Contract No. W81XWH-19-C-0157, Final Report

Contract Number: W81XWH-19-C-0157
Principal Investigator: Forest Sheppard, MD
Report Preparation: Carolyne Falank, PhD and Forest Sheppard, MD

Contracting Institution: Maine Medical Center, Portland ME, USA

Date Notice of Termination was received: 5/6/2022

Effective Date of Termination: 5/9/2022

Extent of completion of performance on the effective date: Major Task 1: *Preclinical studies:* The preclinical studies were completed and reported to the sponsor in 2020. The preclinical studies warranted a change in the clinical research plan and hence IRB and FDA IND; at the beginning of FY21 a contract modification to accommodate this was approved. Major Task 2: *EFIC IRB and FDA IND Approval:* The contracted clinical trial proposal was submitted to our institution's scientific review committee (institutional requirement) and is was then subsequently submitted to our Institutional Review Board. It was currently undergoing revisions during the time of contract termination. We were able to secure an agreement with CSL Behring, manufacturer of RiaSTAP, for this study which allowed us to prepare all documents for FDA IND submission; these documents had been reviewed by our institution and were ready for submission at the time of contract termination.

Furnish notice of termination to each immediate subcontractor and supplier that will be affected by this termination: There are no subcontractors to contact. We did inform CSL Behring who is the supplier of RiaSTAP and an integral part of the FDA IND submission that the contract has been terminated and that we will not be pursuing the clinical trial.

Termination inventory: The TEG6s was in our possession and was mailed out via Fedex on 5/25/2022 and receipt was confirmed to the following address:
Mr. Theo Thomas
U.S. Army Institute of Surgical Research
3698 Chambers Pass, BHT2 Bldg 3610
JBSA Fort Sam Houston, TX 78234

All consumables that were used with the TEG6s kit have been consumed during the preclinical studies and are not available for return.

Contents

Abstract
Hemorrhage is the leading cause of preventable death in trauma. Contemporary treatment for damage control resuscitation (DCR) consists of early hemorrhage control, permissive hypotension, asanguineous fluid minimization and fixed ratio blood product transfusion. Widespread interest in utilizing whole blood in pre-hospital care has increased in both civilian and military medicine. While movement towards the use of pre-hospital blood product administration, fractionated component therapy or whole blood, is accelerating with evidence of survival benefits, the physiologic mechanism(s) underlying the benefits of pre-hospital blood administration remains to be fully elucidated. Perhaps more importantly, the logistical challenge of early blood product administration in the pre-hospital environment (e.g. product cost, storage and transport) is a significant problem. Improvements in survival of traumatic hemorrhage are correlated with increasing levels of fibrinogen. Additionally, increased fibrinogen use has been shown to improve mortality in combat casualties. Thus, fibrinogen is an essential component of the coagulation system and low fibrinogen levels are associated with bleeding, transfusion and poor outcomes. We postulated that the utilization of Fibrinogen Supplemented Albumin Resuscitation as an initial fluid/volume resuscitation in the treatment of traumatic hemorrhage will lead to the beneficial outcomes using RiaSTAP and albumin. Our primary scientific aim was to determine the specifics of reconstituting the solution and determine its stability.

Background: Hemorrhage is the leading cause of preventable death in trauma. In austere environments, traumatic induced hemorrhage is responsible for over 90% of potentially survivable combat deaths prior to medical treatment facility (MTF) arrival and 80% of potentially survivable combat-related deaths after arrival to MTFs.[1,2] In a ten year review of United States military injured service members, casualties were commonly found to be coagulopathic upon presentation to MTFs; thus, the presence of coagulopathy at MTF arrival is associated with higher mortality (6% v. 30%, $p<0.001$, n=3,632).[3] Other reports support this increase in mortality with coagulopathy in combat casualties; mortality increases from 4% to 24% based upon the presence of coagulopathy at arrival to an MTF with a relative risk of death of 5.38 (95% CI, 1.55-11.37; $p<0.001$). The presence of coagulopathy is also directly associated with higher base deficits ($\geq$6).[4] Civilian trauma patients with similar injury severity scores (ISS) demonstrate similar outcomes.[5]

Contemporary treatment for damage control resuscitation (DCR) consists of early hemorrhage control, permissive hypotension, asanguineous fluid minimization and fixed ratio blood product transfusion.[6-8] Pre-hospital casualty care now emphasizes early blood product utilization during evacuation to a military and/or civilian MTF. Currently, the United States military Tactical Combat Casualty Care (TCCC) Guidelines recommend whole blood (WB) as the field resuscitation product of choice if available; WB improves casualty survival as compared to resuscitation with traditional asanqineous fluids, such as Hextend® or crystalloid.[9]

Widespread interest in utilizing whole blood in pre-hospital care has increased in both civilian and military medicine; this is highlighted by the Trauma Hemostasis and Oxygenation Research (THOR) Network at its Remote Damage Control Resuscitation (RDCR) Symposium. Additionally, low titer group O whole blood programs have been established for the U.S. Army's Special Forces and the 75th Ranger Regiment, and the Norwegian Naval Special Operation Commandos.[10-12] Civilian trauma resuscitation strategies increasingly emulate military strategies.

As such, civilian trauma systems have also begun to introduce whole blood and include universal low titer group O whole blood within hospitals and pre-hospital environments.[13-15]

While movement towards the use of pre-hospital blood product administration, fractionated component therapy or whole blood, is accelerating with evidence of survival benefits, the physiologic mechanism(s) underlying the benefits of pre-hospital blood administration remains to be fully elucidated.[16-18] Perhaps more importantly, the logistical challenge of early blood product administration in the pre-hospital environment (e.g. product cost, storage and transport) is a significant problem. Recognizing these factors along with the importance of blood component composition on the coagulation system and the metabolic status of the patient upon arrival to hospital care, the Department of Defense has raised its interest in developing non-blood based multifunctional resuscitation fluids (MRF) that will provide shock/metabolic resuscitation and maintain hemostatic functions.[19]

The Naval Medical Research Unit - San Antonio (NAMRU-SA) has reported that the pre-hospital administration of whole blood (the TCCC gold standard) as compared to Hextend® (currently the most commonly used TCCC fluid) prevents coagulopathy at MTF/hospital arrival, whereas Hextend® provides equivalent restoration of acid-base status. The NAMRU-SA non-human primate study demonstrated that oxygen delivery is likely preload-dependent (e.g. intravascular volume) rather than oxygen carrying capacity-dependent (e.g. hemoglobin) because life-threatening anemia is infrequent in this setting.[20] A prospective study at ten level one trauma centers reported the median interquartile range hemoglobin at hospital arrival was 11.5 g/dL (9.9-13.1).[21]

Notably, resuscitation strategies that maximize the ratio of fresh frozen plasma to red blood cells (RBCs) are associated with lower mortality.[21] Additionally, a ten-year review of transfusions in Operation Enduring and Iraqi Freedom is consistent with the aforementioned civilian observation: Higher ratios of platelets and plasma to pRBCs portended a survival benefit over ratios favoring RBCs.[22] Furthermore, a review at two combat support hospitals during Operation Iraqi Freedom (OIF) revealed that the mean ± SD hemoglobin at MTF arrival for patients who required a massive transfusion was 9.8±2.8 g/dL (n=43) and 10.9±2.7 g/dL (n=196), and survival at both facilities was significantly improved with an increased ratio of fibrinogen administration.[23] If the majority of casualties have a MTF/hospital arrival hemoglobin near or greater than 10 g/dL, and higher survival is associated with higher ratios of plasma, platelets, and fibrinogen to pRBCs ratios, the need for oxygen carrying capacity as part of early resuscitation may not be essential for most patients, at least under current civilian and observed military transport times in Iraq and Afghanistan. Indeed, the concept of using albumin as an early colloid for the resuscitation of traumatic hemorrhagic shock, possibly supplemented with fibrinogen and Tranexamic acid (TXA), is supported not only by the NAMRU-SA group's work, but also by reports from the United States Army Institute of Surgical Research (USAISR).[24, 25]

With respect to a coagulation factor supplemented albumin-based resuscitation strategy for traumatic induced coagulopathy (TIC), two factors stand out: TXA and fibrinogen. TXA is a widely used component in the early treatment of traumatic hemorrhage in combat casualties and has become largely accepted in civilian practice based upon clinical studies documenting survival advantage and relatively low risk.[9, 26, 27] This is mechanistically based on TXA's ability to inhibit

clot lysis, as increased clot fibrinolysis is believed to be a major contributor to morbidity in acute traumatic coagulopathy.[28] The second coagulation component is fibrinogen. Clotting is critically dependent upon fibrinogen. Fibrinogen is cleaved by thrombin to insoluble fibrin strands that are the basis of a stable clot and act as a ligand for platelets, localizing platelet aggregation to the clot. Fibrinogen has been known for over two decades as one of the first coagulation proteins to fall to critically low levels during major blood loss and is an important component of the coagulopathy of trauma. Thus, a significantly lower level of fibrinogen at presentation (1.6 g/L vs. 2.4 g/L; $p<0.001$) is associated with clinical coagulopathy and hypofibrinogenemia, and is an independent predictor of mortality.[29-31] Furthermore, improvements in survival of traumatic hemorrhage are correlated with increasing levels of fibrinogen.[31,32] Additionally, increased fibrinogen use has been shown to improve mortality in combat casualties.[23] Thus, fibrinogen is an essential component of the coagulation system and low fibrinogen levels are associated with bleeding, transfusion and poor outcomes. In addition, fibrinogen is under active investigation in Europe as a stand-alone therapy (similar to how TXA is currently being used).[33]

Based on these observations, we postulate that the utilization of Fibrinogen Supplemented Albumin Resuscitation (FAR) as an initial fluid/volume resuscitation in the treatment of traumatic hemorrhage will lead to the following beneficial outcomes: 1) Low administered volume with maximal intravascular expansion, through osmolar properties, and hence augmented preload to support oxygen delivery and metabolic stabilization; and 2) Early prevention, mitigation and treatment of TIC. Both arrival coagulation status and base deficit correlate with casualty and civilian trauma mortality and hence are valid targets to improve survival.[3-5] Data from human and animal studies support these postulated benefits, and hence the rationale to attempt to improve survival using an albumin based resuscitation fluid that incorporates fibrinogen given in coordination with TXA.[20, 23-27, 32, 33]

Albumin and RiaSTAP™ (fibrinogen concentrate) are FDA approved therapeutics, thus streamlining protocol implementation and avoiding the issues that would complicate the approval and use of a new drug. A prospective, randomized investigation of FAR as an initial resuscitation fluid for patients with severe trauma and traumatic hemorrhagic shock is therefore warranted in order to validate a newly formulated "off-the-shelf" resuscitation strategy that improves outcomes and enhances therapeutic logistical feasible.

Overarching study objective: Was to evaluate of the use of FAR as the resuscitation fluid in severely injured trauma patients.

Hypothesis: The initial resuscitation of trauma patients in severe hemorrhagic shock with FAR will attenuate trauma induced coagulopathy and improve the metabolic recovery from hemorrhagic shock compared to standard of care, crystalloid resuscitation.

Preclinical Study Methodology and Results:

The preclinical studies were designed to elucidate the reconstitution time for 1 vial of RiaSTAP on its own and in addition to being mixed with 25% Albumin. Sequentially with each reconstitution the associated fibrinogen activity would be measured.

Methodology: Sedimentation macros were observed by eye using 25% albumin and 1 vial of CSL Behring RiaSTAP in sterile saline solution.

Results: The reconstitution time for RiaSTAP alone was observed to be: 7 minutes, 8 minutes and 21 minutes for three separate reconstitutions, providing an average reconstitution time of 12 minutes. In the originally proposed RASCAL protocol, this would have equated to an average 12 minute delay until RiaSTAP would have been ready to be added to 25% Albumin in a 500cc infusion bag; effectively delaying the start of the resuscitation by an average excess of 12 minutes. Additionally, though not overly complicated, the multi-step procedure to prepare RASCAL was: Reconstitute RiaSTAP (average 12 minutes), transfer 25% Albumin from bottles to infusion bag, transfer reconstituted RiaSTAP to infusion bag and then hang for infusion was considered a logistical liability even in a hospital Emergency Department setting and not optimal for a forward deployed initial resuscitation fluid in addition to solubility issues of this mixture (Figure 1). During reconstition, sedimentation was observed to happen in all samples when RiaSTAP and 25% Albumin was reconstituted. Sedimentation micros using UV-VIS (405nm fibrinogen, 204nm albumin), and fibrinogen activity using a thromboelastogram (TEG6s) haemonetics hemostasis analyzer system were not able to be completed given that the RiaSTAP reconstitution time alone was longer than expected, variable, inconsistent and when mixed with 25% albumin resulted in significant macro sedimentation (See Figure 1). Thus making this mixture combination not feasible.

Figure 1. Representative image of sedimentation during RiaSTAP reconstitution (25% albumin + RiaSTAP).

Recommendations: Succeeding these preclinical results the project contract was modified and approved to account for the stability findings and non-clinically relevant time of reconstitution that would ultimately delay resuscitation; however, the constituted RASCAL was deemed inappropriate for infusion related to the sedimentation. The new treatment protocol, and contract amendment, was changed to the initiation of 25% Albumin resuscitation while RiaSTAP is reconstituted. Then the transition to RiaSTAP infusion once it is reconstituted followed by resumption of 25% Albumin once RiaSTAP is delivered if a single IV exists, or continuation of

25% Albumin via the initial IV site with parallel infusion of RiaSTAP (once reconstituted) via a 2nd IV (see Figure 2). Specifically this recommendation for the single IV administration, normal saline solution (NSS) and less than 20cc will be used to flush the IV. Both constructs eliminate the logistical and time issues identified in the preclinical studies as well as avoid the identified stability issues while remaining consistent with the scientific foundations of the originally proposed and funded project.

Figure 2. Proposed options for resuscitation using RiaSTAP and 24% albumin A) Single IV Access in which the infusion is done in a series; and B) Dual IV Access where infusions are done in parallel.

Clinical Trial Design

The study will be an EFIC study with patient enrollment occurring under FDA regulation 21CFR50.24. The reported "field" vitals will determine eligibility prior to arrival to the ED; if eligible the patient will be randomized prior to arriving to the ED (Figure 3). Alternatively, at arrival to the ED eligibility will be reevaluated and in addition to vital signs and absolute shock index (SI), will include determination change in SI with an increase in SI $\geq$ 0.3 also making a patient eligible for enrollment. At the time of randomization , 2 vials of RiaSTAP (total of 2.6gm

of fibrinogen) will be reconstituted in a total of 200cc of D5W and administered in series or, if 2 IVs are present, in parallel (once reconstituted) with 500cc of 25% albumin solution for a total volume of 700cc. When the patient arrives to the ED, initial blood samples will be drawn and eligibility (inclusion and exclusion criteria) rapidly confirmed by the Attending Trauma Surgeon, or in their absence the Attending Emergency Medicine Physician; new eligibility and enrollment will also occur if not determined pre-arrival. If new eligibility is determined, patient will be randomized and if randomized to for the study treatment, the 500cc of 25% albumin will be administered in series, or parallel, with 2 vials of RiaSTAP as described above in Figure 2. Those enrolled and randomized to the study (FAR) resuscitation will have any crystalloid infusion stopped and immediately begin receiving FAR; those randomized to crystalloid will proceed with institution CPG guided crystalloid resuscitation.

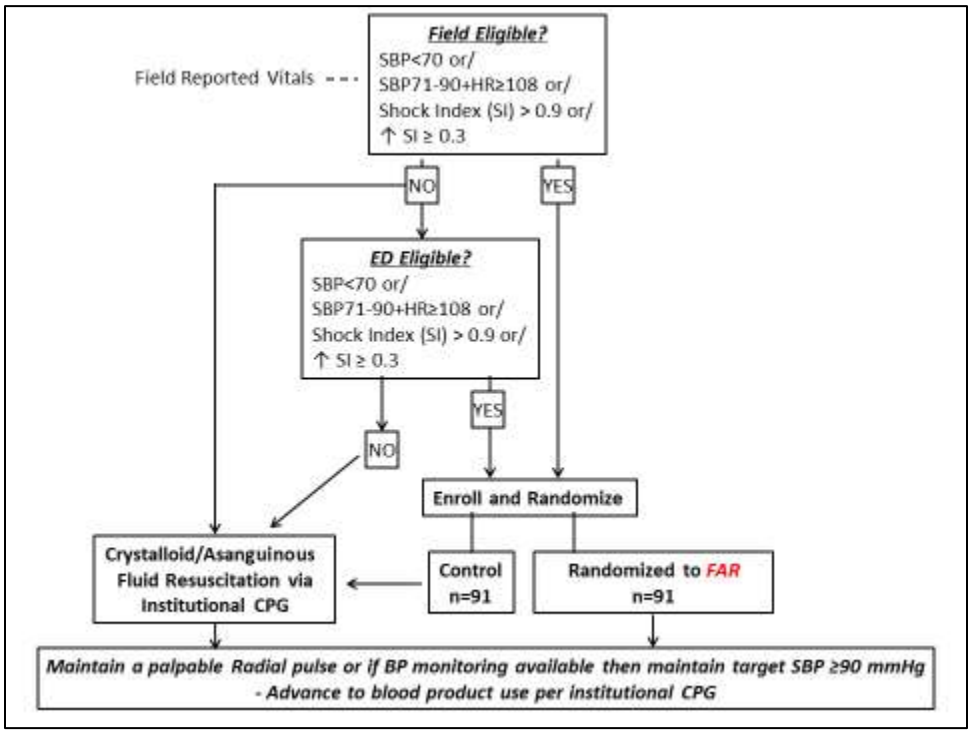

Figure 3. Proposed clinical trial subject eligibility.

Incomplete Tasks

Major Task 2: EFIC IRB and FDA IND Approval: The contracted clinical trial proposal, as per the contract modification in FY-21, was submitted to our institution's scientific review committee (institutional requirement) and is was then subsequently submitted to our Institutional Review Board. It was currently undergoing revisions during the time of contract termination. In

addition, we were able to secure an agreement with CSL Behring, manufacturer of RiaSTAP, for this study which allowed us to prepare all documents for FDA IND submission.

Problems and Complications: The initiation of Major Task 2 was delayed because of the results of the pre-clinical experiments that mandated a change from the originally proposed and contracted RASCAL solution to the separate infusion of 25% Albumin and RiaSTAP. Subsequently, the unforeseen requirement for social distancing, cancellation of public events and closures of business has and continues compound the set back the community outreach required for the EFIC IRB. The COVID-19 pandemic has caused delays and continues to cause delays in initiating the preclinical studies the first year. This has related to supply delivery which appear resolved and currently is due to the requirements for social distancing and continued partial closure of the research facilities for bench studies. This is a fluid situation and we are prepared to initiate as soon as global restrictions are lifted to permit experiments to commence.

Furthermore, the COVID-19 pandemic has resulted in healthcare efforts to be directed completely at the treatment and preparation for infected patient care. This along with the other implications of social distancing, etc. of the pandemic have hindered the finalization of plans and agreements.

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