safety of the Use of Thawed Group A Plasma in Trauma. *Abstract presented at AABB Annual Meeting* 2016.
28. Yazer MH, Jackson B, Sperry JL, Alarcon L, Triulzi DJ, Murdock A. Initial Safety and Feasibility of Cold Stored Uncrossmatched Whole Blood Transfusion in Civilian Trauma Patients. *The journal of trauma and acute care surgery.* 2016.
29. Gruen RL, Jurkovich GJ, McIntyre LK, Foy HM, Maier RV. Patterns of errors contributing to trauma mortality: lessons learned from 2,594 deaths. *Annals of surgery.* 2006;244(3):371-380.