12.1 STUDY INFORMATION

12.1.1 Protocol

Study No.: WLI-4306-HAV

Clinical Investigation – Havana, Cuba

1. **Title:** SeraSeal, A Primary hemostatic Agent, Clinical Study

2. **Principal Investigator:** Ignacio A. Morales Diaz, MD
Mercedes Bandera Ramirez, MD
Jose A. Paraza Corea, MD
Vladimir Curbelo Serrano, MD
Carlos Enrique Pascual Fries, MD
Jose de J. Rego Hernandez, MD
Calixto Valedes Perez, MD
Nelson Chirino Carreno, MD
Rodolfo Arozarena Fundora, MD
Orestes Jose Ponce Gonzdez, MD

3. **Monitors:** Julian Perez Pena, MD
Isis Yera Alos, MD
Liuba Alonso Carbonell, MD

4. **Identification of the Research:** This study focuses on treatment of bleeding wounds

4. **Location:** Joaquin Albarran Hospital, Havana, Cuba
Salvador Allende Hospital, Havana, Cuba
National Institute for Cardio-Vascular Surgery, Havana, Cuba

5. **Research Plan:**

5.1 Purpose: SeraSeal was designed as a primary hemostatic agent to control all forms of bleeding. In this study, SeraSeal is to be applied from the initial incision, and throughout the entire surgical procedure to closing without any intervention of standard surgical methods to control bleeding. Only one delivery system, syringe or spray of SeraSeal is to be used for that particular surgical case without any cross-over.

5.2 Hypothesis or Research Questions or Objectives: The null hypothesis is that SeraSeal will not be more effective than surgical modalities to control bleeding in surgical cases historically known to take more than 6 minutes to control all of the hemorrhages. The alternative hypothesis is that SeraSeal will have a collective time to hemostasis of less than 5 minutes in 95% of the surgical cases.

5.3 Significance: SeraSeal shows promise of being a superior hemostatic agent applicable to wounds, surgical operations in which hemostasis is difficult (hepatic, renal and splenic trauma, debridement, burns, skin grafting, endoscopic procedures), control of epistaxis, esophageal variceal bleeding, bleeding peptic ulcers, and bleeding associated with proctitis and cystitis. For some of these conditions, there is no entirely satisfactory treatment.

5.4 Relevance: The availability of an easy to use, stable, safe, effective hemostatic agent, would be a tremendous advantage to the trauma patient (surgical or accidental), possibly reducing morbidity and mortality significantly, and conserving surgical and blood resources.

5.5 Background and Review of Literature:

Problem with Bleeding Wounds

Bleeding is still a major cause of morbidity and mortality in wounds. Thirteen percent of patients suffering acute trauma, die of bleeding[1-11]. Of the total deaths occurring on the battlefield, eight-five percent are due to blood loss[12]. Particularly vulnerable areas are the face, groin, pelvis and extremities, areas not protected by body armor. In head and neck trauma, uncontrolled bleeding can cause airway compromise and asphxiation[13]. In trauma survivors, inability to control bleeding leads to blood transfusion, increased complication rates, immunosuppression, inability to generate red blood cells, and prolonged time in the hospital. The ideal solution to these problems is to control the bleeding in the first place. The limiting factor in performing burn debridement of chronic wounds results in bleeding, which is essentially an end point for debridement, because there is no satisfactory way of controlling bleeding in granulation tissue. This results in repeated debridement. Bleeding is a major cause of skin graft loss and flap necrosis. Outside of the hospital, the paramedic has only compresses to control bleeding. A quick, effective, and safe device to control bleeding, would be extremely beneficial.

Hemostatic agents in wounds

Commercially available topical thrombogenic agents include microfibillar collagen (Avitene®), collagen sponges (Gelfoam®, Instat®), and fibrin sealants (Tisseel®, AFTA®, ViGuard®). Topical collagen preparations have been available for more than 25 years and have a limited ability to facilitate clotting. When hemorrhage is excessive or the patient's clotting factors are inhibited, they are less effective.

Fibrin Sealants

These are prepared from homolgous, pooled human or bovine fibrinogen, Factor XII, and thrombin that must be combined to make the fibrin. Other components include calcium to provide activation of Factor XIII, fibronectin to aid in adhesion, and an inhibitor of fibrinolysis to prolong fibrin clot life. Fibrin sealants have shown great promise in controlling wound hemorrhage. Fibrin sealants have been shown to reduce operating time, blood loss, and do not appear to interfere with healing[14]. When applied to a ballistic wound as a dry dressing, the sealant reduced blood loss by 64%[15].

Problems with current tissue sealants

Several problems exist with the commercially available fibrin sealants: They are a two part system of fibrin and thrombin, which must be mixed at operation or trauma setting, adding to the operative time and delaying transportation to a stable operative setting. Previously, the FDA withheld approval from European products because they used pooled human plasma as a product source[16]. The fibrin sealants are somewhat difficult to use, as they must be mixed from four components, a process which takes approximately 15 minutes, and then must be used within four hours. If they are to be used endoscopically, they must be placed through a dual luman catheter and mixed in situ[17]. Their viscosity makes application difficult via catheters and in small areas, and limits methods of application such as sprays, or foams, which would be useful for large wounds. The time between mixing and hemostasis can be several minutes, typically 3-5 minutes, further slowing operation repair[18]. The incorporation of fibrin sealants into a dry dressing has reduced some of these problems. The strength of the clot formation, when utilizing a dry dressing, can be sub-optimal. These shortcomings limit the utility of fibrin sealants for hemostasis in the battlefield, emergency response teams such as paramedics, and in the surgical arena.

Bovine topical thrombin in most cases, is a heterolgous plasma thrombin concentrate that has been used for hemostasis since the 1940's. Some commercial bovine thrombin preparations are highly immunogenic, and appear to be associated with an increased risk for adverse clinical outcomes during subsequent surgical procedures[19]. Antibody formation and anaphylaxis (rates 0.5 to 5.8%) have also been seen with Factor V and aprotinin, a polyvalent proteinase inhibitor isolated from bovine lungs, and used alone to enhance clot stability, or in combination with fibrin sealants[20,21]. These antibodies may interfere with the heparin anti-Xa assay,

thereby complicating the monitoring of anticoagulant therapy[22]. The commercially available product, Tisseel VH fibrin sealant, contains bovine aprotinin, resulting in the risk of hypersensitivity[23].

What this study will determine

This study is designed to determine whether SeraSeal is effective as a primary hemostatic agent, and safe in achieving hemostasis in surgical wounds or other emergent hemorrhagic episodes. The effectiveness will be determined by achieving hemostasis after application of the product. The safety will be determined by monitoring outcomes from application: recurrence of bleeding, infection, lack of healing, and presence of hypersensitivity reactions, e.g. pruritus, fever, rash, anaphylaxis.

Characteristics of SeraSeal

SeraSeal is a bovine protein-derived accelerator of hemostasis interacting with the intrinsic and extrinsic pathways. It is obtained from pathogen and prion free cows. SeraSeal can be provided as a solution, spray, foam, incorporated into an absorbable dressing or a non-absorbable removable dressing. Its active ingredients are Factors IIa, VIIa, IXa, and Xa.

Toxicity of SeraSeal

A total dosage level of SeraSeal over 13,000 IU/Kg (2.4 mg protein) in leporids and canines had no adverse effects[24,25]. The total expected treatment levels to be used in this study is less than 1,000 IU/Kg, a thirteen fold lower level than the no toxic effect. Thus, drug related toxicity is not expected to be seen in this clinical study. Cell culture studies reveal that SeraSeal is not directly toxic for any cells tested.

5.6 Research Design and Methods: This clinical trail will be a non-randomized, unblinded study where SeraSeal will be used as a liquid, administered by a syringe (300 IU/0.1ml) or spray (210 IU/pump), and compared to standard surgical methods to control bleeding in historically known surgical cases taking more than 6 minutes to control all of the hemorrhages. The intent-to-treat patients will be placed into either the SeraSeal syringe treatment group, where bleeding from medium to large blood vessels may occur, or the SeraSeal spray treatment group, where only capillary to small blood vessels would be treated with the investigational product.
Patients receiving treatment at Joaquin Albarran Hospital, Salvador Allende Hospital, and National Institute for Cardio-Vascular Surgery, will serve as study volunteers. Prior to enrollment in the study, an initial screening test and subsequent studies will take place, including: informed consent, collection and recording of subject demographic information, medical history, current medications, assessment of inclusion/exclusion criteria, physical examination, with blood draws for hematological assessment for bleeding and time to hemostasis. The location of the study wounds will be skin-skin. The study wound sites will involve bone and soft tissue.

The primary endpoint in this clinical trial is hemostasis. A Secondary endpoint is the number of surgical cases without a therapeutic break from SeraSeal to a standard surgical modality to control the bleeding. There are 10 designated surgical faculty members from General Surgery, Vascular, Obstetrics and Gynecology participating in this study, with a minimum of 10 surgical procedures being studied. All of the participating surgeons are highly skilled and have extensive expertise in their surgical specialty.

In this trial, the dosage level of SeraSeal vial is 300 IU/0.1ml and 210 IU/pump in the spray, with a maximum expected dosage of 10,000 IU, 20 times less than the pre-clinical toxicity studies. After 1-2 minutes, when hemostasis is achieved, the excess SeraSeal will either be removed through suction or absorption onto a surgical sponge. A time to hemostasis is measured from the time when the product is first applied to the wound, until no bleeding or oozing is observed from the wound. A nurse will use a stopwatch to record the time to hemostasis. SeraSeal will be used from the initial incision and through out the entire surgical procedure, and the time to hemostasis for each bleed will be measured, then totaled for the entire surgery. SeraSeal is expected to achieve a total hemostasis time under 5 minutes. Stopping criteria for treatment will be when hemostasis fails to be achieved after 3 applications for a particular wound, the collective time to hemostasis exceeds 5 minutes, the patient experiences a serious adverse event, or at the surgeons discretion. If this should happen, the patient will be switched over to standard surgical practices.

The patients will be monitored for post-operative bleeding and overall wound healing for those wounds that can be observed. A base line hemoglobin (HGB), hematocrit (HCT), prothrombin time (PT), partial thromboplastin time (PTT) be drawn, and measured at 24 to 48 hours after surgery. The patients will be observed and assessed for a minimum of 48 hours after application of SeraSeal for signs of post-operative bleeding, and hypersensitivity to the investigational product throughout the hospitalization period. They will be assessed for, but not limited to, skin rash; wheezing; anaphylaxis; pruritus; prolong coagulation tests; delayed healing or infection through quantitative cultures. In the event there is progression of edema leading to respiratory distress, anaphylaxis, or shock due to blood loss, a crash cart and/or any additional standard and customary treatment(s), will be available. This study will not limit the standard of care performed at the four investigational hospitals. The following components of wound care are "standard practice" at each of the hospitals: daily dressing changes; quantitative cultures when infection may be present; routine blood tests. All enrolled study subjects may request to be withdrawn from the study at any time, and will be terminated from the study and provided standard of care. All adverse events will be recorded according to whether the event was unrelated, unlikely, possibly or probably related to the study treatment.

Table 1: Schedule of Assessments

Test	Pre-Op	Surgery	4-8 Hrs Post-Op	24-48 Hrs Post-Op
Informed Consent	X			
History		X		
Physical Examination	X		X	X
HGB	X		X^{+}	X$^{\#}$
HCT	X		X^{+}	X$^{\#}$
PT	X		X^{+}	X$^{\#}$
PTT	X		X^{+}	X$^{\#}$
ECG	X		X^{+}	X$^{\#}$
Radiological work-up	X		X^{+}	X$^{\#}$
Adverse Events		X	X	X$^{\#}$
Effectiveness		X	X	X
Safety		X	X	X

+ measured as often as needed.
# monitored beyond 48 hours and as needed

5.7 Inclusion/Exclusion Criteria

4.1 Inclusive criteria:

1. Patients over the age of 18
2. Patients of both sexes
3. Patients who are on anticoagulant therapy
4. Patients with a history of blood disorders

4.2 Exclusion criteria:

1. Patients who are pregnant
2. Patients suffering from mental disease
3. Patients with the HIV virus
4. Patients with a history of anaphylaxis
5. Patients receiving treatment with beta-blockers
6. Patients with sensitivity to bovine proteins
7. Patients with signs of infection
8. Patients who during surgery show signs of sepsis
9. Patients with sensitivity to iodine.

5.8 Source of Research Material

5.9 Number of Subjects: A total of 120 subjects, are to participate in this study.

6.0 Recruitment: Subjects will be selected from within each participating surgical department at Joaquin Albarran Hospital, Salvador Allende Hospital, and National Institute for Cardio-Vascular Surgery. The faculty from each hospital will obtain an informed consent, prior to their participation in the study. No promotional fliers are planned. The consent form will be provided by both hospitals in Spanish. Subjects will not be paid for participation in this study.

6.1 Benefits: The possible benefit of the patient's participation in this study is a faster time to clotting, less blood loss, faster wound healing, and less loss of tissue. However, the participant should understand there is no guarantee or promise that they will receive any benefit from this study, other than knowing that the information may help future patients.

6.2 Risks: The risks of application of SeraSeal are theoretical. These risks may be bleeding, infection, and allergic reaction. Anaphylaxis is a possible risk, but the incidence is not clearly known. Aprotinin, a bovine protein derived from the bovine lung, is used in cardiac surgery and is the component of some fibrin sealants. It has an anaphylaxis rate of 2.5% on second exposure, with a mean interval of 1,654 days (range, 16-7,136 days)[26]. SeraSeal contains no aprotinin. Bovine gelatin is a component of some vaccines and anaphylaxis has been reported with this protein[27]. Other bovine proteins for which anaphylaxis and hypersensitivity reactions have been reported, are bovine serum albumin[28], thrombin[29,30], recombinant Factor VIII[31], insulin[32], and tissue sealants[33]. The risk of anaphylaxis appears to be lower for topical devices than injected ones, and repeated administration with a break, as proposed in this study, also appears to have a lower incidence of anaphylaxis[24]. We surmise that anaphylaxis may be a risk of application of SeraSeal. Measures to reduce and treat anaphylaxis is to exclude those patients who are sensitive to bovine proteins from the study, and to provide a crash cart, and/or any additional standard and customary treatment(s). One of the purposes of this study is to determine that risk. Reports of an autoimmune reaction to fibrin sealants containing bovine Factor V mimicking hemophilia have been reported. SeraSeal does not contain Factor V.

6.3 Safeguards for Protecting Subjects: The risks of SeraSeal treatment are minimized by the patient selection process. Should an allergic reaction occur, the participant will be withdrawn from the study and standard of care administered. Physicians, nurses, and technicians, will be on hand for all procedures. Patients will be monitored through routine post-operative care. Should any adverse event occur during treatment, immediate intervention is possible. A crash cart is kept in the treatment room and on the hospital floor. Patient records will be kept confidential.

6.4 Alternatives: Alternatives to SeraSeal are the application of no hemostatic agents, electric cauterization, ligature, pressure dressings, collagen dressings, and the use of fibrin sealants. The application of other dressings containing proteins, carries the risk of allergic reaction.

7.0 Data Analysis:

7.1 Data Collection: There will be one study form for each patient. The surgery form will have all of the outcome data and adverse events. Once the patient's surgical procedure qualifies for the study , the patient will be asked to participate in the clinical trial by the participating investigator surgeon. The only patients that will require special action are the SeraSeal patients. For those patients the need for SeraSeal will be made known only to the attending surgeon and/or fellows, and made available to the OR room. At the time of the pre-op exam, the inclusion/exclusion of participation in the study will be filled out. The monitor board member will collect all data forms and will be responsible for evaluating them for completeness and accuracy, except for time to hemostasis supplied by the surgeon.

7.2 Statistical Analysis: We will compare continuous outcomes such as time to hemostasis (primary outcome), and therapeutical breaks from SeraSeal to standard surgical modalities to control bleeding. This study will use a relatively simple method of analyzing the data generated by employing a non-parametric statistical analysis,

using the chi-square distribution.

For continuous lab outcomes measured on two occasions (base, 24- 48 hours), we will use repeated measure analysis of variance methods to compare mean lab values for conventional versus SeraSeal® overall.

For binary outcomes, (including adverse event outcomes) that are not quantified or that may be dichotomized for clinical convenience and interpretability such as fever (yes or no) or pruritus (yes or no) we will use chi-square methods to compare conventional versus SeraSeal treatment proportions overall.

Sample size: The sample size is based on the primary outcome, total time to hemostasis. We determined that 95% of all surgical cases, the collective time to hemostasis will occur within 5 minutes for a given surgical procedure. Based on an average 175 surgical cases per day, a confidence level of 95%, with a confidence interval of 5, the required total samples size is 120 patients.

8. Duration of Study: Subject participation is 30 days post study procedure. Expected enrollment time is 3 months.

9. Bibliography:

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12.1.3 Monitor Board, Patient Information Sheet, Sample Consent Form

WORTHAM LABORATORIES, INC.

Monitor Board

Julian Perez Peña, M.D.
Ministry of Public Health
Center for the Development of
Pharmacoepidemiology

Isis Belkis Year Alos, M.D.
Ministry of Public Health
Medical and Surgical Investigation Center

Luiba Alonso Carbonell, M.D.
Ministry of Public Health
Medical and Surgical Investigation Center